UNIVERSITAT DE BARCELONA

Noves evidències de la intolerància a la histamina

Sònia Sánchez Pérez

ADVERTIMENT. La consulta d'aquesta tesi queda condicionada a l'acceptació de les següents condicions d'ús: La difusió d'aquesta tesi per mitjà del servei TDX (**www.tdx.cat**) i a través del Dipòsit Digital de la UB (**diposit.ub.edu**) ha estat autoritzada pels titulars dels drets de propietat intel·lectual únicament per a usos privats emmarcats en activitats d'investigació i docència. No s'autoritza la seva reproducció amb finalitats de lucre ni la seva difusió i posada a disposició des d'un lloc aliè al servei TDX ni al Dipòsit Digital de la UB. No s'autoritza la presentació del seu contingut en una finestra o marc aliè a TDX o al Dipòsit Digital de la UB (framing). Aquesta reserva de drets afecta tant al resum de presentació de la tesi com als seus continguts. En la utilització o cita de parts de la tesi és obligat indicar el nom de la persona autora.

ADVERTENCIA. La consulta de esta tesis queda condicionada a la aceptación de las siguientes condiciones de uso: La difusión de esta tesis por medio del servicio TDR (**www.tdx.cat**) y a través del Repositorio Digital de la UB (**diposit.ub.edu**) ha sido autorizada por los titulares de los derechos de propiedad intelectual únicamente para usos privados enmarcados en actividades de investigación y docencia. No se autoriza su reproducción con finalidades de lucro ni su difusión y puesta a disposición desde un sitio ajeno al servicio TDR o al Repositorio Digital de la UB. No se autoriza la presentación de su contenido en una ventana o marco ajeno a TDR o al Repositorio Digital de la UB (framing). Esta reserva de derechos afecta tanto al resumen de presentación de la tesis como a sus contenidos. En la utilización o cita de partes de la tesis es obligado indicar el nombre de la persona autora.

WARNING. On having consulted this thesis you're accepting the following use conditions: Spreading this thesis by the TDX (**www.tdx.cat**) service and by the UB Digital Repository (**diposit.ub.edu**) has been authorized by the titular of the intellectual property rights only for private uses placed in investigation and teaching activities. Reproduction with lucrative aims is not authorized nor its spreading and availability from a site foreign to the TDX service or to the UB Digital Repository. Introducing its content in a window or frame foreign to the TDX service or to the UB Digital Repository is not authorized (framing). Those rights affect to the presentation summary of the thesis as well as to its contents. In the using or citation of parts of the thesis it's obliged to indicate the name of the author.

Departament de Nutrició, Ciències de l'Alimentació i Gastronomia
Facultat de Farmàcia i Ciències de l'Alimentació
Universitat de Barcelona

NOVES EVIDÈNCIES DE LA INTOLERÀNCIA A LA HISTAMINA

Directores:

Dra. M. Carmen Vidal Carou

Dra. M. Luz Latorre Moratalla

Tutora:

Dra. M. Teresa Veciana Nogués

SÒNIA SÁNCHEZ PÉREZ

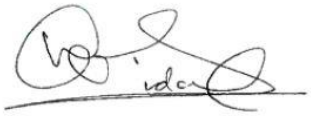
2022

Departament de Nutrició, Ciències de l'Alimentació i Gastronomia
Facultat de Farmàcia i Ciències de l'Alimentació
Universitat de Barcelona

Programa de Doctorat
Alimentació i Nutrició

NOVES EVIDÈNCIES DE LA INTOLERÀNCIA A LA HISTAMINA

Memòria presentada per Sònia Sánchez Pérez
per optar al títol de doctora per la Universitat de Barcelona



Dra. M. Carmen Vidal Carou
Directora

Dra. M. Luz Latorre Moratalla
Directora

Dra. M. Teresa Veciana Nogués
Tutora

Sònia Sánchez Pérez
Doctoranda

SÒNIA SÁNCHEZ PÉREZ
2022

FINANÇAMENT
I SUPORT INSTITUCIONAL

Direcció General de Recerca, Generalitat de Catalunya
SGR-2017-1476

Ministerio de Ciencia e Innovación
Ajut PID2020-117670RB-C21 financiat per MCIN/ AEI/10.13039/501100011033

Universitat de Barcelona. Ajuts predoctorals per a doctorands (APIF-2018)

Institut de Recerca en Nutrició i Seguretat Alimentària (INSA·UB)

Xarxa d'Innovació Alimentària (XIA)

Campus de l'Alimentació de Torribera

AGRAÏMENTS

Aquesta tesi doctoral ha estat possible gràcies a l'esforç de tots els membres del grup de recerca d'Amines i Poliamines Bioactives dels Aliments. Especialment, voldria agrair a les meves directores, la Maricarmen i la Mariluz, la seva constant implicació perquè la tesi arribés a bon port. Maricarmen, gràcies per la teva exigència, per fer-me un lloc en aquest grup i per contar amb mi en els projectes que vindran. A la Mariluz, pel caliu que m'has donat en tot moment i per portar-me de la mà en aquest camí. A la Teresa, per la teva bona disposició i ganes de fer i, a l'Oriol per la teva implicació i dedicació en aquesta tesi com si fos la teva. També, agrair a l'Abel, per sempre compartir els teus coneixements amb mi.

M'agradaria agrair també, al Salva i a la Nelly haver compartit amb mi el vostre "somni europeu" i fer-me una més de la vostra família. A la Judit per aguantar el meu final de tesi i fer-me'l més suportable i, a l'Adriana i al Ricard per compartir les vostres experiències amb mi.

Al lab 36 (la Bea, la Judit, la Berta i l'Alba), per compartir innumerables esmorzars i dinars sempre plens de bon humor. Especialment, agrair a la Bea el bon retrobament que hem tingut després de tants anys i els bons consells que m'has donat quan els he necessitat. En general, agrair a tots els "Torriberos", les diferents sortides a cases rurals, les calçotades, els dinars i els sopars compartits.

Voldria també donar les gràcies al meu company de viatge, el Xabi, per creure en mi i encoratjar-me en tot moment. I, per descomptat a la meva família per estar sempre presents i confiar en tot el faig.

ÍNDEX

Resum | Abstract 1

1. Introducció 7

1.1. Histamina i altres amines biògenes en els aliments . . 8

1.2. Metabolisme de la histamina i altres amines biògenes en l'organisme 15

1.3. Intolerància a la histamina 17

1.3.1. Etiologia de la intolerància a la histamina . . . 19

1.3.1.1. Origen genètic 21

1.3.1.2. Origen patològic 22

1.3.1.3. Influència de fàrmacs i altres substàncies . . 23

1.3.1.4. Disbiosi intestinal 24

1.3.2. Diagnòstic de la intolerància a la histamina . . 25

1.3.2.1. Activitat de l'enzim DAO en sang i en mucosa intestinal 26

1.3.2.2. Identificació de SNPs del gen que codifica l'enzim DAO 27

1.3.2.3. Prova cutània: *histamine 50-skin-prick test* . . 28

1.3.2.4. Test de provocació oral de la histamina . . 28

1.3.2.5. Determinació d'histamina i els seus metabòlits en orina 29

1.3.3. Gestió dietètica de la intolerància a la histamina . . 29

1.3.3.1. Dieta baixa en histamina 29

1.3.3.2. Suplementació amb enzim DAO exogen . . 35

Publicació 1: Concept, aetiology and current diagnostic and treatment approaches of histamine intolerance.

2. Plantejament i objectius 91

3. Resultats · · · · · · · · · **97**

Àmbit 1: Intolerància a la histamina i dieta

3.1. Estudi de la presència d'histamina i altres amines biògenes en aliments d'origen vegetal · · · · · · 103
Publicació 2: Biogenic amines in plant-origin foods: Are they frequently underestimated in low-histamine diets?

3.2. Revisió crítica de les dietes baixes en histamina en base als continguts d'histamina i altres amines biògenes dels aliments exclosos · 125
Publicació 3: Low-histamine diets: Is the exclusion of foods justified by their histamine content?

3.3. Influència d'altres amines biògenes en la degradació de la histamina per part de l'enzim DAO · · · · · 139
Publicació 4: The rate of histamine degradation by diamine oxidase is compromised by other biogenic amines

Àmbit 2: Intolerància a la histamina i microbiota intestinal

3.4. Estudi del perfil i composició de la microbiota intestinal d'individus amb símptomes d'intolerància a la histamina i la seva comparació amb els d'una població sana · · · · · 153
Publicació 5: Intestinal dysbiosis in patients with histamine intolerance.

3.5. Avaluació de potencials canvis en el perfil i composició de la microbiota intestinal en un petit subgrup de pacients al llarg de nou mesos de tractament dietètic de la intolerància a la histamina · · 173
Publicació 6: The dietary treatment of histamine intolerance reduces the abundance of some histamine-secreting bacteria of the gut microbiota in histamine intolerant women. A pilot study.

Àmbit 3: Intoleràncía a la histamina i perfil metabolòmic en orina

3.6. Estudi del perfil d'eliminació d'histamina i 1-metilhistamina en orina en individus amb intoleràncía a la histamina i avaluació del seu comportament al llarg del tractament dietètic d'aquesta intoleràncía · · 193

Publicació 7: 1-methylhistamine as a potential biomarker of food histamine intolerance. A pilot study.

4. Discussió · · · · · · · · **211**

5. Conclusions · · · · · · · · **243**

6. Bibliografia · · · · · · · · **249**

7. Annexes · · · · · · · · **269**

7.1. Contribucions científiques derivades de la tesi doctoral · 269

7.2. Altres contribucions científiques · · · · 273

7.3. Evidències de les comunicacions orals i escrites derivades de la tesi doctoral · · · · · · 279

ÍNDEX DE FIGURES

Figura 1. Síntesi d'histamina a partir de la descarboxilació del seu aminoàcid precursor (histidina) · · · · · · 8

Figura 2. Continguts d'amines biògenes (mg/kg) en diferents tipus d'aliments del mercat espanyol. Dades procedents de la base de dades del grup de recerca · · · · · · 14

Figura 3. Principals símptomes de la intolerància a la histamina i localització dels receptors d'histamina en l'organisme · · · 19

Figura 4. Resum del mecanisme d'acció de l'enzim DAO en l'enteròcit i els factors que poden influir en la seva activitat · · 21

Figura 5. Fases proposades pel seguiment d'una dieta baixa en histamina · · · · · · · 31

Figura 6. Resum dels diferents àmbits d'estudi amb els objectius corresponents i les publicacions derivades · · · 99

Figura 7. Llistat dels aliments que s'exclouen de les dietes baixes en histamina i el número de dietes que proposen la seva exclusió 212

Figura 8. Comparació de l'activitat de l'enzim DAO sobre la histamina sola i sobre la histamina quan es troba amb diferents proporcions de putrescina, cadaverina, tiramina, espermidina o espermina 219

Figura 9. Abundància relativa (%) dels diferents fílums trobats en el grup control i en el grup d'intolerants a la histamina · · 222

Figura 10. Abundància relativa (%) de les diferents famílies trobades en el grup control i en el grup d'intolerants a la histamina 223

Figura 11. Abundància relativa (%) de diferents bacteris amb propietats beneficioses per l'organisme · · · · 224

Figura 12. Abundància relativa (%) de diferents bacteris relacionats amb la formació d'histamina · · · · 225

Figura 13. Diagrama de Venn del total de famílies trobades al llarg del tractament dietètic de la intolerància a la histamina · 230

Figura 14. Evolució de l'abundància relativa (%) de diferents bacteris relacionats amb la formació d'histamina observada en individus intolerants al llarg del tractament dietètic de la intolerància a la histamina 232

Figura 15. Distribució dels nivells d'histamina i 1-methilhistamina (µg/g creatinina) en mostres de primera hora del matí d'individus sans i d'intolerants a la histamina · · · · 236

Figura 16. Distribució dels nivells d'histamina i 1-methilhistamina (µg/g creatinina) en mostres de primera hora del matí d'individus Intolerants amb i sense dèficit de DAO sanguini · · 237

ÍNDEX DE TAULES

Taula 1. Bacteris amb capacitat de formar histamina en els aliments 10

Taula 2. Estudis clínics sobre l'eficàcia d'una dieta baixa en histamina en el tractament de símptomes de la intolerància a la histamina 33

ABREVIATURES

AGCC	Àcids grassos de cadena curta
ALDH	Aldehid deshidrogenasa
DAO	Diamino oxidasa
ECDC	*European Centre for Disease Prevention and Control*
EFSA	*European Food Safety Authority*
ELISA	Assaig immunoabsorbent lligat a enzims
FDA	*Food Drug Administration*
FODMAPs	Oligosacàrids, disacàrids, monosacàrids i poliols fermentables
HDC	L-Histidina descarboxilasa
HDU	Unitats degradadores d'histamina
HNMT	Histamina-N-metiltransferasa
IgE	Immunoglobulina E
JCR	*Journal Citation Reports*
K_M	Constant de Michaelis-Menten
MAO	Monoamino oxidasa
MII	Malaltia inflamatoria intestinal
mU	nmol d'amina degradada per minut
PAO	Poliamino oxidasa
PCoA	Ànalisi de coordinades principals
RASFF	*Rapid Alert System for Food and Feed*
RIA	Radio-immuno assaig
SII	Síndrome d'intestí irritable
SNPs	Polimorfismes de nucleòtids simples
SPE	Extracció en fase sòlida
UHPLC-FL	Cromatografia líquida d'alta eficacia i detecció fluorimètrica

RESUM | ABSTRACT

RESUM

[CAT]

La intolerància a la histamina és una reacció d'hipersensibilitat no al·lèrgica que s'observa com a resultat del consum de quantitats normalment tolerables d'histamina en persones susceptibles, principalment per un dèficit en l'activitat de l'enzim diamino oxidasa (DAO) a nivell intestinal. L'enzim DAO és l'encarregat de degradar la histamina procedent de la dieta, de manera que genera un efecte barrera per prevenir el pas d'aquesta amina al sistema circulatori. Davant d'una situació de deficiència d'aquest enzim, la histamina s'absorbeix a plasma donant lloc al quadre clínic d'aquesta intolerància. Les manifestacions clíniques inclouen un ampli ventall de símptomes gastrointestinals (distensió abdominal, diarrea, dolor abdominal i restrenyiment, entre d'altres) i extra-intestinals (cefalees, taquicàrdia, rinorrea, rinitis, pruïja i envermelliment cutani, entre d'altres). En la darrere dècada, la intolerància a la histamina ha esdevingut un tema d'interès creixent, encara que segueix essent necessari sumar evidència científica que ajudi a definir, diagnosticar i gestionar clínicament aquesta intolerància. L'objectiu general d'aquesta tesi doctoral és aportar noves evidències en relació a aquests aspectes de la intolerància a la histamina.

Un primer objectiu d'aquesta tesi va ser aprofundir en la relació entre la intolerància a la histamina i la dieta, així com estudiar altres factors, a part de la presència d'histamina, que podrien ser la causa de l'aparició de la simptomatologia. A partir d'una revisió crítica de les diferents dietes baixes en histamina disponibles en la literatura científica es va observar una clara falta de consens en quant als aliments que han de ser eliminats en el tractament dietètic de la intolerància a la histamina. A més, de tots els aliments exclosos, únicament el 32% són susceptibles de contenir histamina. L'eliminació d'altres aliments que, en principi, no contenen histamina es podria explicar perquè presenten un elevat continguts d'altres amines, principalment, putrescina. Estudis *in vitro* realitzats en el marc d'aquesta tesi doctoral han demostrat que la presència d'altres amines, putrescina (diamino-butà) i cadaverina (diamino-pentà), redueixen significativament la velocitat de degradació de la histamina per l'enzim DAO.

Respecte a l'etiologia de la intoleràncIa a la histamina, el dèficit de DAO pot tenir un origen genètic o adquirit. Aquest últim pot ser degut a la presència de malalties inflamatòries intestinals que limiten la secreció de la DAO, o bé, a l'acció de certs fàrmacs que poden inhibir-la. Un altre possible origen, del qual encara no hi ha una evidència clara, seria l'existència d'una disbiosi o alteració de la microbiota intestinal en aquests individus. Per tant, un altre objectiu d'aquesta tesi va ser estudiar el perfil i la composició de la microbiota intestinal d'individus amb aquesta intoleràncIa i comparar-los amb els d'una població sana. En aquest sentit, es va observar que els pacients intolerants presentaven una disbiosi intestinal, caracteritzada per una major abundància de bacteris descrits com a formadors d'histamina i una menor proporció d'aquells associats a una bona salut intestinal. A més, el perfil microbià en aquests individus intolerants va diferir significativament i era més heterogeni (beta-diversitat) que el de la població control. Addicionalment, es va estudiar en un petit subgrup de pacients, els possibles canvis en la composició de la microbiota intestinal al llarg de nou mesos de tractament dietètic, observant una disminució significativa de certs bacteris formadors d'histamina (*Morganellaceae*, *Pseudomanadaceae*, *Raoultella*, *Proteus* i *Proteus mirabillis*)

En l'àmbit del diagnòstic es va avaluar la validesa de la determinació del perfil metabòlic de la histamina en orina com a biomarcador d'aquesta intoleràncIa alimentària. Els individus intolerants a la histamina van mostrar un perfil metabòlic de la histamina en orina diferent al del grup control, amb una reducció significativa del principal metabòlit de la histamina en orina, l'1-metilhistamina. Aquests resultats obririen la possibilitat de que es puguin utilitzar com a biomarcadors no invasius de la intoleràncIa a la histamina i que puguin complementar els actuals criteris basats en l'avaluació dels símptomes.

ABSTRACT

[ENG]

Histamine intolerance is a non-allergic hypersensitivity reaction triggered in susceptible individuals by the ingestion of normally tolerable amounts of histamine, mainly due to deficient intestinal activity of the enzyme diamine oxidase (DAO). Responsible for the degradation of dietary histamine, DAO constitutes the main barrier against histamine entry into the systemic circulation. In front of a DAO deficiency, histamine is absorbed into the plasma, provoking the clinical picture of histamine intolerance. Clinical manifestations include a wide range of symptoms, both gastrointestinal (e.g., abdominal distension, diarrhoea, abdominal pain, and constipation) and extraintestinal (e.g., headache, tachycardia, rhinorrhoea, rhinitis, pruritus, and skin redness). Although histamine intolerance has been increasingly studied in the last decade, more scientific evidence is required to help define, diagnose, and clinically manage this condition. Thus, the general aim of this doctoral thesis is to provide new evidence concerning these aspects of histamine intolerance.

The first objective of the thesis was to study in more detail the relationship between histamine intolerance and diet, as well as to investigate factors that could trigger the symptomatology other than the ingestion of histamine. After carrying out a critical review of the different low-histamine diets described in the scientific literature, a clear lack of consensus was observed regarding the foods that should be eliminated in the dietary treatment of histamine intolerance. Another striking observation is that among the excluded foods, only 32% are likely to contain histamine. The elimination of other foods that in principle are histamine-free is based on their high content of other amines, mainly putrescine. *In vitro* studies carried out as part of this doctoral thesis have shown that the presence of other amines, putrescine (diamino-butane) and cadaverine (diamino-pentane), significantly reduces the rate of histamine degradation by DAO enzyme.

Regarding the etiology of histamine intolerance, DAO deficiency can have a genetic origin or be acquired, for example, in people with inflammatory intestinal diseases that

limit DAO secretion, or to the action of certain drugs that can inhibit it. Another possible cause, for which clear evidence is still lacking, is the existence of dysbiosis or alteration of the intestinal microbiota in susceptible individuals. Therefore, another objective of this thesis was to study the profile and composition of the intestinal microbiota of histamine intolerants in comparation with healthy population. In this sense, it was found that intolerant individuals presented intestinal dysbiosis, characterized by a greater abundance of histamine-secreting bacteria and a lower proportion of bacteria associated with good intestinal health. The microbial profile also differed significantly and was more heterogeneous (beta-diversity) in the intolerant than in the control population. Additionally, possible changes in the composition of the intestinal microbiota over nine months of dietary treatment were studied in a small subgroup of patients, observing a significant decrease in certain histamine-secreting bacteria (*Morganellaceae*, *Pseudomanadaceae*, *Raoultella*, *Proteus* and *Proteus mirabillis*).

In the field of diagnosis, the validity of the determination of the metabolic profile of histamine in urine was evaluated as a biomarker of this food intolerance. The metabolic profile in histamine intolerant individuals differed from that of the control group, with significantly lower levels of the main metabolite, 1-methylhistamine. These results open new perspectives for the use of urinary histamine metabolites as non-invasive biomarkers of histamine intolerance and complement the current symptom-based diagnostic criteria.

1. INTRODUCCIÓ

1. INTRODUCCIÓ

Les reaccions adverses als aliments poden classificar-se en reaccions de tipus tòxic, que es donen per l'exposició a quantitats elevades d'un compost de caràcter tòxic present en l'aliment; i les de tipus no tòxic o reaccions d'hipersensibilitat alimentària, en les quals s'engloben tant les al·lèrgies com les intoleràncies alimentàries [1]. Els processos fisiològics implicats en l'al·lèrgia es basen en una resposta immunològica a una proteïna de l'aliment mediada, principalment, per la immunoglobulina E (IgE). En canvi, la intolerància alimentària es caracteritza per una resposta no immunològica provocada per un aliment o un dels seus components a una dosi normalment tolerada per la població sana [2]. Segons la revisió de la nomenclatura de les al·lèrgies i reaccions afins proposada l'any 2004 per la *World Allergy Organitzation*, les intoleràncies alimentaries també poden denominar-se hipersensibilitat alimentària no al·lèrgica [1].

La intolerància alimentaria explicaria la major part de les reaccions d'hipersensibilitat, ja que s'estima que afecta fins a un 20% de la població, mentre que l'al·lèrgia alimentària n'afecta aproximadament al 10% [2–4]. En la literatura científica es troben descrites un ampli ventall d'intoleràncies alimentàries, com per exemple, les causades per diferents mono- i disacàrids com la lactosa, la fructosa o la sacarosa; la intolerància al sorbitol, la malabsorció de FODMAPs (Oligosacàrids, Disacàrids, Monosacàrids i Poliols Fermentables) o la sensibilitat al gluten no celíaca [2,5]. Encara que menys coneguda, la intolerància a la histamina es troba reconeguda des de principis del segle XXI per diferents societats i autoritats científiques de l'àmbit de l'al·lergologia i la seguretat alimentaria com un dels riscos associats a la ingesta d'histamina, dotant-la així d'entitat clínica diferenciada de la ja coneguda intoxicació histamínica [6].

En aquesta introducció es revisarà l'evidència científica disponible fins al moment en relació als continguts d'histamina i altres amines en els aliments i, especialment,

sobre la intoleràncIa a la histamina, que és el principal objecte d'estudi d'aquesta tesi doctoral.

1.1. Histamina i altres amines biògenes en els aliments

La histamina (2-[4-imidazolil]etilamina), descoberta l'any 1910 per Dale i Laidlaw, és una amina biògena heterocíclica amb dos grups amino. A part de les conegudes funcions que té aquesta amina en l'organisme (estimulació dels sucs gàstrics, contracció de cèl·lules del múscul llis, dilatació dels vasos sanguinis, entre d'altres), la histamina també es pot trobar en els aliments. En aquests, es forma, principalment, com a conseqüència de la descarboxilació de la histidina, el seu aminoàcid precursor, per acció de l'enzim L-histidina descarboxilasa (HDC) d'origen bacterià (Figura 1) [6,7]. A part de la histamina, en els aliments també s'hi poden trobar altres amines biògenes, com la tiramina (4-hidroxi-fenetilamina), la putrescina (1,4-diaminobutà) i la cadaverina (1,5-diaminopentà) sintetitzades també, per la descarboxilació microbiana de la tirosina, l'ornitina i la lisina, respectivament. Per tant, l'acumulació d'histamina i altres amines en els aliments requereix de la confluència de diferents factors: a) presència de microorganismes amb capacitat aminogènica (activitat aminoàcid-descarboxilasa), b) disponibilitat dels aminoàcids precursors i c) condicions ambientals favorables pel creixement i l'activitat enzimàtica dels microorganismes [8–10].

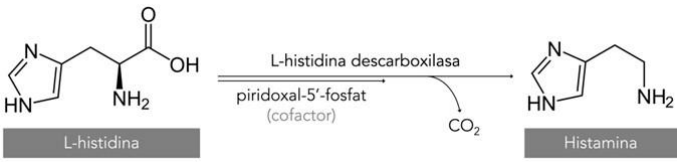


Figura 1. Síntesi d'histamina a partir de la descarboxilació del seu aminoàcid precursor (histidina).

Encara que l'activitat aminoàcid descarboxilasa és una propietat bacteriana soca depenent, s'han identificat un gran número de gèneres, tant bacteris Gram positius com Gram negatius capaços de formar amines (Taula 1) [6,11,12]. En el cas de la histamina, diferents generes bacterians de la família *Enterobacteriaceae*, com *Morganella, Enterobacter, Hafnia, Proteus* i *Photobacterium*, han estat descrits com importants formadors d'histamina, especialment en peixos i derivats [12–15]. En els productes fermentats, com els formatges, els derivats carnis crus-curats fermentats, la cervesa o el vi, a part de certes soques d'enterobacteries, els bacteris de l'àcid làctic són els principals responsables de la formació d'histamina (p. ex. *Lactobacillus hilgardii, Lactobacillus buchnerii i Oenococcus oeni*) (Taula 1) [6,12].

Així, tots aquells aliments alterats microbiològicament com a conseqüència d'una conservació i/o elaboració higiènicament inadequada són susceptibles de presentar grans quantitats d'histamina, i per suposat, d'altres amines. D'altra banda, també poden acumular quantitats importants d'amines biògenes els aliments fermentats/curats degut a la capacitat aminogènica dels bacteris responsables de la fermentació [16,17].

Taula 1. Bacteris amb capacitat de formar histamina en els aliments [6,12–15].

Aliments	Bacteris
Peixos i derivats	*Morganella morganii, Morganella psychrotolerans, Klebsiella pneumonia, Hafnia alvei, Proteus vulgaris, Proteus mirabilis, Enterobacter cloacae, Enterobacter aerogenes, Serratia fonticola, Serratia liquefaciens, Citobacter freundii, Clostridium sp, Pseudomonas fluorescens, Pseudomonas putida, Aeromonas spp., Pleisomonas shigelloides, Photobacterium spp, Photobacterium phosphoreum i Photobacterium psychrotoerans*
Productes fermentats (formatges, derivats carnis crus-curats fermentats, vi i cervesa)	*Lactobacillus buchneri, Oenococcus oeni, Lactobacillus hilgardii, Pediococcus parvulus, Pediococcus damnosus, Lactobacillus bavaricus, Lactobacillus brevis, Lactobacillus curvatus, Lactobacillus parabuchneri, Lactobacillus rossiae, Lactobacillus saerimneri 30a, Enterobacter aerogenes, Enterobacter cloacae, Enterobacter aerogenes,, Escherichia coli, Hafnia alvei, Klebsiellsja oxycata, Enterobacter cloacae, Enterobacter aerogenes, Morganella morganii, Serratia liquefaciens, Tetragenococcus spp i Leuconosctoc spp.*

Degut al seu origen eminentment bacterià, el contingut d'histamina i altres amines en els aliments és molt variable. La Figura 2 mostra, a títol il·lustratiu, els continguts mitjans d'amines biògenes (histamina, tiramina, putrescina i cadaverina) en diferents categories d'aliments del mercat espanyol i procedents de la base de dades del grup de recerca amb més de 1800 mostres d'aliments diferents [7]. Com es pot observar a la figura, els continguts de les diferents amines entre les diferents categories d'aliments són molt variables, i sovint també, dins d'una mateixa categoria (amb coeficients de variació que en molts casos superen el 100 %).

El peix fresc i els seus derivats (semiconserves i conserves de peix) normalment no contenen histamina o en nivells molt baixos. Com es pot veure en la Figura 2.A, el valors mitjans d'histamina i altres amines en peixos i derivats del mercat espanyol, no superaven en cap cas els 15 mg/kg. Per exemple, en el cas de la histamina el 95% de les mostres de peix fresc es trobaven per sota dels 5 mg/kg d'histamina [6].

Aquestes dades coincideixen amb les publicades per l'*European Food Safety Authority* (EFSA) en el seu informe científic sobre l'avaluació del risc d'amines biògenes, on només un 27% de les 6329 mostres de peix fresc i productes derivats procedents de diferents països europeus contenien histamina, i generalment en baixes concentracions (valor mitjà de 2,5 mg/kg) [6]. No obstant això, la falta de frescor en el peix i/o l'aplicació de condicions higiènicament inadequades en els processos d'elaboració dels productes pesquers (conserves i semiconserves) pot conduir a l'acumulació de nivells molt elevats d'histamina. Un exemple són els 657 mg/kg d'histamina trobats en una mostra de sardines en conserva o els 8910 mg/kg registrats per EFSA per aquesta categoria d'aliments [6,7]. És important destacar que quan existeixen problemes en la qualitat higiènica dels aliments, a més d'altes quantitats d'histamina, també es poden formar alts nivells d'altres amines relacionades amb l'activitat descarboxilasa de bacteris alterants, com la cadaverina i la putrescina.

En la carn fresca i en els derivats carnis cuits o curats, a l'igual que en el cas del peix, no s'espera que hi hagi formació d'histamina sempre i quan es mantingui la frescor i unes condiciones higièniques adequades d'aquests productes [18,19].

Per contra, en el cas dels aliments fermentats l'escenari és ben diferent. En aquests tipus d'aliments, l'aparició d'histamina no depèn només de les condicions higièniques de les matèries primeres i/o processos d'elaboració, sinó també de la capacitat aminogènica dels bacteris responsables de la fermentació [18]. A part, l'obtenció d'aquests productes proporciona també unes condicions favorables per la formació d'amines, com fenòmens proteolítics (que augmenten la disponibilitat dels aminoàcids precursors) i la producció d'àcids orgànics dèbils amb la conseqüent disminució del pH [6,20,21]. Com es pot veure en la Figura 2.A, entre les diferents amines biògenes trobades en el productes carnis fermentats i els formatges, la tiramina és l'amina biògena més freqüent i abundant en aquest tipus de productes a causa de l'activitat aminogènica dels bacteris fermentatius de l'àcid làctic [22]. La presència de les diamines putrescina i cadaverina és també bastant

comú, encara que sovint es troben en quantitats més variables que les de tiramina. Pel contrari, la presència d'histamina sol ser menys freqüent i normalment baixa. Tal com mostra la Figura 2.A, els valors mitjans d'histamina en derivats carnis fermentats i formatges es troben al voltant dels 30 mg/kg d'histamina, estant el 85% de les mostres per sota dels 20 mg/kg [7]. No obstant, en alguns casos els nivells d'histamina poden arribar a ser notablement alts, com els 1850 mg/kg reportats en mostres de formatges europeus [6]. Normalment, la presència d'alts continguts d'histamina, generalment acompanyats de quantitats importants de putrescina i cadaverina, sol indicar la presència de bacteris relacionats amb el deteriorament dels aliments [18].

En el cas de les begudes fermentades, com la cervesa i el vi, els continguts d'histamina i tiramina són molt més baixos que els reportats per altres aliments fermentats, amb valor mitjans que no arriben als 5 mg/kg (Figura 2.B). Certament en alguns casos sí s'han assolit nivells propers als 100 mg/kg [23]. En aquests productes, la putrescina pot tenir un origen natural, es troba present en el raïm, el llúpol i la malta, però també pot derivar de l'activitat microbiològica [7]. D'altra banda, la cadaverina pràcticament no s'ha trobat en begudes fermentades, la seva presència en la cervesa, és de forma ocasional.

Des d'una perspectiva legal, només s'han fixat límits tolerables per a la histamina en peix i productes derivats. Per als productes de la pesca procedents d'espècies de peixos associats a un alt contingut d'histidina (particularment peixos de les famílies *Scombridae*, *Clupeidae*, *Engraulidae*, *Coryfenidae*, *Pomatomidae* i *Scomberesocidae*), el Reglament (CE) núm. 2073/2005 de la Comissió, de 15 de novembre de 2005, relatiu als criteris microbiològics aplicables als productes alimentaris, estableix un límit inferior (m) de 100 mg/kg i un límit màxim (M) de 200 mg/kg, i un pla de mostreig de nou mostres. El resultat es considera satisfactori quan es compleixen aquestes tres condicions a la vegada: el valor mig d'histamina de les mostres és inferior a 100 mg/kg; cap mostra no supera els 200 mg/kg i, de les nou mostres, només dues poden tenir un contingut d'histamina d'entre 100 i

200 mg/kg. En els productes de la pesca sotmesos a un tractament de maduració enzimàtica en salmorra, obtinguts a partir d'espècies de peix amb un alt contingut d'histidina, el valor mig màxim permès és 200 mg/kg, el valor màxim individual és 400 mg/kg i només hi pot haver dues mostres entre aquests dos valors en un pla de mostreig de nou mostres [24]. La *Food and Drug Administration* (FDA) dels Estats Units estableix un nivell màxim tolerable de 50 mg/kg d'histamina per tonyina i mahi-mahi o entre 50 i 500 mg/kg d'histamina per les altres espècies marines [25]. En Canadà, Suïssa i Brasil, el límit màxim permès en peix i productes derivats és de 100 mg/kg i a Austràlia i Nova Zelanda és de 200 mg/kg [14].

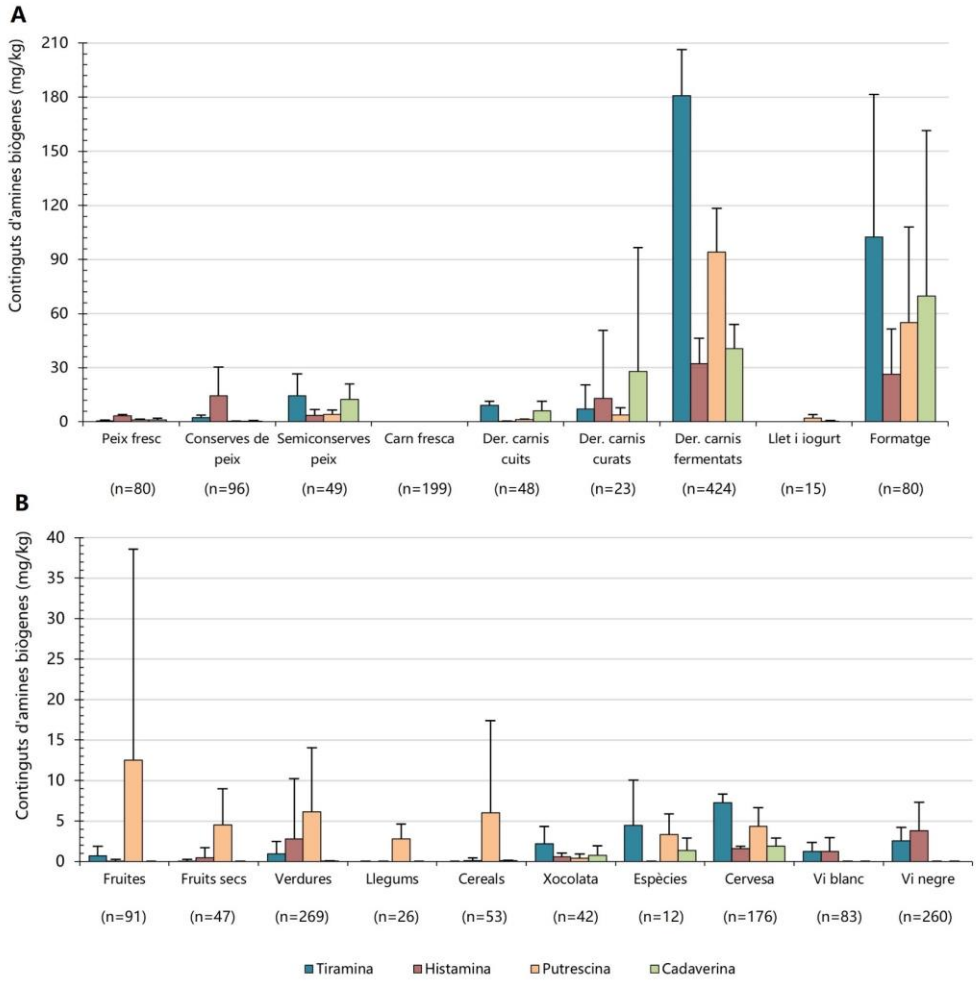


Figura 2. Continguts d'amines biògenes (mg/kg) en diferents tipus d'aliments del mercat espanyol. Dades procedents de la base de dades del grup de recerca.

D'altra banda, en aliments d'origen vegetal com les fruites, fruits secs, hortalisses, llegums i cereals, la putrescina destaca per ser l'amina més freqüent i abundant (Figura 2.B). Per exemple, la putrescina es troba en altes concentracions en aliments com els cítrics, el pebrot verd, el blat de moro, el plàtan, el blat, els brots de soja i els festucs [26]. En molts casos aquesta amina té un origen natural, sent la precursora de les poliamines, espermidina i espermina, que tenen diferents funcions fisiològiques a la planta. En els productes vegetals, la presència d'histamina no és habitual, trobant-se només en un número molt restringit

d'aliments, fet que explicaria que els valors mitjans d'histamina mostrats en la figura siguin molt baixos o pràcticament zero. Els únics vegetals que normalment mostren quantitats significatives d'histamina són els espinacs, l'albergínia i el tomàquet. En aquests aliments, la presència d'aquesta amina sol tenir un origen natural, encara que a vegades també pot ser deguda a una activitat microbiològica [7,27,28]. Lavizzari i col. (2007) ha atribuït alts continguts d'histamina en espinacs a l'activitat de bacteris contaminants durant l'emmagatzematge, pertanyents als grups *Enterobactericeae* i *Pseudomonadaceae* [27]. Malgrat això, són necessaris més estudis per entendre amb més detall l'origen de la histamina en els aliments d'origen vegetal. Els vegetals fermentats com el xucrut també poden acumular alts nivells d'histamina procedents de l'acció microbiana dels bacteris tecnològics utilitzats en la seva elaboració.

1.2. Metabolisme de la histamina i altres amines biògenes en l'organisme

Com que la histamina és una substància sintetitzada de forma endògena que, alhora, també es por trobar en els aliments, sembla coherent pensar que l'organisme humà ha d'estar preparat per a contrarestar tant l'alliberament massiu d'histamina per part dels mastòcits, com els efectes derivats de la ingesta de nivells elevats d'aquesta amina. En l'organisme humà, existeixen dues vies principals per al metabolisme de la histamina:

a) la desaminació oxidativa del grup amino primari a través de l'enzim diamino oxidasa (DAO).

b) la metilació del grup amino secundari de l'heterocicle imidazole de la histamina catalitzada per l'enzim histamina-N-metiltranferasa (HNMT).

L'enzim DAO, també conegut com a histaminasa en l'àmbit de les ciències dels aliments, és una glicoproteïna homodimèrica codificada al cromosoma 7q35, que es troba emmagatzemat en estructures vesiculars de la membrana plasmàtica i és

el responsable de degradar la histamina a nivell extracel·lular. En els mamífers, l'expressió de la DAO es troba limitada a certs teixits específics. La major activitat DAO es localitza en l'intestí prim i el colon ascendent, la placenta i els ronyons [29–31].

L'enzim HNMT és una proteïna codificada al cromosoma 2q22 present en el citosol de les cèl·lules de manera que es capaç de degradar la histamina a nivell intracel·lular. Aquest enzim s'expressa en multitud de teixits de l'organisme humà, com són els ronyons, el fetge, la melsa, la pròstata, els ovaris, en cèl·lules de la medul·la espinal, els bronquis i la tràquea [32].

Tot i que s'ha descrit que la HNMT és altament selectiva per a la histamina, la principal barrera d'absorció de la histamina dietètica a nivell intestinal és l'enzim DAO [29]. A part de la histamina, l'enzim DAO és també capaç de degradar altres amines biògenes, principalment, les diamines putrescina i cadaverina [13,15]. En el cas de la tiramina, encara que el seu catabolisme és a duu a terme, principalment, per l'enzim monoamino oxidasa (MAO), també s'ha descrit que la DAO podria metabolitzar-la encara que en menor mesura [33,34]. Una altra oxidasa intestinal, la poliamino oxidasa (PAO), és l'encarregada de metabolitzar les poliamines, espermidina i espermina, encara que certs autors han atorgat a la DAO certa capacitat per degradar-les [33,35].

La DAO és constantment secretada al lumen intestinal, de manera que en situacions de normalitat, és capaç de degradar la histamina present a nivell intestinal, ja sigui procedent de la dieta o secretada per la microbiota [36–38]. No obstant això, existeixen dos escenaris en els que la capacitat metabòlica de l'enzim DAO es troba compromesa i que poden desencadenar efectes adversos per a la salut:

- **Intoxicació histamínica**: Reacció adversa de tipus tòxic derivada del consum d'aliments amb continguts molt elevats d'histamina que superen la capacitat catabòlica normal de l'enzim DAO. Aquesta intoxicació alimentària, àmpliament coneguda causa anualment brots de gravetat lleu-moderada, generalment pel

consum de productes derivats de la pesca [39]. A nivell europeu els brots d'intoxicació histamínica es troben recollits en els informes anuals de l'EFSA i l'*European Centre for Disease Prevention and Control* (ECDC), així com en el *Rapid Alert System for Food and Feed* (RASFF).

- **Intolerància a la histamina**: Reacció d'hipersensibilitat no al·lèrgica a la histamina dietètica com a conseqüència d'un dèficit en l'activitat DAO a nivell intestinal. A diferència de la intoxicació, aquest trastorn pot aparèixer fruit del consum d'aliments amb contingut normals o, fins i tot, baixos d'histamina, generalment tolerables per a la població sana [6,18,32].

1.3. Intolerància a la histamina

La intolerància a la histamina és el terme freqüentment utilitzat per descriure la reacció adversa que s'observa com a resultat del consum de quantitats normalment tolerables d'histamina en persones susceptibles, principalment per dèficit de DAO. No obstant, també s'han suggerit altres termes com "reacció adversa a la ingesta d'histamina" [40], "histaminosi enteral" o "sensibilitat a la histamina dietètica" [10]. En termes generals, la intolerància a la histamina es pot definir com un desequilibri en la homeòstasi de la histamina degut principalment, a una reduïda capacitat de degradació d'aquesta amina per part de l'enzim DAO a nivell intestinal, el que provoca una acumulació d'histamina en plasma i l'aparició de símptomes diversos [18,29,32,38].

Al llarg de la última dècada, la recerca científica sobre la intolerància a la histamina i el dèficit de DAO ha incrementat significativament. De fet, malgrat que les primeres referències científiques sobre la intolerància a la histamina són de fa més de 30 anys [41–43], gairebé el 85% han aparegut durant els darrers deu anys, generant un degoteig constant de treballs científics que contribueixen a definir i millorar les eines disponibles per gestionar aquesta intolerància alimentària.

És àmpliament conegut que la histamina té un paper important en nombrosos processos fisiològics i patològics. La histamina exerceix els seus efectes a través de la interacció amb quatre receptors específics (H1, H2, H3 i H4) que es troben distribuïts en diferents òrgans i teixits de l'organisme (Figura 3) [29,38,44]. Aquest fet, fa que els efectes adversos observats després de la ingesta d'histamina en persones intolerants, siguin nombrosos i variats i que, per tant, no es pugui definir un quadre clínic concret [10,45]. Aquesta complexitat es posa de manifest en un estudi del 2019 d'un grup investigadors austríacs on de 133 individus diagnosticats amb intoleràncIa a la histamina, el 97% patien tres o més símptomes, normalment afectant diferents òrgans o sistemes, amb un valor mig d'onze símptomes per pacient [46]. A més, aquest estudi va concloure que les manifestacions a nivell del tracte gastrointestinal van ser les més freqüents i de major severitat. Entre elles se'n destaquen, la presència de distensió abdominal en un 92% dels pacients i sensació de plenitud postprandial, diarrea, dolor abdominal i restrenyiment entre el 55% i el 73% dels pacients. L'alta freqüència de símptomes gastrointestinals en individus amb intoleràncIa a la histamina es pot explicar pel fet que el tracte gastrointestinal conté els quatre receptors de la histamina [10]. A part del símptomes gastrointestinals, en aquest estudi també, van ser descrites afectacions del sistema nerviós i de l'aparell cardiovascular com, marejos, cefalees i palpitacions, seguits de símptomes a l'aparell respiratori i dermatològic [46].

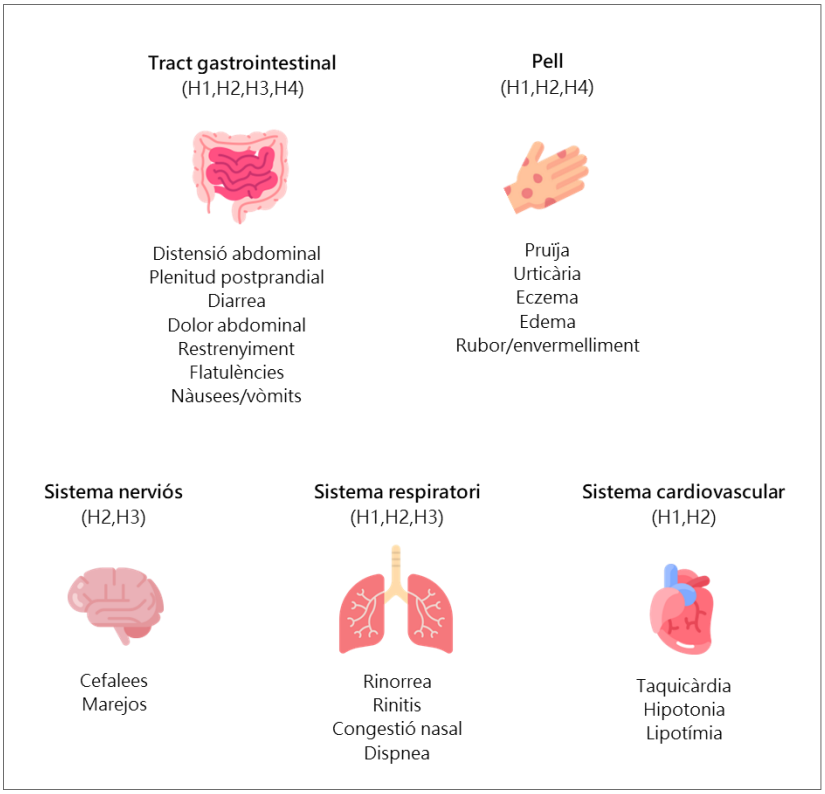


Figura 3. Principals símptomes de la intolerància a la histamina i localització dels receptors d'histamina en l'organisme [10,45].

L'heterogeneïtat de les manifestacions clíniques associades a la intolerància a la histamina fa que el seu diagnòstic sigui complex i per tant, també la determinació de la incidència actual d'aquesta intolerància. Alguns autors han estimat que aquesta incidència podria oscil·lar entre un 1 i un 6% de la població general [29,38,45,47].

1.3.1. Etiologia de la intolerància a la histamina

En la intolerància a la histamina, així com en altres tipus d'intoleràncies alimentàries, l'enzim encarregat de degradar la histamina dietètica es troba inhibit o reduït, provocant, en aquest cas, l'acumulació d'histamina en plasma i l'aparició de símptomes gastrointestinals i extra-intestinals inespecífics. Són nombrosos els

estudis clínics que donen suport al paper de l'enzim DAO com a factor etiològic de la intoleràncIa a la histamina. En tots ells, es relacionen afectacions típiques d'aquesta intoleràncIa, com són les cefalees i els desordres gastrointestinals o dermatològics, amb una prevalença de dèficit de DAO [47–56]. En l'estudi portat a terme per Mušič i col. (2013) es va observar un dèficit de l'enzim DAO en el 80% dels pacients (n=316) amb símptomes associats a intoleràncIa a la histamina (p. ex. urticària, pruïja, diarrea, dolor abdominal, vòmits, restrenyiment, tos, rinitis i cefalea), així com una menor activitat DAO plasmàtica comparada amb un grup control [52]. L'estudi retrospectiu realitzat per Manzotti i col. (2016) van trobar una elevada prevalença de dèficit de DAO (71%) en pacients diagnosticats d'intoleràncIa a la histamina (símptomes gastrointestinals, dermatològics i cefalea) [49]. En un estudi publicat l'any 2021, Mayo-Yáñez i col. també van identificar una elevada prevalença de dèficit de DAO, en aquest cas en individus amb rinitis al·lèrgica persistent (46%). A més, aquest dèficit enzimàtic era més prevalent en el grup de pacients amb una rinitis d'intensitat moderada o severa [56].

Es coneixen tres possibles orígens d'aquesta deficiència de l'enzim DAO, genètic, farmacològic o bé patològic [18,32,47,51]. Més recentment, la idea d'una disbiosi o alteració de la microbiota intestinal com a possible origen de la intoleràncIa a la histamina està guanyant interès (Figura 4) [36].

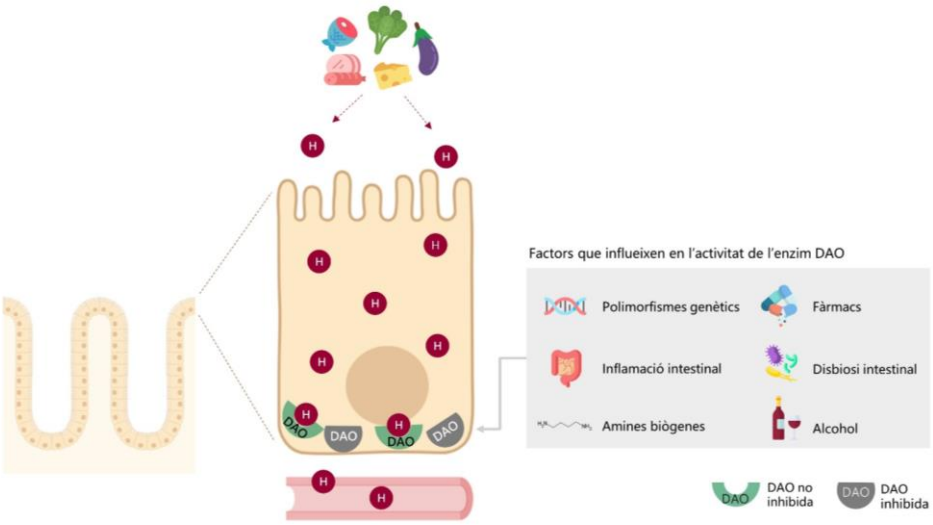


Figura 4. Resum del mecanisme d'acció de l'enzim DAO en l'enteròcit i els diferents factors que poden influir en la seva activitat. Adaptada de Hrubisko i col. (2021).

1.3.1.1. Origen genètic

La presència de variacions genètiques en l'enzim que degrada la histamina podrien ser les responsables de les diferències interindividuals observades en la capacitat per metabolitzar la histamina dietètica [59]. Diversos estudis han confirmat l'existència de més de cinquanta polimorfismes de nucleòtids simples (SNP) que afecten al gen que codifica l'enzim DAO [55,57–60]. En la majoria dels casos, l'efecte d'aquests polimorfismes actua a través de modificacions en la cinètica enzimàtica provocant una elevació de la constant de Michaelis-Menten (K_M), i per tant una reducció en l'activitat degradant de la histamina [59]. Concretament, els SNPs que més afecten la funcionalitat de l'enzim DAO en població caucàsica són rs10156191, rs1049742, rs2268999 i, especialment, rs1049793 [59]. Així mateix, també s'han identificat certes variacions genètiques de l'enzim DAO en individus d'origen asiàtic i africà (rs45558339 i rs35070995, respectivament) [61]. En població espanyola caucàsica, García-Martín i col. (2015) han associat la presència dels SNPs rs10156191 i rs2052129 amb una menor activitat DAO i un major risc de patir migranyes [62]. De la mateixa manera, Meza-Velázquez i col. (2017) han descrit una

major freqüència del polimorfisme rs1049793 en un grup de dones amb migranya [63]. D'altra banda, però, en un estudi més recent dut a terme amb 298 individus amb migranya i 360 individus sans, la freqüència dels polimorfismes genètics (rs1049793, rs10156191 i rs1049742) va ser similar entre ambdós grups [64]. Malgrat que s'han identificat diversos SNPs relacionats amb una menor funcionalitat de l'enzim, encara són necessaris més estudis enfocats a conèixer la relació entre la presència de certes variants genètiques de l'enzim DAO i els fenotips característics de la intolerància a la histamina.

1.3.1.2. Origen patològic

Actualment està augmentant l'evidència que recolza que un dels possibles orígens de la intolerància a la histamina per dèficit de DAO es podria trobar a nivell intestinal. Concretament, la recent revisió realitzada per Schnedl i Enko (2021) detalla la relació existent entre una menor activitat de l'enzim DAO i diverses patologies o trastorns intestinals que afecten la integritat de la mucosa [65].

Per exemple, en malalties inflamatòries intestinals (malaltia de Chron i colititis ulcerosa) i en adenoma colònic s'ha descrit un menor catabolisme de la histamina degut a una afectació de la mucosa derivada d'aquestes patologies [66,67]. De fet, s'ha proposat la determinació de l'activitat enzimàtica de la DAO intestinal com un predictor de dany de la mucosa intestinal [68–71].

Tanmateix, altres estudis també han reportat una reduïda activitat de l'enzim DAO en individus diagnosticats d'intolerància a la lactosa [54,72–75]. En un estudi realitzat per Schnedl i col. (2020) es va observar que els nivells d'hidrogen espirat i la presència de símptomes eren molt més elevats en el grup de pacients amb intolerància a la lactosa i una baixa activitat DAO en comparació als individus amb una activitat DAO normal [74]. Un estudi retrospectiu dut a terme al 2022 per aquest mateix grup d'investigadors va confirmar aquesta relació amb un major nombre de pacients, trobant que la meitat d'un grup de 402 individus intolerants a la lactosa presentaven a més una reducció de l'activitat DAO sèrica. A més aquests pacients amb intolerància a la lactosa i a la histamina van mostrar també majors nivells

d'hidrogen espirat [75]. Segons aquests autors, una alteració de l'estat de la mucosa intestinal seria l'origen d'aquestes intoleràncies alimentàries.

Per últim, dos estudis recents han assenyalat una possible relació entre la sensibilitat al gluten no celíaca i la intolerància a la histamina [76,77]. En un dels estudis es comparen els diferents símptomes gastrointestinals i extra-intestinals associats a la sensibilitat al gluten no celíaca amb els de la intolerància a la histamina identificant grans similituds en els quadres clínics d'ambdós desordres. Griauzdaitė i col. (2020) van reportar que 9 de 10 pacients amb sensibilitat al gluten no celíaca mostraven un dèficit d'activitat de l'enzim DAO [77].

1.3.1.3. Influència de fàrmacs i altres substàncies

Una deficiència de l'enzim DAO també pot tenir un origen adquirit com a conseqüència de l'efecte inhibitori de determinats fàrmacs. Aquest efecte inhibitori és reversible ja que s'elimina quan desapareix l'agent causant [29]. Diversos fàrmacs àmpliament utilitzats per la població general han estat descrits com a potencials inhibidors de l'enzim DAO, com per exemple, l'àcid clavulànic (antibiòtic), el verapamil (antihipertensiu), l'acetilcisteïna (mucolític) i el suxametoni (relaxant muscular), entre d'altres [78–80]. De fet, s'ha estimat que aproximadament el 20% de la població europea pren de forma regular algun d'aquests fàrmacs, provocant un augment important en la susceptibilitat als efectes adversos de la histamina [6]. En molts casos aquest efecte inhibitori podria explicar-se per la similitud estructural que presenten certs fàrmacs amb la forma de la histamina. Aquests fàrmacs serien capaços d'unir-se a la zona activa de l'enzim, provocant una disminució en la degradació enzimàtica de la histamina [80]. Malgrat que a la literatura científica apareixen sovint llargs llistats de fàrmacs als quals se'ls atribueix aquesta capacitat inhibitòria, en general, l'evidència experimental que ho suporta és encara escassa i normalment procedeix d'antics estudis *in vitro* [78–80].

També s'ha descrit que altres substàncies, com certes amines biògenes i l'alcohol, podrien mostrar un efecte inhibitori sobre la degradació de la histamina al competir per les mateixes vies de metabolització [32]. Certs estudis *in vitro* i en animals

d'experimentació apunten la capacitat de certes amines per inhibir la degradació de la histamina per part de l'enzim DAO, al competir també per aquest enzim. No obstant, l'evidència sobre aquesta inhibició competitiva procedeix d'escassos estudis i desactualitzats [81–84]. En el cas de l'alcohol, el seu metabòlit acetaldehid, pot competir amb la histamina per l'enzim aldehid deshidrogenasa (ALDH), el qual també participa en el metabolisme de la histamina, donant lloc a una acumulació d'histamina en plasma [6,17].

Així doncs, la presència de fàrmacs, d'altres amines o d'alcohol pot explicar els diferents graus de reacció entre persones que han consumit aliments amb quantitats similars d'histamina [18].

1.3.1.4. Disbiosi intestinal

Un altre possible origen del qual encara no es disposa de suficient evidència científica seria que una disbiosi intestinal podria afavorir una menor activitat de l'enzim DAO o bé una major formació d'histamina a nivell intestinal en individus intolerants. Encara que durant l'última dècada han aparegut diversos estudis que relacionen determinades patologies o fins i tot intoleràncies alimentàries amb una alteració de la microbiota intestinal, els estudis centrats en la intolerància a la histamina són encara molt escassos. Segons el nostre coneixement només existeix un únic treball, publicat l'any 2018, enfocat en l'estudi sobre la relació entre la intolerància a la histamina i la microbiota intestinal. Aquests autors van reportar diferències en la composició de la microbiota intestinal en pacients amb intolerància a la histamina amb dèficit de DAO comparada amb un grup d'individus sans no intolerants [36]. Concretament, es va observar una major proporció de Proteobacteria, grup bacterià relacionat amb inflamació intestinal, una menor proporció de bifidobacteris i una menor diversitat d'espècies bacterianes en el grup d'individus intolerants. Aquests mateixos autors apunten que aquesta disbiosi podria contribuir a una inflamació de la mucosa intestinal i, en últim terme, afavorir una reducció de l'enzim DAO [36]. D'altra banda, un altre factor que podria tenir rellevància en l'etiologia de la intolerància a la histamina és la presència de soques

bacterianes histaminogèniques a nivell intestinal. La presència d'altes proporcions d'aquest tipus de bacteris en l'intestí humà (p. ex. lactobacillis i enterobacteries, entre d'altres) podria contribuir a un increment dels nivells d'histamina, afavorint així els seus efectes adversos en l'organisme. No obstant, es important considerar que una disbiosi intestinal no seria, per sí sola, la única causa d'aquesta intolerància, sinó que probablement agreujaria els símptomes derivats d'altres causes primàries del dèficit de DAO (genètiques o adquirides).

1.3.2. Diagnòstic de la intolerància a la histamina

En general, pel diagnòstic de les intoleràncies o malabsorcions alimentàries existeixen diferents estratègies o proves analítiques validades que permeten identificar objectivament les persones afectades [45]. En el cas de la intolerància a la histamina, i malgrat els importants avanços en el seu coneixement que s'han produït al llarg dels darrers anys, la inespecificitat dels símptomes i la falta de proves analítiques validades fa que no existeixi un algoritme de diagnòstic de consens [2,32,72,85]

Actualment, el diagnòstic de la intolerància a la histamina s'està portant a terme en base a un examen exhaustiu de la història clínica del pacient. Fonamentalment, es té en compte la presència de 2 o més símptomes típics d'aquesta intolerància i la seva millora o remissió després del seguiment d'una dieta baixa en histamina [2,10,29,32,38,40,45]. La durada d'aquesta dieta per tal de confirmar el diagnòstic no està del tot estipulada, malgrat això alguns autors aconsellen un període d'entre 1 o 2 mesos. Paral·lelament, també és necessari excloure altres possibles intoleràncies, malalties gastrointestinals, al·lèrgies mediades per IgE, l'existència de mastocitosi (mitjançant la determinació de triptasa plasmàtica), així com la presa de fàrmacs inhibidors de la DAO [2].

A part de l'anamnesi, diversos autors han proposat un ventall de proves analítiques que podrien emprar-se com a eines complementàries en el diagnòstic d'aquesta

intoleràncIa. Algunes d'elles, com la determinació de l'activitat DAO sanguínia o la identificació de SNPs, ja s'estan duent a terme en la pràctica clínica, malgrat que encara s'ha d'acabar d'avaluar la seva eficàcia a l'hora d'identificar tots els individus afectats.

1.3.2.1. Activitat de l'enzim DAO en sang i en mucosa intestinal

Una tècnica àmpliament utilitzada pel diagnòstic de la intoleràncIa a la histamina és la determinació de l'activitat DAO sanguínia, basada en la mesura de la quantitat d'histamina que es degrada en una mostra de sang en un temps determinat a través d'un immunoassaig tipus ELISA (*enzyme-linked immunosorbent assay*) o a través d'un radio-immuno assaig (RIA). Valors d'activitat DAO per sota dels 10 U/mL indicarien una possible deficiència d'aquest enzim. No obstant, actualment existeix certa controvèrsia en quant a l'ús de l'activitat DAO sanguínia com a biomarcador de la intoleràncIa a la histamina. De fet existeixen estudis que demostren una relació entre una DAO reduïda i la presència dels símptomes d'aquesta intoleràncIa [47,49,52,86]. Inclús alguns han reportat una relació inversa entre la severitat dels símptomes i l'activitat DAO. Cucca i col. (2022) van observar que els símptomes gastrointestinals i cutanis, sovint associats a cefalees, van ser els més freqüents en un total de 146 pacients amb intoleràncIa a la histamina, la severitat dels quals correlacionava amb el grau de deficiència de l'enzim DAO [86].

D'altra banda, altres autors no han trobat una relació significativa entre la història clínica de símptomes típics d'intoleràncIa a la histamina d'alguns pacients i la seva activitat DAO sanguínia [87–89]. Segons aquests, existeix una variabilitat intraindividual al llarg del dia i entre diferents dies que dificulta la interpretació dels resultats. També és important tenir en compte que els nivells plasmàtics d'activitat DAO podrien no reflectir els nivells de DAO de la mucosa intestinal, que és la que es troba directament implicada en la degradació d'histamina procedent dels aliments [32,45]. A més, actualment, no existeixen dades procedents d'estudis científics en humans que permetin establir els valors de normalitat de la DAO sanguínia en població sana, i que per tant, permetin establir el llindar a partir del

qual es pot considerar un dèficit d'aquesta activitat enzimàtica. De fet, els únics valors existents són els proporcionats pels propis laboratoris comercialitzadors dels testos d'activitat DAO. Segons les societats d'al·lergologia alemanya i suïssa, és necessari seguir investigant en aquest camp abans d'atorgar a l'activitat DAO sanguínia un valor diagnòstic definitiu per a la intolerància a la histamina [85].

Un mètode alternatiu que també s'ha estudiat per avaluar la funcionalitat de la DAO és la mesura de l'activitat d'aquest enzim en la mucosa intestinal, malgrat que és una tècnica més invasiva ja que es realitza mitjançant una biòpsia del teixit colònic. La recerca realitzada en aquest àmbit ha mostrat una reduïda activitat intestinal de l'enzim DAO en individus amb urticària, al·lèrgia alimentària i en persones amb adenoma de colon, juntament amb un increment dels nivells d'histamina [90–94]. Encara que aquesta prova representa un potencial mètode de diagnòstic, són necessaris més estudis que relacionin aquesta activitat enzimàtica amb els símptomes de la intolerància a la histamina.

1.3.2.2. Identificació de SNPs del gen que codifica l'enzim DAO

Degut al possible origen genètic del dèficit de DAO, una de les estratègies pel seu diagnòstic que s'està duent a terme per alguns laboratoris clínics és la determinació dels polimorfisme genètics o SNPs associats a una activitat DAO reduïda. Actualment, existeix la possibilitat de fer un anàlisi no invasiu capaç d'identificar 4 SNPs (rs1049742, rs1049793, rs10156191 i rs2052129) tant en mostres de sang com de mucosa oral [32]. Malgrat la seva potencial utilitat com a biomarcadors de la intolerància a la histamina, encara no hi ha estudis publicats que validin aquest mètode diagnòstic en persones que mostren un fenotip característic de la intolerància a la histamina. Una possible limitació d'aquest mètode de diagnòstic és que no té en compte altres orígens d'una deficiència de l'enzim DAO, especialment el derivat de desordres que afecten el tracte gastrointestinal.

1.3.2.3. Prova cutània: histamine 50-skin-prick test

L'*histamine 50-skin-prick test* és una variant de la prova d'al·lèrgia cutània intradèrmica proposada per Kofler i col. (2011) com a eina pel diagnòstic d'aquesta intoleràncIa. Aquesta prova consisteix en realitzar una lectura de la mida de la fava passats 50 minuts després de l'administració cutània d'histamina, a diferència de la prova d'al·lèrgia cutània habitual en la que aquesta lectura s'acostuma a realitzar passats 20 minuts [95]. En l'estudi de Kofler i col. (2011) van observar que passats aquests 50 minuts, tot i que la mida de la fava no va variar, sí que es van observar diferències en el temps de resolució d'aquesta fava entre els grups estudiats [95]. Darrerament, un altre estudi ha observat una correlació entre aquest retard en la remissió de la fava i una menor activitat de la DAO plasmàtica en individus amb al·lèrgia [96]. Encara que aquesta prova està descrita en la literatura científica, actualment i segons el nostre coneixement, no s'utilitza en la pràctica clínica pel diagnòstic de la intoleràncIa a la histamina.

1.3.2.4. Test de provocació oral de la histamina

Els testos de provocació oral doble cec i control amb placebo s'han descrit com els millors mètodes per verificar una reacció adversa als aliments que a més permeten establir un llindar de tolerància individual [40]. En el cas del test de provocació oral a la histamina, no existeix un procediment establert ni una dosi llindar estandarditzada [40]. Diferents autors han proposat una dosi de provocació de 75 mg d'histamina ja que semblaria ser una dosi inofensiva per individus sans i per ser una quantitat que es podria trobar habitualment en els aliments [97,98]. El fet que la realització d'aquest test s'hagi de sotmetre a supervisió mèdica i hospitalització del pacient conjuntament amb l'alt risc de poder patir efectes adversos greus, fa que la seva aplicació i el seu estudi siguin encara limitats [32].

1.3.2.5. Determinació d'histamina i els seus metabòlits en orina

Actualment, la recerca científica s'està enfocant en el desenvolupament de proves de diagnòstic no invasives que permetin establir un criteri de diagnòstic sòlid i clínicament irrefutable de la intolerància a la histamina per dèficit de DAO. Un d'ells seria l'aplicació de la metabolòmica com a eina per a la identificació de biomarcadors del metabolisme de la histamina en orina. Per a això, Comas-Basté i col. (2017) van desenvolupar i validar un mètode cromatogràfic per determinar de manera ràpida i inequívoca els nivells urinaris d'histamina i el seu metabòlit metilat, la metilhistamina [99]. L'aplicació d'aquest mètode en un petit estudi pilot amb 22 pacients amb intolerància a la histamina va permetre identificar una disminució significativa de la metilhistamina en comparació amb un grup d'individus sans [100]. Cal confirmar aquest resultats amb un major nombre de pacients per validar la utilitat diagnòstica d'aquest paràmetre.

1.3.3. Gestió dietètica de la intolerància a la histamina

El tractament dietètic que s'utilitza per minimitzar o evitar l'aparició dels símptomes relacionats amb la intolerància a la histamina és el seguiment d'una dieta baixa en histamina i/o la suplementació amb enzim DAO exogen [45,101].

1.3.3.1. Dieta baixa en histamina

La dieta baixa en histamina és la principal eina de la qual es disposa actualment per la gestió dietètica de la intolerància a la histamina. Conceptualment, aquestes dietes es basen en l'exclusió dels aliments que els pacients relacionen amb l'aparició de la simptomatologia, principalment, aquells que potencialment poden contenir elevats nivells d'histamina. En la literatura científica existeixen diverses publicacions on s'indiquen els aliments a excloure en el seguiment d'una dieta baixa en histamina [50–52,102–107]. En general, s'eliminen tots aquells aliments susceptibles de contenir histamina com derivats del peix, productes fermentats i alguns productes d'origen vegetal (tomàquet, espinacs i albergínia). No obstant, s'observa que no

existeix un únic tipus de dieta baixa en histamina i que no hi ha un consens en aquells aliments que cal eliminar segons les diferents dietes disponibles en la literatura.

Algunes publicacions diferencien tres fases al llarg del seguiment d'una dieta baixa en histamina (Figura 5) [40,45,85]. La primera fase consisteix en l'eliminació de tots aquells aliments que freqüentment els pacients relacionen amb l'aparició dels símptomes. Aquesta fase és la més curta (fins a 2 setmanes) però també la més restrictiva. El fet que la histamina es trobi àmpliament distribuïda en els aliments i a diferents concentracions fa que per precaució s'hagin d'excloure una gran quantitat de productes. Es important assegurar la frescor dels aliments, sobretot aquells d'origen animal (carns i peixos) ja que la presència d'histamina es relaciona amb una inadequada qualitat higiènica de les matèries primeres i/o dels processos de conservació i/o elaboració [18,22]. En aquesta fase també es tenen en compte factors que puguin influir en l'aparició dels símptomes, com per exemple, el consum d'alcohol o de certs fàrmacs que puguin inhibir l'enzim DAO. En la segona fase de la dieta baixa en histamina s'introdueixen gradualment alguns dels aliments exclosos en la primera, valorant la tolerància de cada pacient. Per últim, en la darrera fase, que té com a objectiu facilitar al màxim l'adherència a la dieta a llarg termini, es continua amb la reintroducció d'aliments exclosos per fer-la el més complerta i equilibrada possible, sempre i quan la tolerància de cada pacient ho permeti.

Fase 1 o fase d'exclusió

Objectiu:
- Minimitzar l'aparició dels símptomes al màxim possible.

Recomanació:
- Reduir la ingesta d'aliments susceptibles de contenir histamina (aliments i begudes fermentades, espinacs, bledes i tomàquets).
- Prioritzar els aliments frescos sobretot aquells d'origen animal (peixos i carns).
- Considerar factors de risc individuals (ingesta d'alcohol, estrès, processos menstruals, ús de fàrmacs, etc.).
- Optimitzar l'aportació de nutrients.
- Suplementar amb enzim DAO exogen.

Duració:
- Entre 10 i 14 dies.

Fase 2 o fase de reintroducció

Objectiu:
- Avaluar la tolerància individual a la ingesta d'histamina.

Recomanació:
- Reintroduir gradualment aquells aliments exclosos en la Fase 1.
- Realitzar, al màxim possible, una dieta complerta i equilibrada.
- Considerar factors de risc individuals (ingesta d'alcohol, estrès, processos menstruals, ús de fàrmacs, etc.).
- Suplementar amb enzim DAO exogen si es necessari.

Duració:
- Fins a 6 setmanes.

Fase 3 o dieta a llarg termini

Objectiu:
- Mantenir una bona qualitat de vida.

Recomanació:
- Seguir introduint aliments exclosos segons la sensibilitat mostrada a la ingesta d'histamina.
- Continuar amb la realització d'una dieta complerta i equilibrada.
- Considerar factors de risc individuals (ingesta d'alcohol, estrès, processos menstruals, ús de fàrmacs, etc.).
- Suplementar amb enzim DAO exogen si es necessari.

Duració:
- Indefinida.

Figura 5. Fases proposades pel seguiment d'una dieta baixa en histamina. Adaptada de Reese i col. (2021) i de Hrubisko i col. (2021).

L'eficàcia del seguiment de les dietes baixes en histamina ha estat avaluada en diferents estudis clínics amb resultats favorables pel que fa a la millora o remissió total dels símptomes associats a la intolerància a la histamina per dèficit de DAO (Taula 2) [50–53,86,102–104,108–114]. Malgrat que el nombre de participants reclutats en aquests estudis és reduït, la taxa d'eficàcia del seguiment d'una dieta baixa en histamina és significativa. En la gran majoria d'aquests treballs (14 de 15) s'ha reflectit una millora de la simptomatologia en més del 75% dels participants. Només en l'estudi reportat per Park i col. (2008) no es van observar millores en cap dels pacients avaluats (Taula 2). En dos treballs recents, en comptes d'avaluar l'efecte de la intervenció dietètica en el percentatge de pacients que van millorar la simptomatologia, han avaluat com influeix aquesta en la duració i la severitat dels símptomes, observant-se millores en ambdós paràmetres (Taula 2). En l'estudi realitzat per Cucca i col. (2022) es va observar una millora del 40% en la severitat dels símptomes en 146 pacients amb intolerància a la histamina després de la intervenció dietètica. A més, aquesta millora en la simptomatologia va ser més gran en aquells individus amb un dèficit de l'enzim DAO [86].

La duració del tractament dietètic aplicat en aquests estudis va ser, en general, de 3-4 setmanes, no apreciant-se una relació positiva entre una major durada i el percentatge d'eficàcia en la millora o remissió de la simptomatologia. Com es pot veure també en la Taula 2, alguns estudis també han avaluat l'efecte de la dieta en altres variables com la histamina i/o l'activitat DAO en sang. Quant a l'activitat de la DAO, els estudis publicats per Steinbrecher i col. (2005), Maintz i col. (2006), Mušič i col. (2013) i Lackner i col. (2019) van assenyalar un augment de l'activitat enzimàtica plasmàtica en més del 50% dels pacients després de la intervenció dietètica, si bé encara no s'ha suggerit cap hipòtesi explicativa. Per contra, Guida i col. (2000), Wagner i col. (2017) i Son i col. (2018) no van detectar canvis en l'activitat de la DAO en sang.

Taula 2. Estudis clínics sobre l'eficàcia d'una dieta baixa en histamina en el tractament de símptomes de la intolerància a la histamina.

	Disseny de l'estudi	n	Simptomatologia	Durada dieta	Resultats de l'estudi	Ref.
Diferents símptomes de la intolerància a la histamina	Estudi prospectiu sobre evolució de simptomatologia	45	28 pacients amb cefalea crònica i 17 amb símptomes dermatològics i respiratoris	4 set.	73% dels pacients amb reducció dels símptomes. Concretament, 68% reducció de la cefalea crònica i 82% de reducció dels altres símptomes	[103]
	Estudi prospectiu sobre evolució de simptomatologia, nivells plasmàtics d'histamina i d'activitat DAO	35	Pacients amb cefalea i altres símptomes (urticària, arrítmia, diarrea i asma)	4 set.	77% dels pacients amb reducció dels símptomes, 73% augment activitat DAO i absència de modificació de la histamina plasmàtica	[108]
	Estudi prospectiu sobre evolució de simptomatologia i activitat DAO en 5 pacients	17	Pacients amb dèficit de DAO, èczema atòpic i altres símptomes (cefalea, rubor i símptomes gastrointestinals)	2 set.	100% dels pacients amb reducció dels símptomes i 60% (tres de cinc) augment de l'activitat DAO.	[50]
	Estudi prospectiu sobre evolució de simptomatologia i activitat DAO	20	Pacients amb dèficit de DAO i símptomes dermatològics, gastrointestinals i respiratoris	6-12 mesos	100% dels pacients amb reducció dels símptomes i 100% augment activitat DAO	[52]
	Estudi retrospectiu sobre evolució de simptomatologia	16	Pacients pediàtrics amb dolor abdominal difús, diarrea, cefalea, vòmit i erupció cutània	4 set.	100% dels pacients amb reducció dels símptomes	[51]
	Estudi prospectiu sobre evolució de simptomatologia i activitat DAO	56	Pacients amb urticària crònica idiopàtica i símptomes gastrointestinals	3 set.	75% dels pacients amb reducció dels símptomes i absència de modificació de l'activitat DAO	[102]
	Estudi retrospectiu sobre evolució de simptomatologia	146	Pacients amb símptomes gastrointestinals, de pell i/o cefalea	No especificat	40% de reducció en la severitat dels símptomes	[86]

Dermatològics	Estudi prospectiu sobre evolució de simptomatologia, nivells plasmàtics d'histamina i d'activitat DAO	29	10 pacients amb urticària crònica i 19 individus control	3 set.	100% dels pacients amb reducció dels símptomes, 100% reducció d'histamina plasmàtica i absència de modificació de l'activitat DAO	[53]
	Estudi prospectiu sobre evolució de simptomatologia i d'ús d'antihistamínics	48	13 pacients amb urticària crònica i 35 pacients control (sense dieta baixa en histamina)	4 set.	Absència de millora en la simptomatologia ni en l'ús d'antihistamínics.	[109]
	Estudi prospectiu sobre evolució de simptomatologia	55	36 pacients amb dermatitis atòpica i 19 individus control	2 set.	33% dels pacients amb reducció de la dermatitis atòpica	[110]
	Estudi retrospectiu sobre evolució de simptomatologia	157	Pacients amb urticària crònica idiopàtica	4 set.	46% dels pacients amb reducció dels símptomes	[111]
	Estudi prospectiu sobre evolució de simptomatologia, nivells plasmàtics d'histamina i d'activitat DAO	22	Pacients amb urticària idiopàtica	4 set.	100% dels pacients amb reducció dels símptomes, 100% reducció d'histamina plasmàtica i absència de modificació de l'activitat DAO	[104]
Gastro-intestinals	Estudi prospectiu sobre evolució de simptomatologia	16	Pacients pediàtrics amb dèficit de DAO i dolor abdominal crònic	4 set.	88% dels pacients amb reducció dels símptomes	[112]
	Estudi retrospectiu sobre evolució de simptomatologia i activitat DAO	63	Pacients amb símptomes gastrointestinals	7-18 mesos	79% dels pacients amb reducció dels símptomes i 52% augment activitat DAO	[113]
Respiratoris	Estudi prospectiu sobre evolució de simptomatologia	18	Pacients pediàtrics amb símptomes lleus d'asma intermitent	8 set.	Reducció de 4 dies en l'aparició dels símptomes i de 19 punts en la severitat dels símptomes	[114]

1.3.3.2. Suplementació amb enzim DAO exogen

En la gestió dietètica de la intolerància a la histamina, la dieta baixa en histamina es pot suplementar oralment amb enzim DAO exogen. Aquest enzim ajudaria a degradar la histamina de la dieta a nivell intestinal [115]. De la mateixa manera que per la dieta, la suplementació amb l'enzim DAO també es modula al llarg de les diferents fases del tractament, per adaptar la dosi a la resposta de cada pacient.

L'any 2017, la Comissió Europea va permetre la comercialització de l'enzim DAO com a complement alimentari o com aliment per a usos mèdics especials [116]. Concretament, aquesta normativa europea autoritza la formulació del complement a base d'extracte proteic de ronyó porcí amb un recobriment entèric per assegurar-ne la integritat durant el seu pas pel medi àcid de l'estomac [116]. Actualment, existeixen diversos complements d'enzim DAO disponibles al mercat, tant a nivell nacional com internacional, tots ells formulats amb la mateixa dosi unitària d'enzim DAO (4,2 mg d'extracte de ronyó porcí per càpsula que contenen 0,3 mg d'enzim DAO).

Actualment, només existeixen cinc estudis d'intervenció on han provat l'eficàcia clínica de la suplementació amb DAO exogen en individus amb símptomes d'intolerància a la histamina. Malgrat que amb certa variabilitat, els investigadors apunten l'eficàcia d'aquest complement per reduir l'aparició i la intensitat dels símptomes. Komericki i col. (2011), Manzotti i col. (2016) i Schnedl i col. (2019) van assajar l'eficàcia de la suplementació amb DAO en pacients amb diferents símptomes gastrointestinals, cardiovasculars, respiratoris, dermatològics i/o neurològics) [49,97,101]. Aquests tres estudis van descriure una millora important en la intensitat o freqüència dels símptomes, encara que la població estudiada (14, 28 i 39 pacients) i el temps d'intervenció (de 2 a 4 setmanes) van ser reduïts. D'altra banda, Yacoub i col. (2018) van observar una reducció significativa en la severitat dels símptomes en 20 pacients amb urticària crònica espontània després de la suplementació amb l'enzim DAO [117]. L'any 2019, un estudi clínic aleatori doble cec va avaluar l'eficàcia de la suplementació amb DAO en pacients amb migranya.

Aquest estudi va considerar un major nombre de pacients (100 pacients) i va obtenir una disminució estadísticament significativa en la durada dels atacs de dolor sense efectes secundaris adversos registrats [118]. Malgrat aquests resultats positius sobre l'ús d'una suplementació amb enzim DAO exogen, són necessaris estudis clínics més ambiciosos amb un disseny experimental rigorós, períodes d'intervenció dietètica més prolongats i una mostra de grandària adequada per a establir l'eficàcia clínica d'aquest tractament dietètic.

Per últim, a més de l'enzim d'origen porcí, alguns estudis han descrit una alta capacitat catalítica de l'enzim DAO d'origen vegetal en la degradació de la histamina [33,34,119]. En concret, s'ha assenyalat que els brots de certes lleguminoses comestibles poden ser fonts interessants d'enzim DAO. Comas-Basté i col. (2020) van demostrar que la germinació de lleguminoses és capaç d'augmentar la capacitat enzimàtica DAO fins a 250 vegades respecte a les llavors sense germinar. A més, en aquest mateix estudi, certes espècies de lleguminosa van mostrar activitats enzimàtiques comparables o inclòs majors que la reportada per extractes de ronyó porcí [115]. La disponibilitat d'un complement DAO extret d'una font vegetal permetria el seu ús en població vegetariana/vegana, així com en aquelles persones amb restriccions religioses al consum de productes derivats del porc.

Fruit de la revisió bibliogràfica realitzada al llarg de la tesi doctoral s'ha publicat un capítol de llibre d'accés obert.

PUBLICACIÓ 1

Concept, aetiology and current diagnostic and treatment approaches of histamine intolerance: A review.

Oriol Comas-Basté, Sònia Sánchez-Pérez, M. Teresa Veciana-Nogués, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou.

Vide Leaf, 2021*,* Prime Achieves in Nutrition, Editor: Eloy A Zepeda-Carrillo.

DOI: 10.37247/PAN.1.2021.10

Prime Archives in Nutrition

Book Chapter

Concept, Etiology and Current Diagnostic and Treatment Approaches of Histamine Intolerance: A Review

Oriol Comas-Basté[1,2,3], Sònia Sánchez-Pérez[1,2,3], Maria Teresa Veciana-Nogués[1,2,3], Mariluz Latorre-Moratalla[1,2,3] and María del Carmen Vidal-Carou[1,2,3]*

[1]Departament de Nutrició, Ciències de l'Alimentació i Gastronomia, Facultat de Farmàcia i Ciències de l'Alimentació, Campus de l'Alimentació de Torribera, Universitat de Barcelona, Spain
[2]Institut de Recerca en Nutrició i Seguretat Alimentària (INSA·UB), Universitat de Barcelona, Spain
[3]Xarxa d'Innovació Alimentària (XIA), Spain

***Corresponding Author:** María del Carmen Vidal-Carou, Departament de Nutrició, Ciències de l'Alimentació i Gastronomia, Facultat de Farmàcia i Ciències de l'Alimentació, Campus de l'Alimentació de Torribera, Universitat de Barcelona, Av. Prat de la Riba 171, 08921 Santa Coloma de Gramenet, Spain

Published **January 11, 2021**

This Book Chapter is a republication of an article published by María del Carmen Vidal-Carou, et al. at Biomolecules in August 2020. (Comas-Basté, O.; Sánchez-Pérez, S.; Veciana-Nogués, M.T.; Latorre-Moratalla, M.L.; Vidal-Carou, M.C. Histamine Intolerance: The Current State of the Art. Biomolecules 2020, 10, 1181.)

How to cite this book chapter: Oriol Comas-Basté, Sònia Sánchez-Pérez, Maria Teresa Veciana-Nogués, Mariluz Latorre-Moratalla, María del Carmen Vidal-Carou. Concept, Etiology and Current Diagnostic and Treatment Approaches of Histamine

Intolerance: A Review. In: Eloy A Zepeda-Carrillo, editor. Prime Archives in Nutrition. Hyderabad, India: Vide Leaf. 2021.

© The Author(s) 2021. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License(http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Funding: This research received no external funding.

Acknowledgments: Sònia Sánchez-Pérez is a recipient of a doctoral fellowship from the University of Barcelona (APIF2018).

Conflicts of Interest: The authors declare no conflict of interest.

Abstract

Histamine intolerance, also referred to as enteral histaminosis or sensitivity to dietary histamine, is a disorder associated with an impaired ability to metabolize ingested histamine that was described at the beginning of the 21st century. Although interest in histamine intolerance has considerably grown in recent years, more scientific evidence is still required to help define, diagnose and clinically manage this condition. This book chapter will provide an updated review on histamine intolerance, mainly focusing on its etiology and the existing diagnostic and treatment strategies. In this work, a glance on histamine intoxication will also be provided, as well as the analysis of certain uncertainties historically associated to histamine intoxication outbreaks that may be better explained by the existence of interindividual susceptibility to ingested histamine.

Keywords

Histamine; Food Intolerance; Histamine Intolerance; Histaminosis; Histamine Intoxication; Diamine Oxidase (DAO); Low-Histamine Diet; Food Supplement

Introduction

In 2011 the European Food Safety Authority (EFSA) issued a scientific report warning that the levels of biogenic amines found in foods marketed in European Union countries may still entail a consumer health risk [1]. Among them, histamine has the highest toxic potential, along with tyramine, and is therefore of great interest in terms of food safety. First described more than 60 years ago, the deleterious effects of excessive histamine ingestion were initially referred to as scombroid fish poisoning or scombrotoxicosis, as they were associated with the consumption of fish in this family, but the condition is now known as histamine intoxication or histamine poisoning. In recent years, another disorder associated with histamine intake, arising from an enzymatic deficiency, has been described. The inability of certain individuals to metabolize histamine in the intestine, resulting in sensitivity to normal or even low histamine levels in food, may help to explain some of the uncertainties historically associated with histamine intoxication.

During the last decade, histamine intolerance has gained social and scientific recognition, with a significant increase in the interest of researchers to investigate this disorder. This review aims to analyze the pathophysiological relevance of dietary histamine, giving special focus to the adverse effects derived from histamine intake and, in particular, to the state of the art concerning the etiology, diagnosis and treatment of histamine intolerance.

Histamine

Histamine (2-[4-imidazolyl]ethylamine) is a bioactive amine that is synthesized by decarboxylation of its precursor amino acid, histidine, in an enzymatic reaction first described by Windaus and Vogt in 1907 involving L-histidine decarboxylase (EC 4.1.1.22) (Figure 1) [2]. Due to its chemical structure and number of functional groups, histamine can be defined as a heterocyclic diamine with an imidazole ring and ethylamine (i.e. an organic compound that provides a functional group in the form of a primary amine) [1,3].

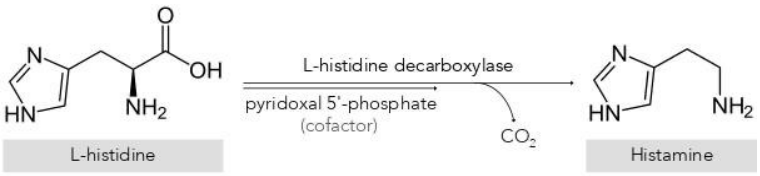


Figure 1: Synthesis of histamine by decarboxylation of its precursor amino acid.

The physiological and pathophysiological effects of histamine on the body were first described in 1910 by Dale and Laidlaw, two pioneering researchers who studied the functions of this organic compound at the Wellcome Physiological Research Laboratories [4–6]. Specifically, histamine is synthesized and stored in high concentrations in secretory granules, mainly in basophils and mast cells, and also in gastric enterochromaffin cells, lymph nodes and the thymus [1,7]. Functionally, this amine is involved in various immune and physiological mechanisms, stimulating gastric acid secretion, inflammation, smooth muscle cell contraction, vasodilation and cytokine production, among other processes [8–11]. In addition, histamine functions as a neurotransmitter, being synthesized by neurons located in the posterior region of the hypothalamus whose axons extend through the brain [12]. These wide-ranging physiological effects occur by interaction with four G-protein-coupled receptors with seven transmembrane domains (H1, H2, H3, and H4), which activate signal transduction pathways upon perceiving their ligand, histamine [7,12].

Two main histamine metabolic pathways are known in humans, involving the enzymes diamine oxidase (DAO) and histamine-N-methyltransferase (HNMT) (Figure 2) [10,11,13]. DAO (EC 1.4.3.22), also called histaminase or amiloride-binding protein, is a copper-dependent amino oxidase encoded by the AOC1 gene located on chromosome 7 (7q34-36) [14–16]. This functional enzyme, a homodimer with two isoforms, catalyzes the oxidative deamination of the primary amine group of histamine[14,16,17]. On the other hand, histamine can be metabolized to 1-methylhistamine by the enzyme HNMT (EC 2.1.1.8), a small monomeric protein encoded by a gene located on chromosome

2q22.1 [18]. HNMT catalyzes the methylation of the secondary amine group of the histamine imidazole aromatic heterocycle by a reaction requiring the S-adenosyl methionine co-substrate as a methyl group donor [11,13,19].

Thus, depending on its location, the histamine present in the body is deaminated or methylated by the action of the enzymes DAO and HNMT, respectively [1,10,20]. DAO is a secretory protein stored in vesicular structures of the plasma membrane and is responsible for the degradation of extracellular histamine [1,15]. In mammals, the expression of DAO is restricted to certain tissues, mainly the small intestine, ascending colon, placenta, and kidneys [14,21]. In the intestine, DAO activity increases progressively from the duodenum to the ileum and is located mainly in the intestinal villi [22]. In contrast, the enzyme HNMT is expressed in a wide range of human tissues, above all in the kidneys and liver, and also the spleen, colon, prostate, ovaries, spinal cord cells and the trachea and respiratory tract [10,13]. HNMT is a cytosolic protein responsible for the inactivation of intracellular histamine and can be synthesized in the cell itself or incorporated from the extracellular space by binding to a receptor or by membrane transporters [7,18]. Regarding substrates, HNMT is highly selective for histamine, whereas DAO can also metabolize other biogenic amines such as putrescine and cadaverine, although it shows a preference for histamine [14,16,23]. The affinity of DAO and HNMT for histamine is very similar, although the latter shows a slightly lower Michaelis-Menten enzymatic constant (kM: 6-13 μmol/L) than DAO (kM: 20 μmol/L) [10].

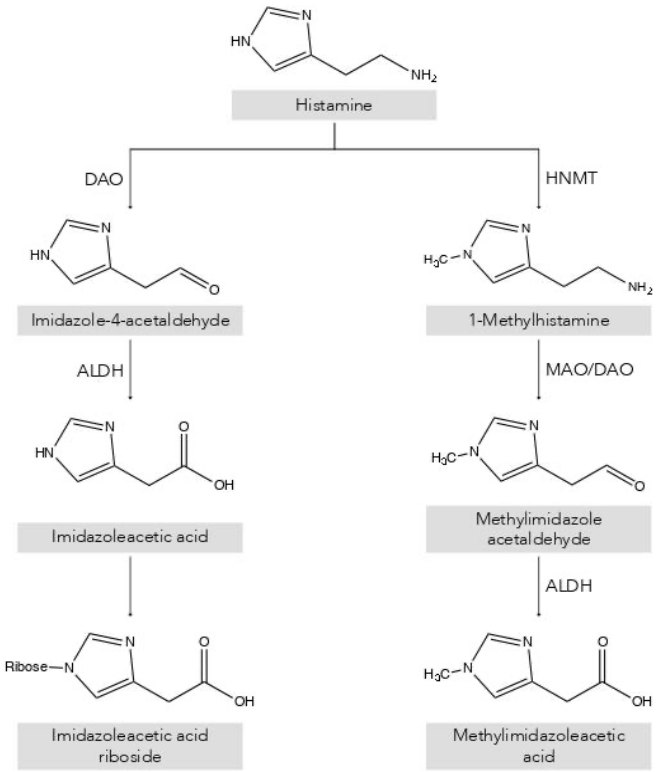


Figure 2: Histamine metabolism in humans. DAO: diamine oxidase; HNMT: histamine-N-methyltransferase; ALDH: aldehyde dehydrogenase; MAO: monoamine oxidase.

The gateway for dietary histamine in the body is the intestinal epithelium. Therefore, although HNMT is also present in the gastrointestinal tract, the more highly expressed DAO plays the major role in protecting the body against exogenous histamine, whether originating from ingested food or generated by the intestinal microbiota [24–26]. The protective effect of DAO has been demonstrated in animal experimentation models that were administered aminoguanidine for irreversible and selective DAO inhibition followed by a dose of histamine [24,27,28]. The development of anaphylaxis symptoms in DAO-inhibited pigs and sheep compared to control groups indicates that the enzyme exerts a significant barrier effect against the absorption of

exogenous histamine into the systemic circulation [1,13,19,24,29]. The HNMT enzyme ranks second to DAO in protecting against the absorption of dietary histamine from the intestinal lumen but appears to be more effective against intravenously or intradermally supplied histamine [13,30].

Histamine in Foods

Histamine is present in a wide range of foods in highly variable concentrations, which are the main exogenous source of this compound [31]. The main route for histamine formation in food is decarboxylation of histidine through the action of L-histidine decarboxylase, an enzyme of bacterial origin [32,33]. Apart from histamine, food can also contain other biogenic amines, mainly tyramine (4-hydroxy-phenethylamine), putrescine (1,4-diaminobutane) and cadaverine (1,5-diaminopentane), which are formed through enzymatic deamination of the amino acids tyrosine, ornithine (and/or agmatine) and lysine, respectively [31,34]. The accumulation of these compounds in food is the result of the transformation of amino acids by microorganisms and depends on various factors, such as the availability of the precursor amino acids and environmental conditions favorable for growth and/or the bacterial decarboxylase activity [31,34,35].

These decarboxylation reactions have been described as a survival strategy for microorganisms in acidic environments, as well as an alternative source of metabolic energy in situations of suboptimal substrate availability [1,9]. This enzymatic activity in bacteria is a species- and strain-dependent property [32]. Several gram-positive and gram-negative bacteria responsible for microbial spoilage or fermentative processes in food are able to produce histamine [1,36]. Specifically, the Enterobacteriaceae species *Hafnai aluei*, *Morganella morganii* and *Klebsiella pneumonia* have been identified as some of the most prolific histamine-forming bacteria in fish [9,37]. On the other hand, in cheeses, fermented meat, vegetable derivatives, and fermented beverages, various lactic acid bacteria have also been described as histamine-producing microorganisms (e.g. *Lactobacillus hilgardii*, *Lactobacillus buchnerii*, *Lactobacillus curvatus* and *Oenococcus oeni*) as well as certain strains of Enterobacteriaceae [1,38,39].

Foods that potentially contain high levels of histamine are: a) those microbiologically altered, such as fish and meat, or derived products that may have been preserved or processed in unsuitable hygienic conditions; and b) fermented products, in which the bacteria responsible for the fermentation process may also have aminogenic capacity [3,40]. Table 1 shows histamine content in the different food categories from the Spanish market [31].

Table 1: Histamine content in different food categories. Adapted from [31].

Food		Histamine content (mg/kg)			
	n	Mean (SD)	Median	Minimum	Maximum
Fruits, vegetables and plant-based products					
Fruits	136	0.07 (0.20)	ND	ND	2.51
Nuts	41	0.45 (1.23)	ND	ND	11.86
Vegetables	98	2.82 (7.43)	ND	ND	69.72
Legumes	11	ND	ND	ND	ND
Cereals	28	0.12 (0.33)	ND	ND	0.89
Chocolate	25	0.58 (0.44)	0.17	0.16	0.56
Spices	12	ND	ND	ND	ND
Alcoholic beverages					
Beer	176	1.23 (2.47)	0.70	ND	21.60
White wine	83	1.24 (1.69)	0.45	0.10	13.00
Red wine	260	3.81 (3.51)	1.90	0.09	55.00
Fish and seafood products					
Fresh fish	136	0.79 (0.71)	ND	ND	36.55
Canned fish	96	14.42 (16.03)	5.93	ND	657.05
Semi-preserved fish	49	3.48 (3.37)	2.18	ND	34.90
Meat and meat products					
Fresh meat	6	ND	ND	ND	ND
Cooked meat	48	0.30 (0.26)	ND	ND	4.80
Cured meat	23	12.98 (37.64)	0.80	ND	150.00
Dry-fermented sausages	209	32.15 (14.22)	8.03	ND	357.70
Dairy products					
Unripened cheese	20	ND	ND	ND	ND
Raw milk cheese	20	59.37 (106.74)	18.38	ND	389.86
Pasteurized milk cheese	20	18.05 (38.23)	4.59	ND	162.03

ND: not detected.

Uncertainties associated with Histamine Poisoning: A Paradigm Shift towards Histamine Intolerance

Although histamine has important physiological functions in the body, it can pose a health risk when ingested in high levels [41]. The proper functioning of histamine degradation systems is key in preventing its accumulation. Histamine intoxication, a kind of food poisoning, may occur after the consumption of foods with an unusually high histamine content that overpowers the degradation mechanisms (generally higher than 500 mg/kg) [1,3,42].

Historically, histamine intoxication has also been termed scombroid fish poisoning or the mahi-mahi flush because of its repeated association with the consumption of fish in the Scombridae and Scomberesocidae families (e.g. tuna, herring and mackerel) [43]. Histamine was first identified in 1946 as the causative agent of the toxic effects of consuming poorly transported tuna, and for a long time histamine poisoning was associated almost exclusively with the consumption of spoiled fish [44,45]. Over the years, the World Health Organization (WHO) has recommended the use of the term histamine intoxication to better designate this pathology, as it can be caused by marine species from other families (e.g. Clupeidae, Engraulidae, Coriphaenidae and Pomatomidae) and even other foods, such as cheese [43]. A meta-analysis carried out in 2018 of the different scientific reports of histamine intoxication between 1959 and 2013 established that the causative food in 98% of cases was fish, the remaining percentage being attributed to cheese [46]. Currently, international health administrations consider histamine intoxication to be one of the main problems of global food security, both for its effects on human health and its impact on trade [47,48].

Histamine intoxication is characterized by occurring in outbreaks and having a short incubation period (i.e. 20-30 minutes post-ingestion), with symptoms that are generally of low/moderate severity and remit in a few hours [3]. The symptoms are closely linked to the various physiological functions of histamine in the

body, affecting the skin (e.g. redness, rash, urticaria, pruritus, edema and local inflammation), the gastrointestinal tract (e.g. nausea, vomiting and diarrhea) and the hemodynamic (hypotension) and neurological (e.g. headache, palpitations and tingling) bodily functions [1,41]. The symptomatic similarity of histamine intoxication with allergy means it is likely to be under-diagnosed [43,48,49]. The diagnosis of histamine intoxication is based primarily on the determination of elevated plasma histamine levels and/or the identification of an ingested food with an unusually high histamine content [13]. In general, an outbreak of histamine poisoning tends to involve more than one individual, lasts a short period of time and a particular causative food is identified [38].

In terms of incidence, the data available for the European Union shows an increase in histamine intoxication outbreaks in the last ten years, unlike other types of food poisoning, and with an almost hegemonic predominance of fish as the causative agent (over 90% of cases) [42,50]. The most recent data from the EFSA and European Center for Disease Prevention and Control (ECDC) show that in 2017 there was a 22% increase in outbreaks compared to the previous year [50]. Specifically, in 2017 there were a total of 117 outbreaks of histamine intoxication involving 572 people, 9% of whom required hospitalization. Fortunately, no deaths have been attributed to histamine poisoning over the past decade [42]. The same trend is observed in the information provided by the European Union Food and Feed Warning System (RASFF), with a progressive rise in the number of cases of histamine poisoning linked to tuna consumption in 2014-2017 and a particular high increase in 2017 [3].

Although histamine intoxication has been extensively studied in recent decades, unresolved questions remain, concerning, for example, the variable histamine concentrations in the foods triggering outbreaks, or the heterogeneity in the degree and type of adverse effects [46]. Furthermore, the fact that oral administration of histamine in doses equivalent to those normally found in foods causing illness does not produce the same range

and/or severity of symptoms, is a paradox that has led to multiple hypotheses [30].

Several authors have proposed that alcohol and certain food components, such as other biogenic amines, may have a potentiating effect on histamine toxicity [13,48]. Amines such as putrescine and cadaverine, which are usually found in foods along with histamine, can also act as DAO substrates. It has therefore been suggested that these amines could weaken the protective barrier against dietary histamine by competitively interacting with degradation enzymes in the intestine [3,49]. Other possible potentiators are alcohol and its metabolite acetaldehyde, as they compete with histamine for the enzyme ALDH, which is simultaneously involved in histamine and alcohol metabolism [1,32]. The potentiation effect of these components could help explain the differences in absorption of the same dose of histamine when ingested in isolation or in a food matrix [48,49]. The FAO and WHO have acknowledged that the involvement of potentiators can alter the threshold dose for toxicity, and they recommend that future studies focus on clarifying the ambiguities in the pathogenesis of histamine intoxication [30].

Finally, several authors have reported considerable interindividual variability in histamine tolerance, which has been demonstrated in intervention studies [1,3,10,13]. After the oral administration of the same histamine dosage, not all participants showed symptoms, and those who did varied in symptom type and severity and even had different blood histamine levels [48,51,52]. These results indicate the existence of population subgroups with greater sensitivity and clinical responses to histamine, likely linked to a diminished histamine degradation capacity, which could explain some of the historical uncertainties associated with histamine intoxication outbreaks [1]. Without disputing the clinical entity of histamine intoxication, the paradigm shift lies precisely in moving the focus from food to the human body, maintaining histamine as the causative agent, but focusing on how each person is able to respond to the intake of variable levels of histamine from food. Thus, histamine intolerance is the clinical condition that

describes the inability of certain individuals to degrade histamine and results in the onset of symptoms caused by its accumulation in the blood (Figure 3).

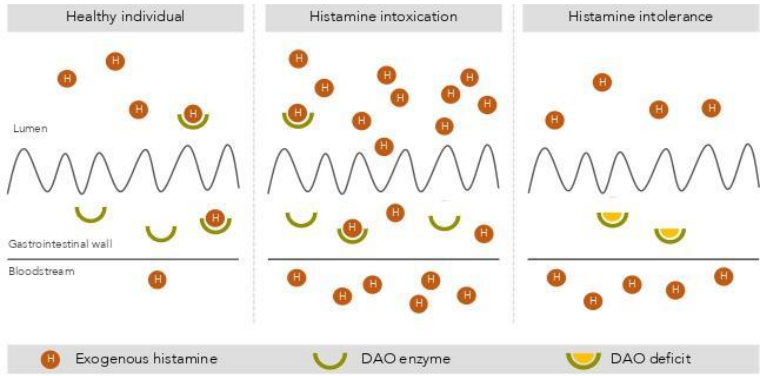


Figure 3: Intestinal degradation of histamine by the DAO enzyme in three different situations: a healthy individual, histamine intoxication and histamine intolerance. Adapted from [13].

Histamine Intolerance

According to the 2003 review of allergy nomenclature by the World Allergy Organization, adverse reactions to food without an immunological basis should be referred to as non-allergic food hypersensitivity, in order to clearly differentiate them from food allergies initiated by a specific immune mechanism [53]. Non-allergic food hypersensitivity is commonly known as food intolerance, a response triggered by a food or any of its components at a dose normally tolerated by the healthy population [54]. While the prevalence of food allergies is estimated at 1-2% in adults, currently almost 20% of the westernized world's population suffers from some type of food intolerance, with lactose intolerance being the most common [54].

Histamine intolerance, also referred to as enteral histaminosis or sensitivity to dietary histamine, can be defined as a disorder arising from a reduced histamine degradation capacity in the intestine due to an impaired DAO activity, leading to its

accumulation in plasma and the appearance of adverse effects [11,41,55].

Regarding DAO enzyme, it was firstly identified back in 1929 by Charles H. Best in autolysing lung tissue, which he called histaminase because of its ability to degrade histamine [56]. Years later, given its ability to also degrade other diamines, as described above, the more accurate designation of DAO was proposed [57,58]. Beyond its role in the intestinal degradation of histamine in humans, DAO is also present in microorganisms, plants and animals, where it also catalyzes the oxidative deamination of the primary amino group of histamine into its corresponding aldehyde, concomitantly producing stoichiometric amounts of ammonia and hydrogen peroxide (Figure 4) [14,59,60].

Figure 4: Oxidative deamination of histamine by the DAO enzyme.

Although the first scientific references to histamine intolerance date from more than 20 years ago, it is significant that almost 80% are from the last decade, reflecting the growing interest of researchers in this disorder (Figure 5). In 2011, EFSA already considered histamine intolerance as one of the risks associated with histamine intake, clinically differentiating it from histamine intoxication [1]. In a subsequent joint report, the WHO and FAO emphasized that the no observed adverse effect level (NOAEL) established for histamine was only valid for healthy people, and not for members of susceptible populations, such as those with histamine intolerance [30]. EFSA concluded that only foods with histamine levels below the detection limits are safe for individuals with histamine intolerance [1].

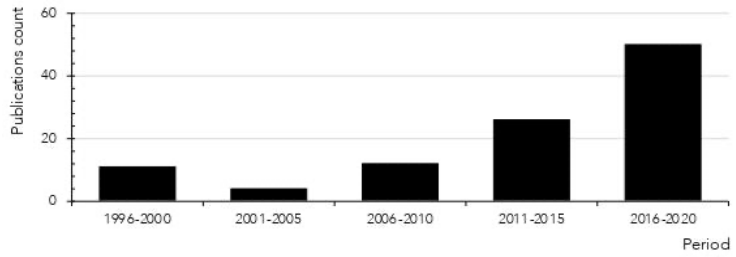


Figure 5: Count of scientific publications containing the key words histamine intolerance or histaminosis according to a search performed through the PubMed search engine at the MEDLINE bibliographic database (Search performed in july 2020).

Clinical manifestations of histamine intolerance consist of a wide range of non-specific gastrointestinal and extra-intestinal symptoms, due to the ubiquitous distribution of the four histamine receptors in different organs and tissues of the body (Figure 6) [10,13,54,61]. In a very recently published study, a team of Austrian researchers comprehensively analyzed the symptoms experienced by 133 patients diagnosed with histamine intolerance [62]. The most frequent and severe manifestations were gastrointestinal, with abdominal distension observed in 92% of patients and postprandial fullness, diarrhea, abdominal pain and constipation in 55-73%. Impairments of the nervous and cardiovascular systems, such as dizziness, headaches and palpitations, were recorded in second place, followed by respiratory and dermatological symptoms. Highlighting the complexity of the clinical picture of histamine intolerance, combinations of three or more symptoms involving different organs were recorded in 97% of cases, with an average of 11 symptoms per patient. The low specificity and complex variability of symptoms undoubtedly contribute to the current difficulty in achieving consensus on the diagnostic criteria for histamine intolerance, as will be discussed in detail below [13]. A lack of data also makes it difficult to determine the current incidence of this condition, although some authors have estimated that it affects 1-3% of the population, a percentage that will possibly increase as more knowledge and diagnostic tools for histamine intolerance become available [10,13,63].

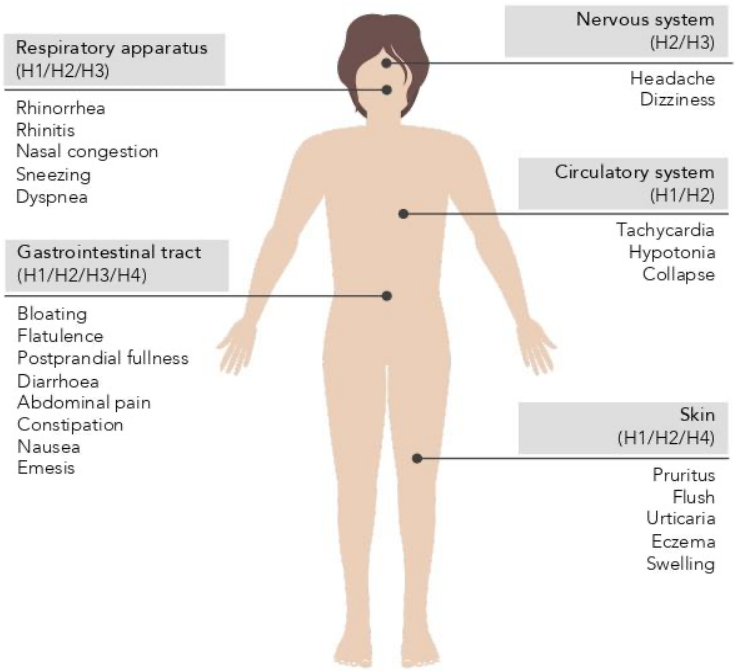


Figure 6: Main symptoms of histamine intolerance and possibly corresponding histamine receptors [10,64].

The Etiology of Histamine Intolerance

As mentioned in previous sections, the main barrier against exogenous histamine in the intestines is the DAO enzyme, which prevents its passage into the systemic circulation [10,13,65]. Numerous clinical studies have provided data on the prevalence of low plasma DAO levels in individuals showing symptoms of histamine intolerance, mainly headaches and gastrointestinal or dermatological disorders [66]. Although certain studies have limitations, either in the design or number of participants, the majority point to an association between symptoms and DAO deficiency, establishing a general trend that supports the key role of DAO in the etiology of these disorders. A DAO deficiency that predisposes a population subgroup to histamine intolerance may have a genetic, pathological, or pharmacological origin [1,41].

Regarding the genetic background of histamine intolerance, several studies have analyzed in depth the polymorphisms in genes encoding the enzymes L-histidine decarboxylase, DAO and HNMT, as well as the different histamine receptors. More than 50 non-synonymous single-nucleotide polymorphisms (SNPs) in the DAO-encoding gene have been identified, some of which can produce a protein with altered activity and lead to symptoms of histamine intolerance [67–72]. Specifically, the most relevant SNPs affecting DAO enzyme functionality in Caucasian individuals are rs10156191, rs1049742, rs2268999, and especially rs1049793 [69,71]. On the other hand, it has also been identified an SNP in the promoter region of the gene that causes a lower transcriptional activity of the DAO-encoding gene (rs2052129), as well as several genetic variations responsible for enzyme deficiency in people of Asian or African origin (rs45558339 and rs35070995, respectively) [67,72]. In most cases, the effect of these genetic variations on DAO functionality is through changes in enzyme kinetics, the resulting increase in KM causing a reduction in the rate of histamine degradation [69]. In parallel, three SNPs have been identified as responsible for enhanced DAO enzyme activity (rs2071514, rs1049748 and rs2071517) [72]. There is also evidence of DAO mutations in patients with certain cardiovascular, gastrointestinal and nervous system pathologies, although with contradictory results regarding positive/negative effects [68].

DAO deficiency can also be an acquired condition, caused by certain pathologies or interaction with drugs. Several inflammatory bowel pathologies affecting mucosal integrity are known to result in impaired DAO activity, the degree of which can be correlated with the severity of mucosal damage [73–75]. Thus, DAO activity has been proposed as a marker of integrity of the intestinal mucosa. Miyoshi et al. demonstrated that DAO activity can be a useful predictor of intestinal mucosal damage in patients receiving chemotherapy [76]. Additionally, DAO deficiency has also been linked to certain functional gastrointestinal disorders, such as carbohydrate malabsorption and non-celiac gluten sensitivity (NCGS) [63,73,77–79]. Enko et al. found that a concomitant reduction in DAO and lactase enzyme activities could be a consequence of mucosal damage in

the small intestine due to gastrointestinal disorders (e.g. gastroenteritis, irritable bowel syndrome, short bowel syndrome and gastrointestinal surgery) [73]. Moreover, patients with lactose intolerance and plasma DAO deficit showed higher end-expiratory H2 levels and the appearance of more symptoms during the H2 breath test in comparison with lactose-intolerant individuals with normal DAO activity [79]. More recently, two works have suggested a potential relationship between a reduced DAO activity and the presence of NCGS. Schnedl et al. based this relationship on the broad parallelism between the symptomatology of NCGS and histamine intolerance while the pilot study conducted by Griauzdaite et al. reported a strong association between reduced DAO activity and the presence of NCGS, although with a reduced number of patients [77,78]. In fact, Griauzdaite et al., found out that 9 of 10 patients with NCGS had decreased serum DAO activity levels [78]. This recently indicated relationship between both disorders, NCGS and histamine intolerance, should be further explored as it may be of interest for the correct clinical management of affected patients.

Finally, DAO deficiency can be a temporary and reversible condition, caused by the inhibitory effect of substances such as biogenic amines and alcohol, as discussed above, as well as several widely used drugs (Table 2) [1,10]. It has been estimated that approximately 20% of the European population regularly take DAO-inhibiting drugs, which significantly increases the number of people susceptible to the adverse effects of dietary histamine [28]. In vitro experimental results show a potent inhibitory effect (greater than 90%) of chloroquine, a historical antimalarial active ingredient, and clavulanic acid, a β-lactam antibiotic widely used in combination with amoxicillin [80]. A significant inhibition of the enzymatic activity has also been observed with the antihypertensive drug verapamil and the histamine H2 receptor antagonist cimetidine, although the clinical use of the latter is currently anecdotal [23,80]. Other substances have also shown an inhibitory effect, albeit to a lesser extent (Table 2) [23,80,81]. In most cases, the structural similarity of the cited drugs with histamine could explain their potential to bind to the active site of DAO and reduce its

enzymatic activity [23]. Along the same lines, substances with an inhibitory effect on other enzymes involved in any of the metabolic pathways of histamine in the body (i.e. HNMT, ALDH and MAO) may act as a trigger of histamine hypersensitivity [82].

Table 2: Active ingredients with an experimentally demonstrated inhibitory effect on the DAO enzyme [23,28,80,81].

Active ingredient	Indication
Cloroquine	Antimalarial
Clavulanic acid	Antiobiotic
Colistimethate	Antiobiotic
Cefuroxime	Antiobiotic
Verapamil	Antihypertensive
Clonidine	Antihypertensive
Dihydralazine	Antihypertensive
Pentamidine	Antiprotozoal
Isoniazid	Antituberculous
Metamizole	Analgesic
Diclofenac	Analgesic and anti-inflammatory
Acetylcysteine	Mucoactive
Amitriptyline	Antidepressant
Metoclopramide	Antiemetic
Suxamethonium	Muscle relaxant
Cimetidine	Antihistamine (H2 antagonist)
Prometazina	Antihistamine (H1 antagonist)
Ascorbic acid	Vitamin C
Thiamine	Vitamin B1

Prevalence of DAO Deficit in Persons with Symptoms Related to Histamine Intolerance

Several studies have evaluated the prevalence of DAO deficit in plasma of individuals with symptoms of histamine intolerance and/or diagnosed with certain chronic disorders.

Mušič et al. found DAO deficiency in 80% of 316 adult patients showing various symptoms associated with histamine intolerance (e.g. urticaria, pruritus, diarrhea, abdominal pain, vomiting, constipation, cough, rhinitis and headache), as well as significantly lower plasma DAO activity compared to the control group [83]. Similarly, in a retrospective study, Manzotti et al.

evaluated DAO activity in 14 patients with a confirmed diagnosis of histamine intolerance who showed mainly gastrointestinal and dermatological symptoms, but also headaches [84]. In this case, patients showed a high prevalence of DAO deficit (71%) and a significantly lower mean DAO activity compared to healthy volunteers. A lower percentage of DAO deficiency in histamine- intolerant patients (24%) was reported by Pinzer et al. [63]. Those patients featured elevated histamine levels and constantly reduced DAO activities throughout the day.

In a study focused only on headache symptoms, Steinbrecher and Jarisch reported DAO deficiency in 23 of 27 patients (85%) [85]. In parallel, the authors described a significant increase in DAO activity after patients followed a low-histamine diet for four weeks, along with a remission or reduction in frequency of headaches in almost 90% of individuals. More recently, Izquierdo et al. studied the prevalence of DAO deficit in 137 patients diagnosed with a confirmed migraine diagnosis and in a control group of 61 non-migraine individuals [66]. In this study, a high prevalence of DAO deficiency was observed in the migraine group (87%) and with a mean DAO activity significantly lower in comparison with that obtained from control volunteers. However, the prevalence of DAO deficiency in the control population amounted up to 44%, what was attributed to the fact that certain individuals could present other symptoms associated with histamine intolerance or DAO deficiency other than migraine. Another study with 44 migraine patients reported a 60% prevalence of DAO deficiency and a significant co-presence of certain gastrointestinal disorders, such as celiac disease and NCGS [78].

In the field of dermatological symptomatology, several studies have monitored plasma DAO activity in patients with eczema, chronic idiopathic urticaria, and atopic dermatitis. Overall, the reported prevalence of DAO deficiency ranges from 19 to 57%, with the exception of the study by Worm et al., who did not detect statistically significant differences in plasma DAO activity between control patients and those with atopic dermatitis [86–89].

Finally, regarding gastrointestinal symptoms, Honzawa et al. assessed the clinical significance of plasma DAO activity levels in 98 patients suffering inflammatory bowel disease [90]. This study showed that DAO activity in blood was significantly lower in patients with Crohn's disease and ulcerative colitis compared to the control population, suggesting its potential importance as a marker of intestinal permeability. In a pediatric population under 15 years of age, Rosell-Camps et al. determined DAO deficiency in 88% of patients with abdominal pain, diarrhea and vomiting [91]. In contrast, in a more recent study by a group of Austrian researchers, DAO deficiency was found only in 8% of 394 children with chronic abdominal pain [92].

To date, little data is available on the prevalence of this enzymatic deficiency related to gender, and it is inconclusive. Klockler et al. found no differences in plasma DAO activity between men and women, although the number of individuals considered was scarce (n = 28) [93]. Likewise, the study performed by Izquierdo et al. reported similar percentages of DAO deficiency in migraine women (83%) and men (90%) [66]. On the contrary, García-Martín et al. did describe differences in plasma DAO activity by gender, with the prevalence of this enzyme deficiency being higher in women [94]. Significant fluctuations in DAO activity values have also been reported in women associated with different stages of the menstrual cycle [94,95].

One factor that could explain the discordance among the prevalence data of DAO deficit in patients with disorders associated with histamine intolerance is that the parameter considered in all of them was serum DAO activity, which, a priori, would not reflect an enzymatic deficiency derived from certain intestinal pathologies. Overall, in spite of the varying percentages in DAO deficiency, the currently available studies seem to indicate an etiological relationship between DAO deficiency and certain symptoms or disorders related to histamine intolerance. Nevertheless, more studies are needed to assess the clinical significance of the determination of plasma DAO activity as well as to develop new diagnostic methods

aimed at identifying individuals with histamine intolerance due to DAO deficiency.

Diagnosis of Histamine Intolerance

Despite significant advances in the understanding of histamine intolerance, reaching a consensus on a diagnostic algorithm remains a pending challenge. The inspecificity of symptoms and lack of validated diagnostic tools prompts many affected individuals to go ―doctor shopping‖, that is, to consult several medical specialists in search of an explanation and solution for their varied symptomatology [13,63]. In the absence of a consensual and clinically validated diagnosis, Figure 7 shows a schematic summary of the diagnostic algorithm for histamine intolerance based on the available scientific evidence reviewed below.

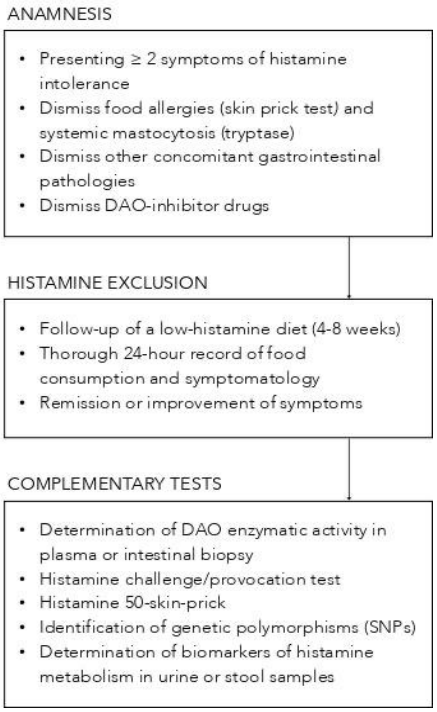


Figure 7: Summary of the described approaches to the diagnosis of histamine intolerance.

The combination of diagnostic criteria currently in use includes the appearance of typical clinical manifestations and the exclusion of other related disorders [10,13,54]. All the authors who have proposed a diagnostic algorithm for histamine intolerance emphasize the need to initially rule out other potential causes of symptoms associated with an increase in plasma histamine [10,13,54]. For this purpose, it is advisable to carry out an intradermal skin allergy test (i.e. skin prick test) to discard IgE sensitization caused by food allergy, and to measure plasma tryptase to exclude an underlying systemic mastocytosis [10]. It is also important to know whether the patient is taking any medication with a possible inhibitory effect on DAO activity [55]. If these conditions are negative, the appearance of two or more typical symptoms of histamine intolerance and their improvement or remission after the following of a low-histamine diet (i.e. a diet excluding foods that, a priori, contain high histamine levels) will confirm the diagnosis of histamine intolerance [10,54,96,97]. In the diet follow-up, a thorough 24-hour record of all the foods consumed and symptoms experienced is recommended in order to establish a relationship, if any, between a food and the onset of symptoms [10,13]. The duration of the low-histamine diet to confirm the diagnosis is not clearly stipulated, although some studies suggest a period of 4 to 8 weeks [54,97]. In addition to the diet, testing the effect of antihistamine treatment on symptoms has also been proposed, although its usefulness once dietary histamine is removed is unclear [10,54].

Once it has been established that dietary histamine is responsible for the intolerance-associated symptoms, the diagnosis of this disorder is virtually confirmed. A range of non-validated complementary tests have also been proposed by several authors with the aim of obtaining a marker to confirm the diagnosis [97]. However, it has to be taken into account that not all of the tests consider the different origins of DAO deficiency (i.e. genetic, pathological, or pharmacological). Thus, a genetic origin would lead to a reduction of the DAO enzymatic activity in the whole organism. Likewise, the pharmacological blockade of DAO would take place in all tissues where the drug is distributed after entering the systemic circulation, although in a punctual manner

upon the substance presence. Lastly, the scope of a DAO deficit due to intestinal pathologies would be limited to the local intestinal environment.

Due to the genetic background of DAO deficiency, one of the strategies for the diagnosis could be the determination of genetic polymorphisms (SNPs) that characterize the population genetically susceptible to histamine [54]. Currently, there is already the possibility of performing a non-invasive genetic analysis capable of identifying three of the SNPs associated with reduced DAO activity (i.e. rs10156191, rs1049742 and rs1049793) from a sample of the oral mucosa, although evidence-based studies on the diagnosis potential of this test are still needed. It is important to note that this test will only reflect the existence of a genetic DAO deficiency.

The most studied, and possibly also the most controversial, is the determination of plasma DAO activity. This analytical test consists of measuring the amount of histamine degraded in a blood sample in a given time interval. Two types of commercial testing kits are currently available on the market, one consisting of an ELISA-type immunoassay, and the other a radioimmunoassay using radioactively labeled putrescine [83,84]. The evidence for the validity of blood DAO activity measurements for the diagnosis of histamine intolerance is neither abundant nor conclusive. Some studies have proposed that determining blood DAO activity may be helpful in identifying subjects with symptoms associated with histamine intolerance [63,83,84]. In contrast, three studies did not find a significant relationship between the clinical history of patients with typical symptoms of histamine intolerance and blood DAO activity values, concluding that this technique cannot be recommended as a diagnostic tool in routine clinical practice until studies have validated its effectiveness [98–100]. Moreover, the work performed by Schnoor et al. also reported a high interassay variation in DAO activity values that made the proper classification of histamine intolerant subjects impossible [100]. This controversy is described in a joint article published in 2017 by the German and Swiss allergology societies, which emphasizes the need for more research before giving plasma

DAO activity a definitive diagnostic value for histamine intolerance [97]. A variant of the intradermal skin allergy test called the histamine 50-skin-prick test was also proposed by Kofler et al. to diagnose histamine intolerance [101]. In this technique, the results were read after 50 minutes (as opposed to the usual 20 minutes) and showed that, although the size of the wheal did not differ between the histamine intolerant and control groups, the time course was significantly different. Patients with symptoms of intolerance showed a delayed remission of the wheal induced by cutaneous administration of histamine, signaling a reduced degradation ability. The same results were obtained in a study recently published by Wagner et al., who re-evaluated this skin test as a diagnostic tool of histamine intolerance, also observing a correlation between the delay in wheal disappearance and a lower plasma DAO activity [102].

Both the determination of plasma DAO activity and the histamine 50-skin-prick test could be suitable tests to identify a DAO deficiency from genetic or pharmacological origin, but they would not be useful to determine a deficit secondary to certain intestinal diseases.

On the contrary, there are certain alternatives, such as the intestinal biopsy, the histamine provocation test or the histamine metabolomics in urine that could make it possible to diagnose histamine intolerance due to DAO deficiency without excluding any of the possible etiological causes.

The measurement of intestinal DAO activity by a colon biopsy during endoscopic procedures has been studied as a possible diagnostic marker. The few available studies have shown a reduced intestinal DAO catabolic activity in patients with recurrent urticaria, food allergy and colon adenoma, accompanied by an increase in histamine levels [103–106]. Although this test has interesting diagnostic potential, more studies are needed to validate its clinical significance and its relationship with the symptoms of histamine intolerance [97]. If proven, this diagnostic test would be very adequate since this disorder originates from a reduced ability of the intestinal DAO enzyme to cope with dietary histamine.

Histamine challenge/provocation test has also been proposed by some authors as a diagnostic tool for intolerance, which would, at the same time, establish the individual tolerance threshold. This double-blind, placebo-controlled test involves oral administration of histamine and requires patient medical supervision and hospitalization. In the study by Wöhrl et al., half of the healthy volunteers developed symptoms after the administration of a solution containing 75 mg of histamine [107]. In contrast, the results of a multicenter study by Komericki et al. using the same oral dose of histamine indicated the challenge test was unreliable for diagnosing histamine intolerance due to a lack of intraindividual reproducibility of symptoms after two different provocation tests [108]. The application of this procedure is still limited because of the risk of serious adverse side effects and the absence of a standardized dose of histamine and properly established protocol [97].

Finally, in recent years, efforts have been made to identify a non-invasive marker to establish a solid and clinically irrefutable diagnostic criterion for histamine intolerance due to DAO deficiency. Currently, the application of metabolomics as a tool for the identification of biomarkers of histamine metabolism in urine is also being challenged as a possible new diagnostic strategy [11]. The hypothesis is that individuals with histamine intolerance could have a different excretion profile of histamine and its metabolites in urine than normal individuals. For this purpose, Comas-Basté et al. have recently proposed a chromatographic approach that allows for determining in a fast and unequivocally manner the urinary levels of histamine and its methylated metabolite, methylhistamine [11]. It is still necessary to validate the potential diagnostic utility of this approach in patients with histamine intolerance, as well as complementing the excretion profile with other histamine metabolites to obtain a more accurate image of the possible alterations produced in this intolerance.

Treatment Approaches to Histamine Intolerance

Currently, the main strategy to avoid the symptoms of histamine intolerance is to follow a low-histamine diet. Supplementation

with exogenous DAO has recently been postulated as a complementary treatment to enhance dietary histamine degradation in intolerant individuals who have a deficiency of this enzyme in the intestine [109,110].

Low-Histamine Diet

A low-histamine or histamine- free diet has been proposed as the main strategy for the preventive treatment of histamine intolerance [10,54,82,111]. Conceptually, these diets exclude a number of foods that patients associate with the onset of symptoms, primarily those that may contain high levels of histamine [82]. However, there is no a single dietary recommendation of a low-histamine diet. As it may be seen in Table 3, there is no coincidence in all the foods excluded in the different low-histamine diets found in the literature [10,87,91,112–118].

Table 3: Foods excluded in the different low-histamine diets found in the literature [10,87,91,112–118].

Foods excluded by low-histamine diets		
<20%*	**20-60%***	**>60%***
Milk	Shellfish	Cured and semi-cured cheese
Lentils	Eggs	Grated cheese
Chickpeas	Fermented soy derivatives	Oily fish
Soybeans	Eggplant	Canned and semi-preserved oily fish derivatives
Mushrooms	Avocado	Dry-fermented meat products
	Banana	Spinach
	Kiwi	Tomatoes
	Pineapple	Fermented cabbage
	Plum	Citrus
	Nuts	Strawberries
	Chocolate	Wine
		Beer

* Percentage of low-histamine diets from the literature that are excluding each foodstuff.

Histamine is widely distributed in different food categories and in highly variable concentrations, as its accumulation is influenced by multiple factors [3,119]. In fresh foods such as fish

and meat, and in some derived products, the presence of histamine is due to a lack of freshness or an inadequate hygienic quality of raw materials and/or production processes [31]. For this reason, meat and fish can be consumed in the framework of a low-histamine diet, as long as their freshness is ensured. In contrast, fermented products are systematically excluded, due to a high probability of containing histamine [31]. Other foods such as spinach, eggplant and tomatoes should also be avoided for the same reason. In general, all these above-mentioned foods are unanimously eliminated in most published low-histamine diets (Table 3).

On the other hand, there are certain foods that a priori do not contain histamine but that patients associate with the appearance of symptoms. For these foods there is much more variability when it comes to their exclusion from low-histamine diets (Table 3). The exclusion of foods could be based on their content of other biogenic amines, such as putrescine and cadaverine, which act as competitive substrates for DAO and may therefore inhibit intestinal degradation of histamine if present in significant quantities [1,82]. Thus, the onset of symptoms after the consumption citrus fruits, mushrooms, soybeans, bananas and nuts may be due to high levels of other amines, specially putrescine [82]. These diets may also exclude certain foods free of histamine and with too low concentrations of other amines to justify their exclusion. This is the case, for example, of papaya, kiwi, strawberries, pineapple and plums, which have reported to trigger the release of endogenous histamine, although the mechanism responsible has not yet been elucidated [8,13].

The effectiveness of a low-histamine diet has been demonstrated in clinical studies, which report favorable results in terms of improvement or total remission of symptoms frequently associated with histamine intolerance and DAO deficiency (Table 4). As shown in Table 4, over the past three decades, various clinical studies have assessed the effect of a low-histamine diet on the evolution of various symptoms, mainly dermatological, gastrointestinal, and neurological, including cases with more than one type. Although most studies have involved only a small group of patients (mean of 38 per study,

with a minimum of 10 and maximum of 157), they report an efficacy rate for the diet ranging from 33% to 100%. Specifically, 10 of the 13 studies reviewed found an improvement in symptoms in more than 50% of patients who followed the diet; two studies show success rates of less than 50% (33% and 46%) and only one did not observe any beneficial effects (Table 4). Most of the studies involved patients with dermatological symptoms, primarily chronic idiopathic urticaria, atopic dermatitis, and eczema. In this field, a recent systematic literature review included a total of 1,668 patients with chronic urticaria undergoing different exclusion diets, including a low-histamine, pseudoallergen-free (i.e. without preservatives and artificial colors present in processed foods or aromatic compounds from certain natural products) and the fish exclusion diets [120]. Overall, following any of the exclusion diets resulted in the total or partial remission of symptoms in 4.9% and 37.5% of patients, respectively. A low-histamine diet for an average of 3 weeks resulted in one of the highest remission rates. Despite the promising results of a low-histamine diet for the treatment of dermatological conditions, scientific societies of dermatology still consider this exclusion diet of unproven utility pending randomized, double-blind, placebo-controlled clinical trials to confirm its effectiveness [121].

In general, the duration of the dietary treatment considered in the different clinical studies ranges from 3 to 4 weeks, and no positive relationship could be established between a longer duration and the success rate in symptom remission (Table 4). As may also be seen in this table, some studies have also assessed the effect of diet on other variables, such as plasma histamine levels or plasma DAO activity [83,85–87,112,122,123]. Regarding DAO activity, the studies published by Steinbrecher et al., Maintz et al., Mušič et al. and Lackner et al. all point out an increase in plasma enzymatic activity in more than 50% of patients after the dietary intervention, although no explanatory hypothesis has been yet suggested [83,85,86,123]. In contrast, Guida et al., Wagner et al and Son et al., reported no changes in serum DAO activity [87,112,122]. The inconsistency of these data highlights the need to develop more research in this specific field before conclusions can be drawn.

Table 4: Clinical studies on the efficacy of a low-histamine diet for the treatment of symptoms of histamine intolerance.

Design and outcomes of the study	Number of patients and symptoms	Duration	Percentage of patients with improvement in the study outcomes	Reference
Prospective study with evaluation of the evolution of the symptomatology	28 patients with chronic headache and 17 with other dermatological and respiratory symptoms	4 weeks	68% reduction in chronic headache and 82% reduction in other symptoms	[124]
Prospective study with evaluation of the evolution of symptoms, plasma histamine levels and DAO activity	10 patients with chronic idiopathic urticaria and 19 control individuals	3 weeks	100% reduction of symptoms, 100% reduction of plasma histamine and no changes in DAO activity	[122]
Prospective study with evaluation of the evolution of symptoms, plasma histamine levels and DAO activity	35 patients with headache and other symptoms (urticaria, arrhythmia, diarrhea and asthma)	4 weeks	77% reduction in symptoms, 73% increase in DAO activity and no changes in plasma histamine levels	[85]
Prospective study with evaluation of the evolution of symptoms and DAO activity (in 5 of the patients)	17 patients with DAO deficiency, atopic eczema and other symptoms (headache, flushing and gastrointestinal symptoms)	2 weeks	100% reduction in symptoms and 60% (three out of five) increase in DAO activity	[86]
Prospective study with evaluation of the evolution of symptoms and the use of antihistamine drugs	13 patients with chronic idiopathic urticaria and 35 control patients (without diet)	4 weeks	Lack of improvement in symptoms and no changes in the use of antihistamines	[125]
Prospective study with evaluation of the evolution of the symptomatology	36 patients with atopic dermatitis and 19 control individuals	2 weeks	33% reduction in symptoms	[88]
Prospective study with evaluation of the evolution of the symptomatology and DAO activity	20 patients with DAO deficiency and dermatological, gastrointestinal and respiratory symptoms	6-12 moths	100% reduction of symptoms and 100% increase of DAO activity	[83]
Retrospective study with evaluation of the evolution of the symptomatology	16 pediatric patients with diffuse abdominal pain, diarrhea, headache, vomiting and rash	4 weeks	100% reduction of symptoms	[91]
Prospective study with evaluation of the evolution of the symptomatology	16 pediatric patients with chronic abdominal pain and DAO deficiency	4 weeks	88% reduction of symptoms	[92]

Retrospective study with evaluation of the evolution of the symptomatology	157 patients with chronic idiopathic urticarial	4 weeks	46% reduction of symptoms	[126]
Prospective study with evaluation of the evolution of the symptomatology and DAO activity	56 patients with chronic idiopathic urticaria and gastrointestinal symptoms	3 weeks	75% reduction in symptoms and no changes in DAO activity	[87]
Prospective study with evaluation of the evolution of symptoms, plasma histamine levels and DAO activity	22 patients with chronic idiopathic urticarial	4 weeks	100% reduction in symptoms, 100% reduction in plasma histamine levels and no changes in DAO activity	[112]
Retrospective study with evaluation of the evolution of the symptomatology and DAO activity	63 patients with gastrointestinal symptoms	7-18 months	79% reduction in symptoms and 52% increase in DAO activity	[123]

Exogenous DAO Supplementation

Similar to the current treatment for lactose intolerance, the possibility of oral supplementation with exogenous DAO has been proposed by several authors to facilitate dietary histamine degradation [13,127]. Improving intestinal DAO activity would allow a less restrictive diet, which could include foods with a tolerable dose of histamine [10,61]. In this context, in the update of the official list of novel foods in 2017, the European Commission gave the green light to the marketing of a DAO supplement as food supplement or as food for special medical purposes [128]. Specifically, European regulations authorize the formulation of porcine kidney protein extract with an enteric coating to ensure its integrity during its passage through the gastric environment [128]. In this specific regulation, the minimum DAO enzymatic capacity required for the supplement is determined through a radioextractionassay (REA). This technique, based on the radioactive labeling of putrescine and the scintillation counting of its consumption, is advantageous in terms of rapidity and sensitivity but it was mainly conceived to be applicable to serum samples. Comas-Basté et al. have recently developed a rapid and reliable UHPLC-FL method for the *in vitro* determination of DAO activity specifically for the analysis of unpurified complex matrixes, such as porcine kidney extract and DAO supplements [129]. This methodological approach is based in the direct determination of histamine degradation and overcomes certain drawbacks in terms of matrix interferences and handling of radioactive materials.

Porcine kidneys are the main source of DAO enzyme according to the literature. Several studies have demonstrated the capacity of this product to degrade histamine and other biogenic amines in vitro [57,129–133]. A wide variability of the DAO capacity of porcine kidney extracts has been reported (with values ranging from 0,1 to more than 100 mU/mg), depending on the purification grade applied to the matrix and/or the amine compound used as reaction substrate [57,129–133]. In fact, many of these works sought the selective purification of the DAO enzyme to design biosensors for the biorecognition of biogenic amines as indicators of freshness in foods [134]. More recently, two studies have been published specifically focused in

investigating the in vitro DAO activity of porcine kidney protein extract expressly used as active ingredient to formulate food supplements for the preventive treatment of histamine intolerance [129,130]. Comas-Basté et al. studied the in vitro enzymatic activity of 13 different production batches of porcine kidney protein extract used in the elaboration of food supplements, reporting a low influence of the raw material (porcine kidney) on the DAO activity of the extract, with a mean value of 0,23 ± 0.01 mU/mg [129]. Later, Kettner et al. obtained a porcine kidney crude extract with an in vitro DAO activity of 0.5 ± 0.06 mU/mg and described a 10-fold increase of this enzymatic activity through the application of several consecutive purification steps [130].

Regarding the food supplement, divergent results have also been reported, since while certain authors discard its enzymatic capacity, DAO activity values ranging from 0.04 to 0.20 mU/mg have also been described for different commercial products available in the market [129,130]. Overall, these works coincide in the need to identify alternative sources to the porcine kidney DAO for exogenous supplementation in histamine-intolerant individuals.

A higher catalytic capacity of DAO enzymes of plant origin in degrading certain amino substrates has been described by some authors in comparison with those of animal origin [129,135–137]. Specifically, the germinated sprouts of certain edible legumes have been pointed out as interesting sources of DAO enzyme. Germination is a physiological process that has been described as capable of increasing the DAO enzymatic capacity of sprouts up to 250 times compared to ungerminated seeds [61,138]. The increased presence of DAO enzyme in legume sprouts could be associated with the importance of hydrogen peroxide, by-product of the deamination reaction, in cell wall structuring, lignification and mobilization of seed reserves during germination [139–142]. In fact, it has been demonstrated that the germination of legume seeds for a period of 6-8 days in darkness provides the optimal environment to maximize the DAO activity of this plant-origin matrix [61,138,143]. From a commercial point of view, having a plant source of this enzyme would expand the target of this novel food for vegetarian/vegan population, as well as religious restrictions on the consumption

of pork products. In addition, obtaining DAO enzyme from legumes would be a practice in accordance with the current call for action of the Sustainable Development Goals.

Nowadays, only 5 published intervention studies (4 of them in the last 5 years) have tested the clinical efficacy of exogenous DAO supplementation in patients with symptoms of histamine intolerance (Table 5). Although with some variability, the available research points to the effectiveness of DAO supplements in reducing the appearance and intensity of symptoms. However, it is difficult to compare the different studies since they differ in the design, the enzyme dosage, the intervention time and the measurement of efficacy outcomes. Komericki et al., Manzotti et al., and Schnedl et al. assayed the efficacy of DAO supplementation in patients with diverse symptoms associated with histamine intolerance (gastrointestinal, cardiovascular, respiratory, dermatological and/or neurological complaints) [84,108,109]. All three studies reported an important improvement in the intensity or frequency of symptoms, although they involved a short-sized population (14, 28 and 39 patients) and/or a reduced intervention time (from two to four weeks). Moreover, Schnedl et al. also evaluated the changes in plasmatic DAO activity, reporting a slight increase in 61% of patients during the intervention, which the authors linked to a possible improvement in the integrity of the intestinal mucosa due to the supplementation [109].

The clinical trials developed by Yacoub et al. and Izquierdo-Casas et al. were focused on a single disorder related to histamine intolerance (chronic spontaneous urticaria and migraine, respectively) [110,144]. Yacoub et al. considered 20 patients with chronic spontaneous urticaria, who showed a significant reduction in the severity of the complaint according to the Urticaria Activity Score (UAS-7) [144]. Regarding migraine, the randomized double-blind clinical trial conducted by Izquierdo et al. considered a larger number of patients (100 patients) and obtained a statistically significant decrease in the duration of pain attacks with no recorded adverse side effects [110]. However, the authors did not find statistical differences when considering other research outputs, such as the frequency and intensity of pain.

Table 5: Studies on the efficacy of DAO enzyme supplementation for the treatment of symptoms of histamine intolerance.

Design	Number of patients and symptoms	Duration of DAO supplementation	Efficacy outcomes	Reference
Randomized, double-blind, placebo-controlled, cross-over provocation study using histamine-containing and histamine-free tea in combination with DAO capsules or placebo	39 patients with histamine intolerance (headache and gastrointestinal and skin complaints)	-	Statistically significant reduction of histamine-associated symptoms compared to placebo.	[108]
Retrospective study with evaluation of the clinical response to DAO supplementation	14 patients with diagnosis of histamine intolerance (headache and gastrointestinal, cardiovascular, respiratory and skin complaints)	2 weeks	Reduction of at least one of the reported symptoms in 93% of patients.	[84]
Double-blind, placebo-controlled, cross-over study	20 patients with chronic spontaneous urticarial	1 month	Significant reduction of 7-Day Urticaria Activity Score (UAS-7) and slight significant reduction of daily antihistamine dose.	[144]
Randomized, double-blind, placebo-controlled clinical trial	100 patients with episodic migraine and serum DAO deficit	1 month	Significant decrease in the duration of migraine attacks and decrease in triptans intake.	[110]
Open-label interventional pilot study	28 patients with histamine intolerance (gastrointestinal, cardiovascular, respiratory and skin complaints) and reduced serum DAO values	1 month of intervention and 1 month of follow-up	Significant improvement in frequency and intensity of all symptoms. 61% of patients showed slightly increase in serum DAO values. During the follow-up period (without DAO supplementation) the symptoms sum scores increased and DAO levels decreased.	[109]

Overall, despite the promising results, more ambitious clinical studies with a rigorous experimental design, longer treatment periods and a properly sized sample are essential to establish the clinical efficacy of this treatment.

Conclusions and Perspectives

Histamine intolerance is currently a clinical entity of increasing interest, which can appear due to the intake of histamine from foods, mainly caused by a deficiency of the DAO enzyme at the intestinal level. Novel knowledge and study of histamine intolerance has helped clarify many of the uncertainties that were classically associated with histamine intoxication. Etiologically, various SNPs have been identified in the gene encoding the DAO enzyme related to lower enzyme activity. Moreover, certain inflammatory bowel diseases that limit enzyme secretion or some DAO-inhibiting drugs have also been identified as possible causes of DAO deficiency. This intolerance manifests through a plethora of non-specific gastrointestinal and extraintestinal symptoms.

The diagnosis of histamine intolerance is usually performed after ruling out allergic symptoms and by the presence of at least two clinical manifestations and their improvement or remission after following a low-histamine diet. Various complementary tests are currently being proposed to improve the diagnosis of this intolerance based, among others, on determining the DAO activity in blood or intestinal biopsy samples or on identifying genetic or metabolic urinary markers by non-invasive techniques.

The clinical management is carried out mainly through the follow-up of a low-histamine diet, although there is no consensus on the list of foods to be excluded. Even so, there are different clinical studies that show the efficacy of this dietary intervention in improving the quality of life of patients with symptoms of histamine intolerance. Oral supplementation with exogenous DAO enzyme from porcine kidney is also being used to enhance the intestinal capacity to degrade dietary histamine. Although few works have assayed the clinical efficacy of this preventive treatment, promising results have been obtained so far. Research

is currently also being made to identify new sources of DAO enzyme, especially of plant origin, due to its higher catalytic capacity and other potential productive and commercial advantages.

In this context, it is necessary to keep promoting the multidisciplinary study of this disorder, both from basic (i.e. analytical chemistry, food science, physiology and biochemistry) and clinically applied research, meant to increase the scientific base and the currently available diagnostic and treatment strategies for histamine intolerance.

References

1. EFSA Panel on Biological Hazards (BIOHAZ) Scientific Opinion on risk based control of biogenic amine formation in fermented foods. EFSA J. 2011; 9: 1–93.
2. Windaus A, Vogt W. Synthese des Imidazolyl-äthylamins. Berichte der Dtsch. Chem. Gesellschaft. 1907; 40: 3691–3695.
3. Comas-Basté O, Luz Latorre-Moratalla M, Sánchez-Pérez S, Teresa Veciana-Nogués M, del Carmen Vidal-Carou M. Histamine and Other Biogenic Amines in Food. From Scombroid Poisoning to Histamine Intolerance. In: Proestos C, editor. Biogenic Amines. London: IntechOpen. 2019.
4. Dale HH, Laidlaw PP. The physiological action of β iminazolylethylamine. J. Physiol. 1910; 41: 318–344.
5. Tansey EM. The wellcome physiological research laboratories 1894-1904: The home office, pharmaceutical firms, and animal experiments. Med. Hist. 1989; 33: 1–41.
6. Riley JF. Histamine and Sir Henry Dale. Br. Med. J. 1965; 1: 1488–1490.
7. Panula P, Chazot PL, Cowart M, Gutzmer R, Leurs R, et al. International union of basic and clinical pharmacology. XCVIII. histamine receptors. Pharmacol. Rev. 2015; 67: 601–655.
8. Vlieg-Boerstra BJ, van der Heide S, Oude Elberink JNG, Kluin-Nelemans JC, Dubois AEJ. Mastocytosis and adverse reactions to biogenic amines and histamine-releasing foods: what is the evidence? Neth. J. Med. 2005; 63: 244–249.
9. Russo P, Spano G, Arena MP, Capozzi V, Fiocco D, et al. Are

consumers aware of the risks related to biogenic amines in food. Curr Res. Technol. Edu. Top. Appl. Microbiol. Microb. Biotechnol. 2010; 1087–1095.
10. Maintz L, Novak N. Histamine and histamine intolerance. Am J Clin Nutr. 2007; 85: 1185–1196.
11. Comas-Basté O, Latorre-Moratalla ML, Bernacchia R, Veciana-Nogués MT, Vidal-Carou MC. New approach for the diagnosis of histamine intolerance based on the determination of histamine and methylhistamine in urine. J. Pharm. Biomed. Anal. 2017; 145: 379–385.
12. Worm J, Falkenberg K, Olesen J. Histamine and migraine revisited: mechanisms and possible drug targets. J. Headache Pain. 2019; 20: 30.
13. Kovacova-Hanuskova E, Buday T, Gavliakova S, Plevkova J. Histamine, histamine intoxication and intolerance. Allergol. Immunopathol. (Madr). 2015; 43: 498–506.
14. Elmore BO, Bollinger JA, Dooley DM. Human kidney diamine oxidase: heterologous expression, purification, and characterization. J. Biol. Inorg. Chem. 2002; 7: 565–679.
15. Schwelberger HG, Feurle J, Houen G. Mapping of the binding sites of human diamine oxidase (DAO) monoclonal antibodies. Inflamm. Res. 2018; 67: 245–253.
16. Finney J, Moon HJ, Ronnebaum T, Lantz M, Mure M. Human copper-dependent amine oxidases. Arch. Biochem. Biophys. 2014; 546: 19–32.
17. Boehm T, Pils S, Gludovacz E, Szoelloesi H, Petroczi K, et al. Quantification of human diamine oxidase. Clin. Biochem. 2017; 50: 444–451.
18. Schwelberger HG, Feurle J, Houen G. Mapping of the binding sites of human histamine N-methyltransferase (HNMT) monoclonal antibodies. Inflamm. Res. 2017; 66: 1021–1029.
19. Schwelberger HG. Histamine intolerance: Overestimated or underestimated? Inflamm. Res. 2009; 58: 51–52.
20. Jarisch R, Wantke F, Raithel M, Hemmer W. Histamine and biogenic amines. In Histamine Intolerance: Histamine and Seasickness. Berlin: Springer. 2015; 3–43.
21. Gludovacz E, Maresch D, De Carvalho LL, Puxbaum V, Baier LJ, et al. Oligomannosidic glycans at asn-110 are essential for secretion of human diamine oxidase. J. Biol.

Chem. 2018; 293: 1070–1087.
22. Elsenhans B, Hunder G, Strugala G, Schümann K. Longitudinal pattern of enzymatic and absorptive functions in the small intestine of rats after short-term exposure to dietary cadmium chloride. Arch. Environ. Contam. Toxicol. 1999; 36: 341–346.
23. McGrath AP, Hilmer KM, Collyer CA, Shepard EM, Elmore BO, et al. Structure and inhibition of human diamine oxidase. Biochemistry. 2009; 48: 9810–9822.
24. Boehm T, Reiter B, Ristl R, Petroczi K, Sperr W, et al. Massive release of the histamine-degrading enzyme diamine oxidase during severe anaphylaxis in mastocytosis patients. Allergy Eur. J. Allergy Clin. Immunol. 2019; 74: 583–593.
25. Maršavelski A, Petrović D, Bauer P, Vianello R, Kamerlin SCL. Empirical Valence Bond Simulations Suggest a Direct Hydride Transfer Mechanism for Human Diamine Oxidase. ACS Omega. 2018; 3: 3665–3674.
26. Smolinska S, Jutel M, Crameri R, O'Mahony L. Histamine and gut mucosal immune regulation. Allergy. 2014; 69: 273–281.
27. Sjaastad ÖV. Potentiation by aminoguanidine of the sensitivity of sheep to histamine given by mouth. Effect of aminoguanidine on the urinary excretion of endogenous histamine. Q. J. Exp. Physiol. Cogn. Med. Sci. 1967; 52: 319–330.
28. Sattler J, Häfner D, Klotter HJ, Lorenz W, Wagner PK. Food-induced histaminosis as an epidemiological problem: Plasma histamine elevation and haemodynamic alterations after oral histamine administration and blockade of diamine oxidase (DAO). Agents Actions. 1988; 23: 361–365.
29. Klocker J, Mätzler SA, Huetz GN, Drasche A, Kolbitsch C, et al. Expression of histamine degrading enzymes in porcine tissues. Inflamm. Res. 2005; 54: S54-57.
30. FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization) Public Health Risks of Histamine and other Biogenic Amines from Fish and Fishery Products. Meeting report, World Health Organization. 2013.
31. Bover-Cid S, Latorre-Moratalla ML, Veciana-Nogués MT, Vidal-Carou MC. Processing Contaminants: Biogenic

Amines. In Encyclopedia of Food Safety. Amsterdam: Elsevier. 2014; 2: 381–391.
32. Vidal-Carou MC, Veciana-Nogués MT, Latorre-Moratalla ML, Bover-Cid S. Biogenic Amines: Risks and Control. In: Toldrá F, Hui Y, Astiasarán I, Sebranek J, Talon R, editors. Handbook of Fermented Meat and Poultry. Oxford: John Wiley & Sons, Ltd. 2014; 413–428.
33. Doeun D, Davaatseren M, Chung MS. Biogenic amines in foods. Food Sci. Biotechnol. 2017; 26: 1463–1474.
34. Gardini F, Özogul Y, Suzzi G, Tabanelli G, Özogul F. Technological factors affecting biogenic amine content in foods: A review. Front. Microbiol. 2016; 7.
35. Latorre-Moratalla ML, Bover-Cid S, Bosch-Fusté J, Vidal-Carou MC. Influence of technological conditions of sausage fermentation on the aminogenic activity of L. curvatus CTC273. Food Microbiol. 2012; 29: 43–48.
36. Linares DM, MartÍn MC, Ladero V, Alvarez MA, Fernández M. Biogenic amines in dairy products. Crit. Rev. Food Sci. Nutr. 2011; 51: 691–703.
37. Visciano P, Schirone M, Tofalo R, Suzzi G. Histamine poisoning and control measures in fish and fishery products. Front. Microbiol. 2014; 5.
38. Ladero V, Calles-Enriquez M, Fernandez M, A Alvarez M. Toxicological Effects of Dietary Biogenic Amines. Curr. Nutr. Food Sci. 2010; 6: 145–156.
39. Latorre-Moratalla ML, Bover-Cid S, Talon R, Aymerich T, Garriga M, et al. Distribution of aminogenic activity among potential autochthonous starter cultures for dry fermented sausages. J. Food Prot. 2010; 73: 524–528.
40. Naila A, Flint S, Fletcher G, Bremer P, Meerdink G. Control of biogenic amines in food - Existing and emerging approaches. J. Food Sci. 2010; 75: R139–R150.
41. Latorre-Moratalla ML, Comas-Basté O, Bover-Cid S, Vidal-Carou MC. Tyramine and histamine risk assessment related to consumption of dry fermented sausages by the Spanish population. Food Chem. Toxicol. 2017; 99: 78–85.
42. European Food Safety Authority (EFSA) Assessment of the incidents of histamine intoxication in some EU countries. Technical report. EFSA Support. Publ. 2017; 14: 1–37.
43. Taylor SL. Histamine poisoning associated with fish, cheese,

and other foods. Geneve: World Health Organization Press. 1985; 46.
44. Legroux R, Levaditi J, Segond L. Methode de mise en evidence de l'histamine dans les aliments causes d'intoxications collectives a l'aide de l'inoculation au cobaye. C. R. Biol. 1946; 140: 863–864.
45. Legroux R, Levaditi J, Bouidin G, Bovet D. Intoxications histaminiques collectives consecutives a l'ingestion de thon frais. Presse Med. 1964; 54: 545–546.
46. Colombo FM, Cattaneo P, Confalonieri E, Bernardi C. Histamine food poisonings: A systematic review and meta-analysis. Crit. Rev. Food Sci. Nutr. 2018; 58: 1131–1151.
47. FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization) Histamine in Salmonids. Joint FAO/WHO literature review. Geneva: World Health Organization. 2018.
48. Lehane L, Olley J. Histamine fish poisoning revisited. Int. J. Food Microbiol. 2000; 58: 1–37.
49. Hungerford JM. Scombroid poisoning: A review. Toxicon. 2010; 56: 231–243.
50. European Food Safety Authority (EFSA), European Centre for Disease Prevention and Control (ECDC) The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. EFSA J. 2018; 16.
51. Van Gelderen CEM, Savelkoul TJF, van Ginkel LA, van Dokkum W. The effects of histamine administered in fish samples to healthy volunteers. Clin. Toxicol. 1992; 30: 585–596.
52. Motil KJ, Scrimshaw NS. The role of exogenous histamine in scombroid poisoning. Toxicol. Lett. 1979; 3: 219–223.
53. Johansson SGO, Bieber T, Dahl R, Friedmann PS, Lanier BQ, et al. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. J. Allergy Clin. Immunol. 2004; 113: 832–836.
54. Tuck CJ, Biesiekierski JR, Schmid-Grendelmeier P, Pohl D. Food Intolerances. Nutrients 2019; 11.
55. Amon U, Bangha E, Küster T, Menne A, Vollrath IB, et al. Enteral histaminosis: Clinical implications. Inflamm. Res.

1999; 48: 291–295.
56. Best CH. The disappearance of histamine from autolysing lung tissue. J. Physiol. 1929; 67: 256–263.
57. Mondovì B, Rotilio G, Finazzi A, Scioscia-Santoro A. Purification of pig-kidney diamine oxidase and its identity with histaminase. Biochem. J. 1964; 91: 408–415.
58. Wolvekamp MCJ, de Bruin RWF. Diamine Oxidase: An Overview of Historical, Biochemical and Functional Aspects. Dig. Dis. 1994; 12: 2–14.
59. Schwelberger HG, Bodner E. Purification and characterization of diamine oxidase from porcine kidney and intestine. Biochim. Biophys. Acta - Protein Struct. Mol. Enzymol. 1997; 1340: 152–164.
60. Herbert T. Diamine Oxidase. J. Biol. Chem. 1950; 188: 125–136.
61. Comas-Basté O, Latorre-Moratalla ML, Rabell-González J, Veciana-Nogués MT, Vidal-Carou MC. Lyophilised legume sprouts as a functional ingredient for diamine oxidase enzyme supplementation in histamine intolerance. LWT. 2020; 125: 109201.
62. Schnedl WJ, Lackner S, Enko D, Schenk M, Holasek SJ, et al. Evaluation of symptoms and symptom combinations in histamine intolerance. Intest. Res. 2019; 17: 427–433.
63. Pinzer TC, Tietz E, Waldmann E, Schink M, Neurath MF, et al. Circadian profiling reveals higher histamine plasma levels and lower diamine oxidase serum activities in 24% of patients with suspected histamine intolerance compared to food allergy and controls. Allergy Eur. J. Allergy Clin. Immunol. 2018; 73: 949–957.
64. Schnedl WJ, Lackner S, Enko D, Schenk M, Mangge H, et al. Non-celiac gluten sensitivity: people without celiac disease avoiding gluten—is it due to histamine intolerance? Inflamm. Res. 2018; 67: 279–284.
65. Aschenbach JR, Honscha KU, Von Vietinghoff V, Gäbel G. Bioelimination of histamine in epithelia of the porcine proximal colon of pigs. Inflamm. Res. 2009; 58: 269–276.
66. Izquierdo-Casas J, Comas-Basté O, Latorre-Moratalla ML, Lorente-Gascón M, Duelo A, et al. Low serum diamine oxidase (DAO) activity levels in patients with migraine. J. Physiol. Biochem. 2018; 74: 93–99.

67. García-Martín E, Ayuso P, Martínez C, Blanca M, Agúndez JAG. Histamine pharmacogenomics. Pharmacogenomics. 2009; 10: 867–883.
68. Kucher AN. Association of Polymorphic Variants of Key Histamine Metabolism Genes and Histamine Receptor Genes with Multifactorial Diseases. Russ. J. Genet. 2019; 55: 794–814.
69. Ayuso P, García-Martín E, Martínez C, Agúndez JAG. Genetic variability of human diamine oxidase: Occurrence of three nonsynonymous polymorphisms and study of their effect on serum enzyme activity. Pharmacogenet. Genomics. 2007; 17: 687–693.
70. García-Martín E, García-Menaya J, Sánchez B, Martínez C, Rosendo R, et al. Polymorphisms of histamine-metabolizing enzymes and clinical manifestations of asthma and allergic rhinitis. Clin. Exp. Allergy. 2007; 37: 1175–1182.
71. Kaur S, Ali A, Siahbalaei Y, Ahmad U, Nargis F, et al. Association of Diamine oxidase (DAO) variants with the risk for migraine from North Indian population. Meta Gene. 2019.
72. Maintz L, Yu CF, Rodríguez E, Baurecht H, Bieber T, et al. Association of single nucleotide polymorphisms in the diamine oxidase gene with diamine oxidase serum activities. Allergy Eur. J. Allergy Clin. Immunol. 2011; 66: 893–902.
73. Enko D, Meinitzer A, Mangge H, Kriegshaüser G, Halwachs-Baumann G, et al. Concomitant Prevalence of Low Serum Diamine Oxidase Activity and Carbohydrate Malabsorption. Can. J. Gastroenterol. Hepatol. 2016; 2016: 1–4.
74. Mondovi B, Fogel WA, Federico R, Calinescu C, Mateescu MA, et al. Effects of amine oxidases in allergic and histamine-mediated conditions. Recent Pat. Inflamm. Allergy Drug Discov. 2013; 7: 20–34.
75. Fukudome I, Kobayashi M, Dabanaka K, Maeda H, Okamoto K, et al. Diamine oxidase as a marker of intestinal mucosal injury and the effect of soluble dietary fiber on gastrointestinal tract toxicity after intravenous 5-fluorouracil treatment in rats. Med. Mol. Morphol. 2014; 47: 100–107.
76. Miyoshi J, Miyamoto H, Goji T, Taniguchi T, Tomonari T, et al. Serum diamine oxidase activity as a predictor of

gastrointestinal toxicity and malnutrition due to anticancer drugs. J. Gastroenterol. Hepatol. 2015; 30: 1582–1590.
77. Schnedl WJ, Enko D. Considering histamine in functional gastrointestinal disorders. Crit. Rev. Food Sci. Nutr. 2020; 1–8.
78. Griauzdaitė K, Maselis K, Ţvirblienė A, Vaitkus A, Jančiauskas D, et al. Associations between migraine, celiac disease, non-celiac gluten sensitivity and activity of diamine oxidase. Med. Hypotheses. 2020; 142: 109738.
79. Enko D, Kriegshäuser G, Halwachs-Baumann G, Mangge H, Schnedl WJ. Serum diamine oxidase activity is associated with lactose malabsorption phenotypic variation. Clin. Biochem. 2017; 50: 50–53.
80. Leitner R, Zoernpfenning E, Missbichler A. Evaluation of the inhibitory effect of various drugs / active ingredients on the activity of human diamine oxidase in vitro. Clin. Transl. Allergy. 2014; 4: P23.
81. Sattler J, Hesterberg R, Lorenz W, Schmidt U, Crombach M, et al. Inhibition of human and canine diamine oxidase by drugs used in an intensive care unit: Relevance for clinical side effects? Agents Actions. 1985; 16: 91–94.
82. Sánchez-Pérez S, Comas-Basté O, Rabell-González J, Veciana-Nogués M, Latorre-Moratalla M, et al. Biogenic Amines in Plant-Origin Foods: Are They Frequently Underestimated in Low-Histamine Diets? Foods. 2018; 7: 205.
83. Mušič E, Korošec P, Šilar M, Adamič K, Košnik M, et al. Serum diamine oxidase activity as a diagnostic test for histamine intolerance. Wien. Klin. Wochenschr. 2013; 125: 239–243.
84. Manzotti G, Breda D, Di Gioacchino M, Burastero SE. Serum diamine oxidase activity in patients with histamine intolerance. Int. J. Immunopathol. Pharmacol. 2016; 29: 105–111.
85. Steinbrecher I, Jarisch R. Histamin und kopfschmerz. Allergologie. 2005; 28: 85–91.
86. Maintz L, Benfadal S, Allam JP, Hagemann T, Fimmers R, et al. Evidence for a reduced histamine degradation capacity in a subgroup of patients with atopic eczema. J. Allergy Clin. Immunol. 2006; 117: 1106–1112.

87. Wagner N, Dirk D, Peveling-Oberhag A, Reese I, Rady-Pizarro U, et al. A Popular myth – low-histamine diet improves chronic spontaneous urticaria – fact or fiction? J. Eur. Acad. Dermatology Venereol. 2017; 31: 650–655.
88. Worm M, Fiedler EM, Dölle S, Schink T, Hemmer W, et al. Exogenous histamine aggravates eczema in a subgroup of patients with atopic dermatitis. Acta Derm. Venereol. 2009; 89: 52–56.
89. Cho HJ, Cho SI, Kim HO, Park CW, Lee CH. Lack of association of plasma histamine with diamine oxidase in chronic idiopathic urticaria. Ann. Dermatol. 2013; 25: 189–195.
90. Honzawa Y, Nakase H, Matsuura M, Chiba T. Clinical significance of serum diamine oxidase activity in inflammatory bowel disease: Importance of evaluation of small intestinal permeability. Inflamm. Bowel Dis. 2011; 17: 23–25.
91. Rosell-Camps A, Zibetti S, Pérez-Esteban G, Vila-Vidal M, et al. Intolerancia a la histamina como causa de síntomas digestivos crónicos en pacientes pediátricos. Rev. Esp. Enfermedades Dig. 2013; 105: 201–207.
92. Hoffmann KM, Gruber E, Deutschmann A, Jahnel J, Hauer AC. Histamine intolerance in children with chronic abdominal pain. Arch. Dis. Child. 2013; 98: 832–833.
93. Klocker J, Perkmann R, Klein-Weigel P, Mörsdorf G, Drasche A, et al. Continuous administration of heparin in patients with deep vein thrombosis can increase plasma levels of diamine oxidase. Vascul. Pharmacol. 2004; 40: 293–300.
94. García-Martín E, Ayuso P, Martínez C, Agúndez JAG. Improved analytical sensitivity reveals the occurrence of gender-related variability in diamine oxidase enzyme activity in healthy individuals. Clin. Biochem. 2007; 40: 1339–1341.
95. Hamada Y, Shinohara Y, Yano M, Yamamoto M, Yoshio M, et al. Effect of the menstrual cycle on serum diamine oxidase levels in healthy women. Clin. Biochem. 2013; 46: 99–102.
96. Jarisch R. Histamin-Intoleranz. In: Thieme, editor. Histamin und Seekrankheit. 2nd ed., Stuttgart. 2004.
97. Reese I, Ballmer-Weber B, Beyer K, Fuchs T, Kleine-Tebbe

J, et al. German guideline for the management of adverse reactions to ingested histamine. Guideline of the German Society for Allergology and Clinical Immunology (DGAKI), the German Society for Pediatric Allergology and Environmental Medicine (GPA), the German Asso. Allergo J Int. 2017; 26: 72–79.

98. Töndury B, Wüthrich B, Schmid-Grendelmeier P, Seifert B, Ballmer-Weber B. Histamine intolerance: Is the determination of diamine oxidase activity in the serum useful in routine clinical practice? Allergologie. 2008; 31: 350–356.
99. Kofler H, Aberer W, Deibl M, Hawranek T, Klein G, et al. Diamine oxidase (DAO) serum activity: Not a useful marker for diagnosis of histamine intolerance. Allergologie. 2009; 32: 105–109.
100. Schnoor HSJ, Mosbech H, Skov PS, Poulsen LK, Jensen BM. Diamine oxidase determination in serum. Low assay reproducibility and misdassification of healthy subjects. Allergo J. 2013; 22: 108–111.
101. Kofler L, Ulmer H, Kofler H. Histamine 50-Skin-Prick Test: A Tool to Diagnose Histamine Intolerance. ISRN Allergy. 2011; 2011: 1–5.
102. Wagner A, Buczyłko K, Zielińska-Bliźniewska H, Wagner W. Impaired resolution of wheals in the skin prick test and low diamine oxidase blood level in allergic patients. Postep. Dermatologii i Alergol. 2019; 36: 538–543.
103. Lessof MH, Gant V, Hinuma K, Murphy GM, Dowling RH. Recurrent urticaria and reduced diamine oxidase activity. Clin. Exp. Allergy. 1990; 20: 373–376.
104. Raithel M, Küfner M, Ulrich P, Hahn EG. The involvement of the histamine degradation pathway by diamine oxidase in manifest gastrointestinal allergies. In Proceedings of the Inflammation Research. Springer. 1999; 48: 75–76.
105. Kuefner MA, Schwelberger HG, Weidenhiller M, Hahn EG, Raithel M. Both catabolic pathways of histamine via histamine-N-melhyl-transferase and diamine oxidase are diminished in the colonic mucosa of patients with food allergy. Inflamm. Res. 2004; 53.
106. Kuefner MA, Schwelberger HG, Hahn EG, Raithel M.

Decreased histamine catabolism in the colonic mucosa of patients with colonic adenoma. Dig. Dis. Sci. 2008, 53, 436–442.
107. Wöhrl S, Hemmer W, Focke M, Rappersberger K, Jarisch R. Histamine intolerance-like symptoms in healthy volunteers after oral provocation with liquid histamine. Allergy Asthma Proc. 2004; 25: 305–311.
108. Komericki P, Klein G, Reider N, Hawranek T, Strimitzer T, et al. Histamine intolerance: Lack of reproducibility of single symptoms by oral provocation with histamine: A randomised, double-blind, placebo-controlled cross-over study. Wien. Klin. Wochenschr. 2011; 123: 15–20.
109. Schnedl WJ, Schenk M, Lackner S, Enko D, Mangge H, et al. Diamine oxidase supplementation improves symptoms in patients with histamine intolerance. Food Sci. Biotechnol. 2019; 28: 1779–1784.
110. Izquierdo-Casas J, Comas-Basté O, Latorre-Moratalla ML, Lorente-Gascón M, Duelo A, et al. Diamine oxidase (DAO) supplement reduces headache in episodic migraine patients with DAO deficiency: A randomized double-blind trial. Clin. Nutr. 2019; 38: 152–158.
111. San Mauro Martin I, Brachero S, Garicano Vilar E. Histamine intolerance and dietary management: A complete review. Allergol. Immunopathol. (Madr). 2016; 44: 475–483.
112. Son JH, Chung BY, Kim HO, Park CW. A histamine-free diet is helpful for treatment of adult patients with chronic spontaneous urticaria. Ann. Dermatol. 2018; 30: 164–172.
113. Joneja JMV, Carmona-Silva C. Outcome of a histamine-restricted diet based on chart audit. J. Nutr. Environ. Med. 2001; 11: 249–262.
114. Pediatrii K, Alergologii Dziecięcej N, Kacik J. Objawy pseudoalergii a zaburzenia metabolizmu histaminy Symptoms of pseudoallergy and histamine metabolism disorders. Pediatr. i Med. Rodz. 2016; 12: 234–241.
115. Böhn L, Störsrud S, Törnblom H, Bengtsson U, Simré M. Self-Reported Food-Related Gastrointestinal Symptoms in IBS Are Common and Associated With More Severe Symptoms and Reduced Quality of Life. Am. J. Gastroenterol. 2013; 108: 634–641.
116. Lefèvre S, Astier C, Kanny G. Histamine intolerance or

false food allergy with histamine mechanism. Rev. Fr. Allergol. 2017; 57: 24–34.
117. Ede G. Histamine intolerance: Why freshness matters? J. Evol. Heal. 2017; 2: 11.
118. Swiss Interest Group Histamine Intolerance (SIGHI) Therapy of Histamine Intolerance Available online at: https://www.histaminintoleranz.ch/en/introduction.html
119. Vidal-Carou MC, Veciana-Nogués MT, Latorre-Moratalla ML. Intolerancia a la histamina e hipersensibilidad a aditivos alimentarios. In: Salas-Salvadó J, Bonadai Sanjaume A, Trallero-Casañas R, Saló i Solà ME, Burgos-Peláez R, editors. Nutrición y dietética clínica. Barcelona: Elsevier. 2019; 535–540.
120. Cornillier H, Giraudeau B, Samimi M, Munck S, Hacard F, et al. Effect of diet in chronic spontaneous urticaria: A systematic review. Acta Derm. Venereol. 2019; 99: 127–132.
121. Zuberbier T, Aberer W, Asero R, Abdul Latiff AH, Baker D, et al. The EAACI/GA2LEN/EDF/WAO guideline for the definition, classification, diagnosis and management of urticaria. Allergy Eur. J. Allergy Clin. Immunol. 2018; 73: 1393–1414.
122. Guida B, De Martino CD, De Martino SD, Tritto G, Patella V, et al. Histamine plasma levels and elimination diet in chronic idiopathic urticaria. Eur. J. Clin. Nutr. 2000; 54: 155–158.
123. Lackner S, Malcher V, Enko D, Mangge H, Holasek SJ, et al. Histamine-reduced diet and increase of serum diamine oxidase correlating to diet compliance in histamine intolerance. Eur. J. Clin. Nutr. 2019; 73: 102–104.
124. Wantke F, M, G, Jarisch R. Histamine-free diet: treatment of choice for histamine-induced food intolerance and supporting treatment for chronical headaches. Clin. Exp. Allergy Exp. Allergy. 1993; 23: 982–985.
125. Park C, Park C, Choi J, Lee H. A study of elimination diet for chronic idiopathic urticaria in Korea. J. Am. Acad. Dermatol. 2008; 58: AB38.
126. Siebenhaar F, Melde A, Magerl M, Zuberbier T, Church MK, et al. Histamine intolerance in patients with chronic spontaneous urticaria. J. Eur. Acad. Dermatology Venereol. 2016; 30: 1774–1777.

127. Parker AM, Watson RR. Lactose Intolerance. In Nutrients in Dairy and Their Implications for Health and Disease. Amsterdam: Elsevier. 2017; 205–211.
128. European Commission Commission Implementing Regulation (EU) 2017/2470 of 20 December 2017 establishing the Union list of novel foods in accordance with Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. Off. J. Eur. Union. 2017; 351: 72–201.
129. Comas-Basté O, Latorre-Moratalla ML, Sánchez-Pérez S, Veciana-Nogués MT, Vidal-Carou MC. In vitro determination of diamine oxidase activity in food matrices by an enzymatic assay coupled to UHPLC-FL. Anal. Bioanal. Chem. 2019; 411: 7595–7602.
130. Kettner L, Seitl I, Fischer L. Evaluation of porcine diamine oxidase for the conversion of histamine in food relevant amounts. J. Food Sci. 2020; 85.
131. Mondovì B, Rotilio G, Costa MT, Finazzi-Agrò A, Chiancone E, et al. Diamine oxidase from pig kidney. Improved purification and properties. J. Biol. Chem. 1967; 242: 1160–1167.
132. Floris G, Fadda MB, Pellegrini M, Corda M, Agro' AF. Purification of pig kidney diamine oxidase by gel-exclusion chromatography. FEBS Lett. 1976; 72: 179–181.
133. Bouvrette P, Male KB, Luong JHT, Gibbs BF. Amperometric biosensor for diamine using diamine oxidase purified from porcine kidney. Enzyme Microb. Technol. 1997; 20: 32–38.
134. Kivirand K, Rinken T. Biosensors for Biogenic Amines: The Present State of Art Mini-Review. Anal. Lett. 2011; 44: 2821–2833.
135. Blemur L, Le TC, Marcocci L, Pietrangeli P, Mateescu MA. Carboxymethyl starch/alginate microspheres containing diamine oxidase for intestinal targeting. Biotechnol. Appl. Biochem. 2016; 63: 344–353.
136. Pietrangeli P, Federico R, Mondovì B, Morpurgo L. Substrate specificity of copper-containing plant amine oxidases. J. Inorg. Biochem. 2007; 101: 997–1004.
137. Masini E, Bani D, Marzocca C, Mateescu MA, Mannaioni PF, et al. Pea seedling histaminase as a novel

therapeutic approach to anaphylactic and inflammatory disorders: A plant histaminase in allergic asthma and ischemic shock. Sci. World J. 2007; 7: 888–902.

138. Yang R, Chen H, Gu Z. Factors Influencing Diamine Oxidase Activity and γ-Aminobutyric Acid Content of Fava Bean (Vicia faba L.) during Germination. J. Agric. Food Chem. 2011; 59: 11616–11620.
139. Torrigiani P, Serafini-Fracassini D, Fara A. Diamine oxidase activity in different physiological stages of Helianthus tuberosus tuber. Plant Physiol. 1989; 89: 69–73.
140. Joseph P, Srivastava SK. Photoregulation of Diamine Oxidase from Pea Seedlings. J. Plant Physiol. 1995; 146: 108–114.
141. Laurenzi M, Tipping AJ, Marcus SE, Knox JP, Federico R, et al. Analysis of the distribution of copper amine oxidase in cell walls of legume seedlings. Planta. 2001; 214: 37–45.
142. Tavladoraki P, Cona A, Angelini R. Copper-containing amine oxidases and FAD-dependent polyamine oxidases are key players in plant tissue differentiation and organ development. Front. Plant Sci. 2016; 7.
143. Kivirand K, Rinken T. Purification and properties of amine oxidase from pea seedlings. Proc. Est. Acad. Sci. Chem. 2007; 56: 164–171.
144. Yacoub MR, Ramirez GA, Berti A, Mercurio G, Breda D, et al. Diamine Oxidase Supplementation in Chronic Spontaneous Urticaria: A Randomized, Double-Blind Placebo-Controlled Study. Int. Arch. Allergy Immunol. 2018; 176: 268–271.

2. PLANTEJAMENT I OBJECTIUS

2. PLANTEJAMENT I OBJECTIUS

En els darrers anys s'ha observat un increment significatiu en la incidència d'intoleràncies alimentàries en les societats desenvolupades, entre elles la intolerància a la histamina. Es tracta de trastorns molt discapacitants que causen una pèrdua notable de la qualitat de vida dels individus que les pateixen. La intolerància a la histamina és un trastorn d'interès creixent, tant per a la societat com per a la comunitat científica, tot i així, encara es requereixen esforços en la investigació per tal d'aprofundir en el coneixement d'aquesta intolerància.

En aquest context, aquesta tesi doctoral pretén aportar **noves evidències en relació amb la intolerància a la histamina**. La tesi es divideix en tres àmbits d'estudi i, per cadascun d'ells, es presenten algunes de les incerteses que existeixen actualment i els objectius plantejats per donar-les resposta.

Àmbit 1. Intolerància a la histamina i dieta

Actualment, la principal estratègia per evitar l'aparició dels símptomes de la intolerància a la histamina és el seguiment d'una dieta baixa en histamina. Fins al moment, la seva eficàcia s'ha demostrat en una quinzena d'estudis clínics (amb certes limitacions metodològiques), amb millora o remissió de la simptomatologia associada. Conceptualment, aquest tipus de dietes exclouen aquells aliments que els pacients relacionen amb l'aparició de la simptomatologia. Per tant, amb l'objectiu de realitzar una gestió dietètica correcta de la intolerància a la histamina és necessari conèixer quins aliments contenen o poden contenir aquesta amina i si la seva exclusió en aquest tipus de dietes està justificada.

Incerteses:

- La informació disponible sobre el contingut d'histamina i altres amines biògenes en aliments no fermentats d'origen vegetal és escassa, especialment si es compara amb els d'origen animal.

- No existeix, a dia d'avui, un únic tipus de recomanació dietètica pel tractament de la intolerància a la histamina i no hi ha un consens entre les diferents dietes baixes en histamina disponibles a la literatura sobre quins aliments s'haurien d'eliminar.
- Malgrat les dietes baixes en histamina exclouen aliments que poden contenir quantitats elevades d'histamina, també n'eliminen d'altres que, *a priori*, no contenen o contenen nivells molt baixos d'aquesta amina.

Per tant, l'objectiu d'aquesta part de la tesi doctoral és **aprofundir en la relació entre la intolerància a la histamina i la dieta, així com estudiar altres factors, a part de la presència d'histamina, que podrien ser la causa de l'aparició de la simptomatologia**. Aquest coneixement permetrà el disseny de pautes alimentàries més adequades pel tractament preventiu d'aquest trastorn, millorant, en últim terme, la qualitat de vida d'un sector important de la població.

Objectius específics:

Objectiu 1. Estudiar la presència d'histamina i altres amines biògenes en aliments d'origen vegetal.

Objectiu 2. Revisar críticament les dietes baixes en histamina descrites en la literatura en base als continguts d'histamina i altres amines biògenes dels aliments exclosos d'aquestes dietes.

Objectiu 3. Avaluar la influència d'altres amines biògenes en la degradació de la histamina per part de l'enzim DAO.

Àmbit 2. Intolerància a la histamina i microbiota intestinal

Darrerament han aparegut diversos estudis que relacionen certes intoleràncies i desordres gastrointestinals amb una alteració de la microbiota intestinal. En aquest sentit, s'ha plantejat la hipòtesi que una disbiosi a nivell intestinal podria estar també implicada en l'etiologia de la intolerància a la histamina. Per exemple, un sobrecreixement d'espècies bacterianes amb capacitat de formar histamina explicaria una major acumulació d'aquest compost a nivell intestinal que podria superar la capacitat metabolitzadora de la DAO. A més, també es planteja que el tractament dietètic de la intolerància a la histamina podria modificar el perfil o composició de la microbiota intestinal en individus amb aquesta intolerància.

Incerteses:

- Es desconeix el paper de la microbiota intestinal en la intolerància a la histamina i si el seu perfil o composició difereix significament del de la població sana.
- La gestió dietètica de la intolerància a la histamina ha mostrat eficàcia en la millora de la simptomatologia de la intolerància a la histamina, però no es disposa d'informació sobre el seu efecte en la composició de la microbiota intestinal.

En aquesta segona part de la tesi es planteja iniciar l'estudi del **rol de la microbiota intestinal en la intolerància a la histamina**.

Objectius específics:

Objectiu 4. Estudiar el perfil i la composició de la microbiota intestinal d'individus amb símptomes d'intolerància a la histamina i comparar-los amb els d'una població sana.

Objectiu 5. Avaluar potencials canvis en el perfil i composició de la microbiota intestinal en un petit subgrup de pacients al llarg de nou mesos del tractament dietètic de la intolerància a la histamina.

Àmbit 3. Intolerància a la histamina i perfil metabòlic en orina

La intolerància a la histamina per dèficit de DAO pot hipotèticament donar lloc a canvis en el perfil metabòlic urinari de la histamina. De fet, resultats previs del grup de recerca han identificat diferències en la concentració urinària del metabòlit metilat de la histamina (1-metilhistamina) en els individus amb intolerància a la histamina en comparació amb un grup control.

Incertesa:

- Malgrat les dades preliminars indiquen una reducció de l'excreció urinària de la 1-metilhistamina en individus amb símptomes d'intolerància a la histamina, encara no es pot afirmar que aquesta alteració del perfil de metabolits pot esdevenir un biomarcador no invasiu d'aquesta intolerància.

En el marc d'aquesta tesi doctoral es pretén **avaluar la validesa de la determinació del perfil metabòlic de la histamina en orina com a biomarcador d'aquesta intolerància alimentària**. Els resultats d'aquest estudi podrien ser de gran interès pel diagnòstic d'individus amb intolerància a la histamina, complementant o substituint els actuals mètodes de diagnòstic.

Objectiu específic:

Objectiu 6. Estudiar si els individus amb intolerància a la histamina mostren un perfil d'excreció de la histamina en orina significativament diferent al dels individus sans. Addicionalment, també es planteja avaluar, en un petit subgrup de pacients, el comportament dels nivells urinaris d'histamina i el seu metabòlit, 1-metilhistamina, durant nou mesos del tractament dietètic de la intolerància a la histamina.

3. RESULTATS

3. RESULTATS

Aquest apartat s'organitza en tres seccions, atenent els diversos àmbits d'estudi plantejats en els objectius d'aquesta tesi doctoral (Figura 6). Els resultats de la tesi es troben recollits en un total de set publicacions incloent sis articles científics i un capítol de llibre.

Cada article es presenta precedit per un breu resum que sintetitza els objectius, la metodologia i els principals resultats obtinguts.

Figura 6. Resum dels diferents àmbits d'estudi amb els corresponents objectius i publicacions derivades.

ÀMBIT 1

Intolerància a la histamina i dieta

Objectiu 1: Estudiar la presència d'histamina i altres amines biògenes en aliments d'origen vegetal.

Objectiu 2: Revisar críticament les dietes baixes en histamina descrites en la literatura en base als continguts d'histamina i altres amines biògenes dels aliments exclosos d'aquestes dietes.

Objectiu 3: Avaluar la influència d'altres amines biògenes en la degradació de la histamina per part de l'enzim DAO.

3.1. Estudi de la presència d'histamina i altres amines biògenes en aliments d'origen vegetal

PUBLICACIÓ 2

Biogenic amines in plant-origin foods: Are they frequently underestimated in low-histamine diets?

Sònia Sánchez-Pérez, Oriol Comas-Basté, Judit Rabell-González, M. Teresa Veciana-Nogués, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou.

Foods, 2018, 7, 205.

Índex d'impacte (JCR: 2018): 3,011

Posició en l'àrea "Food Science & Technology": Q2 (36/135)

COMUNICACIÓ ESCRITA 1

Sònia Sánchez-Pérez, Oriol Comas-Basté, Nelly Carolina Muñoz-Esparza, M. Teresa Veciana-Nogués, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou. **Aminas biógenas en alimentos de origen vegetal: ¿Estan frecuentemente subestimadas en las dietas bajas en histamina?.** *15ª Reunión Anual de la Sociedad Española de Seguridad Alimentaria (SESAL)*, 2019, Alicante.

COMUNICACIÓ ESCRITA 2

Sònia Sánchez-Pérez, Oriol Comas-Basté, Salvador Hernández-Macias, Nelly Carolina Muñoz-Esparza, M. Teresa Veciana-Nogués, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou. **Histamine-free diets: What about legumes?.** *XII Conferència Internacional de Dieta Mediterrània*, 2018, Barcelona.

PLANTEJAMENT I OBJECTIU

Les dietes baixes en histamina utilitzades per a la gestió dietètica de la intoleràncIa a la histamina exclouen principalment els aliments que els pacients relacionen amb el desencadenament dels símptomes. Per tal de realitzar un correcte tractament dietètic és necessari conèixer quins aliments contenen histamina i altres amines.

Tradicionalment, els continguts d'histamina i altres amines han estat àmpliament descrits en productes com el peix i els seus derivats i en productes fermentats (formatges, derivats carnis fermentats, xucrut, vi i cervesa), en els quals la seva presència es pot atribuir a l'activitat aminogènica tant de microorganismes alterants com d'alguns amb una funció tecnològica. No obstant, la informació disponible sobre els continguts d'amines biògenes en productes no fermentats d'origen vegetal és molt més escassa.

L'objectiu d'aquest treball va ser estudiar la presència d'histamina i altres amines biògenes en aliments no fermentats d'origen vegetal, ja que alguns d'ells són exclosos amb relativa freqüència en les dietes baixes en histamina.

METODOLOGIA

Es va realitzar una recerca exhaustiva dels continguts d'amines biògenes en aliments no fermentats d'origen vegetal (verdures/hortalisses, fruites i cereals) en la literatura científica, utilitzant les bases de dades *PubMed* i *Web of Science* i incloent tots els articles publicats a partir de l'any 1990. A més, també es van incloure els continguts d'histamina i altres amines procedents de la pròpia base de dades del grup de recerca, amb un total de 52 aliments d'origen vegetal del mercat espanyol [7].

RESULTATS

La cerca bibliogràfica va proporcionar un total de 20 estudis que reportaven dades sobre continguts d'amines biògenes en aliments no fermentats d'origen vegetal. Segons aquesta revisió, els únics productes que contenien nivells significatius d'histamina van ser l'albergínia (4,2 - 100,6 mg/kg), els espinacs (9,5 - 69,7 mg/kg) i el tomàquet (<0,05 - 17,1 mg/kg) mostrant una gran variabilitat en els seus continguts tant entre mostres d'un mateix estudi com de diferents estudis. En el cas dels espàrrecs, la carbassa i les bledes, la presència d'aquesta amina ha estat descrita puntualment per alguns autors i sempre en quantitats extremadament baixes (<2 mg/kg).

Per contra, la putrescina es va trobar en tots els aliments d'origen vegetal revisats, principalment en el pebrot verd, el blat de moro, la soja, els pèsols, els cítrics, el plàtan i els fruits secs, assolint valors de fins a 200 mg/kg. El germen de blat va ser el cereal que més va destacar en continguts de putrescina, amb valors mitjans de fins a 62 mg/kg. Les amines tiramina i cadaverina es van trobar en un major número d'aliments que la histamina, encara que sovint en concentracions més baixes, rarament excedint els 10 mg/kg. És destacable que els aliments que contenien histamina també acostumaven a tenir tiramina, com en el cas de l'albergínia, el tomàquet, els espàrrecs, les bledes i l'alvocat.

TAKE-HOME MESSAGE

La histamina no es troba àmpliament distribuïda entre els aliments no fermentats d'origen vegetal. Els tomàquets, l'albergínia i els espinacs són els que sovint la contenen i, per tant, seran els que *a priori* s'haurien de tenir en compte en la gestió dietètica de la intolerància a la histamina. L'exclusió d'alguns aliments vegetals no fermentats a les dietes baixes en histamina sembla, per tant, no estar en general justificada pel seu contingut d'histamina.

foods

MDPI

Review

Biogenic Amines in Plant-Origin Foods: Are they Frequently Underestimated in Low-Histamine Diets?

Sònia Sánchez-Pérez [1,2,3], Oriol Comas-Basté [1,2,3], Judit Rabell-González [1,2,3], M. Teresa Veciana-Nogués [1,2,3], M. Luz Latorre-Moratalla [1,2,3] and M. Carmen Vidal-Carou [1,2,3,*]

1 Departament de Nutrició, Ciències de l'Alimentació i Gastronomia, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona (UB), Av. Prat de la Riba 171, 08921 Santa Coloma de Gramenet, Spain; soniasanchezperez@ub.edu (S.S.-P.); oriolcomas@ub.edu (O.C.-B.); juditrabellgonzalez@hotmail.com (J.R.-G.); veciana@ub.edu (M.T.V.-N.); mariluzlatorre@ub.edu (M.L.L.-M.)

2 Institut de Recerca en Nutrició i Seguretat Alimentària (INSA·UB), Universitat de Barcelona (UB), Av. Prat de la Riba 171, 08921 Santa Coloma de Gramenet, Spain

3 Xarxa de Referència en Tecnologia dels Aliments de la Generalitat de Catalunya (XaRTA), C/ Baldiri Reixac 4, 08028 Barcelona, Spain

* Correspondence: mcvidal@ub.edu; Tel.: +34-934-033-786

Received: 8 November 2018; Accepted: 12 December 2018; Published: 14 December 2018

Abstract: Low-histamine diets are currently used to reduce symptoms of histamine intolerance, a disorder in histamine homeostasis that increases plasma levels, mainly due to reduced diamine-oxidase (DAO) activity. These diets exclude foods, many of them of plant origin, which patients associate with the onset of the symptomatology. This study aimed to review the existing data on histamine and other biogenic amine contents in nonfermented plant-origin foods, as well as on their origin and evolution during the storage or culinary process. The only plant-origin products with significant levels of histamine were eggplant, spinach, tomato, and avocado, each showing a great variability in content. Putrescine has been found in practically all plant-origin foods, probably due to its physiological origin. The high contents of putrescine in certain products could also be related to the triggering of the symptomatology by enzymatic competition with histamine. Additionally, high spermidine contents found in some foods should also be taken into account in these diets, because it can also be metabolized by DAO, albeit with a lower affinity. It is recommended to consume plant-origin foods that are boiled or are of maximum freshness to reduce biogenic amine intake.

Keywords: histamine; putrescine; tyramine; cadaverine; biogenic amines; histamine intolerance; low-histamine diet; plant-origin foods; culinary process; storage conditions

1. Introduction

In recent years, various diets have been proposed for the treatment of histamine intolerance [1–8]. These diets, known as low- or free-histamine diets, usually exclude foods that patients associate with the onset of intolerance symptoms. Such foods tend to be rich in histamine, but some, surprisingly, are not usually regarded as sources of this amine.

As described in the literature and scientific reports issued by the European Food Safety Authority (EFSA) and a joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) committee, histamine intolerance (also called food histaminosis or food histamine sensitivity) is a disorder associated with increased plasma histamine levels and is recognized as clinically different from the more established histamine intoxication [9,10]. Although in both cases, histamine is the causative agent, the etiology of the disorders differs. Intoxication appears after the consumption of foods with unusually high histamine concentrations, while intolerance is due to a

deficiency in histamine metabolism, so that symptoms may be triggered even by the intake of low amounts [1,9–11].

Diamine oxidase (DAO) is the main enzyme responsible for the metabolism of histamine and other amines at the intestinal level, and impaired DAO activity is one of the main causes of histamine intolerance [1,12,13]. This enzymatic deficit may have its origins in genetic mutations. Different polymorphisms of a single nucleotide in the gene that encodes this enzyme (*AOC1* on chromosome 7) have been associated with lower DAO activity [14–16]. The deficit may also be due to acquired causes such as inflammatory bowel diseases that block the secretion of DAO [1,3,12], or to the inhibitory action of drugs, some of them with a very widespread use (e.g., acetylcysteine, clavulanic acid, metoclopramide, verapamil) [1,17]. Another enzyme involved in histamine metabolization is monoamine oxidase (MAO) [13]. Therefore, MAO inhibitor drugs, such as selegiline or rasagiline, could also favor the plasmatic accumulation of histamine and the onset of symptoms of histamine intolerance. In addition, the presence of other biogenic amines, mainly putrescine and cadaverine, may compromise the intestinal degradation of histamine by enzymatic competition with DAO [9].

The symptoms of histamine intolerance are numerous and highly variable, due to the effects and functions of histamine in multiple organs and systems of the body. They include gastrointestinal (abdominal pain, diarrhea, vomiting), dermatological (urticaria, dermatitis, or pruritus), respiratory (rhinitis, nasal congestion, and asthma), cardiovascular (hypotonia and arrhythmias), and neurological (headaches) symptoms, and it is common for more than one disorder to occur simultaneously [1,11,12]. Several clinical studies have shown that patients with a potential diagnosis of histamine intolerance or with a diagnosis of migraine, intestinal, or dermatological diseases (atopic dermatitis, eczema, or chronic urticaria) have a higher prevalence of DAO deficits compared to the control population [3,6,18–28].

In order to carry out a correct dietary treatment of histamine intolerance, it is necessary to know what foods may contain this amine and what factors influence its accumulation. Likewise, it is also important to consider the occurrence of other amines that are also metabolized by the DAO enzyme. In contrast to plant-origin foods, there is more available information on the contents of histamine and other amines in fish and fish derivatives and all types of fermented products (cheeses, sausages, sauerkraut, wines, beer), in which their presence is attributed to the aminogenic activity of spoilage microorganisms and also to fermentative microorganisms [9,10,29]. Therefore, the freshness of the food and the hygienic conditions of the raw materials and manufacturing processes, as well as the adequate selection of starter cultures without decarboxylase activity, are of vital importance to avoid or reduce the formation of these compounds [9,29–31].

Due to the information available on the contents of biogenic amines in nonfermented plant-origin foods being scarce, the aim of this study was to review the existing data on the contents of histamine and other biogenic amines in these types of products, as well as their origin and evolution during storage or cooking.

2. Methods

A selective search of scientific literature dealing with biogenic amine contents in nonfermented plant-origin foods, including vegetables, fruits, and cereals, was performed. The bibliographic search was carried out in the PubMed and Web of Science databases using the following keywords: “histamine”, “biogenic amines”, “tyramine”, “putrescine”, “cadaverine”, “plant-origin food”, “food samples”, “storage”, “cooking”, “fruit”, “vegetable”, “legume”, “cereal”, “spinach”, “eggplant”, “tomato”, “citrus”, “modified atmosphere packaging”, and “microbial decarboxylase activity”. Original analytical studies, reviews, and table compilations of content in food were included. Articles published before 1990 were excluded from this review.

Apart from data obtained from the literature, data on the biogenic amine content of plant-origin foods from our own database of Spanish market products were also used. Specifically, histamine, tyramine, putrescine, and cadaverine contents of 25 types of vegetables, 19 fruits, and 8 cereals were included.

3. Content of Biogenic Amines in Plant-Origin Foods

In this section, the contents of biogenic amines (histamine, tyramine, putrescine, and cadaverine) in different plant-origin foods are reviewed, using our own database and data from studies published by other authors. A total of 20 studies reporting data on biogenic amine contents in such foods were found. Most provided data on putrescine contents (normally together with the polyamines spermine and spermidine, not dealt with in this section), and only a few included other amines, such as histamine, tyramine, and cadaverine.

3.1. Vegetables and Legumes

Table 1 shows the contents of biogenic amines in different types of vegetables and legumes (nonfermented).

The only products found to contain significant levels of histamine were eggplant, spinach, and tomato, each showing a great variability in content, both in samples from the same study and among different studies. Histamine values ranged from 4.2 to 100.6 mg/kg in eggplant, from 9.5 to 69.7 mg/kg in spinach, and from not detected to 17.1 mg/kg in tomato. In the case of asparagus, pumpkin, and chard, histamine was found in only a few samples and at very low levels (<2 mg/kg).

Histamine occurs naturally in certain foods [29,32], which explains why it was recorded in practically all samples of spinach, eggplant, and tomato. The variability observed may have been due to botanical variety, as reported by Kumar et al. [33] for eggplant. However, as occurs in foods of animal origin, the presence of high contents of histamine and other amines in plant-origin products could also be associated with microbial activity [29,32,34]. Lavizzari et al. [32] attributed the high contents of histamine in spinach to the activity of contaminating bacteria during storage, belonging mainly to the groups Enterobacteriaceae and Pseudomonadaceae. There is currently a need for more research to understand in more detail the origin of histamine in plant foods such as spinach, eggplant, and tomatoes.

Tyramine has been found in more foods than histamine, although in lower concentrations, in no case exceeding 10 mg/kg. It should be noted that histamine-containing foods also contained tyramine (eggplants, tomatoes, spinach, chard, and asparagus). Although there is very little information about the origin of tyramine in nonfermented vegetables, its presence seems to be associated with microbial aminogenic activity. The ability to form tyramine has been reported for bacteria of the genus *Enterococcus* isolated from plants and fruits, mainly *E. faecium*, *E. mundtii*, and *E. casseliflavus* [35].

Putrescine has been detected in all the studied vegetables and legumes, although its content varied greatly among foods and sometimes also within the same product. In most vegetables and legumes, the average values ranged from 1 to 25 mg/kg. However, some samples of green pepper, eggplant, sweet corn, green and purple beans, spinach, tomato ketchup, soybeans, and peas had strikingly high putrescine contents, in some cases exceeding 200 mg/kg (Table 1). The putrescine found in food can have a dual origin. In plant-origin foods, low contents of this amine generally have a physiological source, as it performs different functions in plants, as do the polyamines spermidine and spermine, ranging from the activation of organogenesis to protection against stress [34,36,37]. On the other hand, the presence of putrescine is also associated with the decarboxylase activity of different groups of spoilage bacteria, mainly Enterobacteriaceae and *Clostridium* spp. [36]. According to Kalač et al. [38], the high amounts of putrescine found in frozen peas are due to bacterial activity in the period between harvesting and freezing or during thawing. However, high putrescine contents cannot always be attributed to bacterial decarboxylase activity. Toro-Funes et al. [39] have suggested that the considerable levels of putrescine found in soybean sprouts arise from the germination process, as this amine is a plant growth factor. In general, based on the available information, and due to the great variability in the reported contents, it is difficult to establish to what degree the presence of putrescine in plant-origin products can be considered physiological or the result of bacterial activity.

Cadaverine, like tyramine, has been described in few vegetables and legumes and in relatively low concentrations, with average values that in no case exceeded 8 mg/kg. The values reported by Nishimura et al. [40] in onion (29 mg/kg) and tofu (18 mg/kg) were an exception.

3.2. Fruits and Nuts

Table 2 shows the content of biogenic amines in different types of fresh fruits, fruit juices, and nuts. There were fewer publications reporting amine data for this type of food than for vegetables and legumes. In general, the contents were low, putrescine being in many cases the only amine found (in addition to the polyamines spermidine and spermine).

Avocado and kiwi, and grapefruit, orange, and pineapple juices, are the only products in this category for which the presence of histamine has been reported, but not in all studies. The 23 mg/kg of histamine in avocado reported by Jarisch et al. [12] stands out, although no relevant information about its possible origin was provided. A study conducted by Preti et al. [41] concluded that the presence of histamine in grapefruit, orange, and pineapple juices is due to a lack of hygienic quality during processing or storage, since this amine is not found in the original fresh fruit.

Similarly, very few fruits contained tyramine, and levels have always been low (Table 2). Avocado and plum stand out for their content of this amine, although in no case has it exceeded 7 mg/kg.

Putrescine has been found in practically all the fruits and nuts, with the highest levels in orange, orange juice, mandarin, grapefruit, grapefruit juice, banana, passion fruit, and pistachio. The range of contents of this amine in citrus fruits and their juices has been very broad, varying from not detected to as high as 200 mg/kg. Suggested explanations for this variability have included different origins, cultivation, and transport and storage conditions [41–44]. As reported by Gonzalez-Aguilar et al. [45], the contents of putrescine in mandarin (flavedo) can be increased by a drop in temperature before harvesting and by damage of mechanical origin. Its presence in most of the samples, unaccompanied by high levels of other amines (related to bacterial activity), seemed to indicate that, with some exceptions, putrescine in fruits has a physiological origin. To confirm this, it would be necessary to carry out more studies analyzing the fruit at the moment of collection. The only fruits reported as having no putrescine were avocado and plum, although interestingly, these did contain histamine and tyramine.

The only fruits with a notable content of cadaverine were bananas and sunflower seeds, for which Nishimura et al. [40] reported average levels of 11 and 22 mg/kg, respectively, although these data were from the analysis of only two samples.

3.3. Cereals and Derivatives

Table 3 shows the contents of biogenic amines in cereals and some derivatives such as breakfast cereals, pasta, and bread. The quantitative information available on amines in cereals is very limited. In principle, these foods do not contain amines other than putrescine, which has a physiological origin [36]. The only standout source of putrescine is wheat germ, which, like soya bean sprouts, has a high rate of cell division, in which putrescine and polyamines play a significant role [36].

Table 1. Biogenic amine contents (mg/kg fresh weight) found in vegetables and legumes. Data are presented as average (standard deviation) and range (minimum–maximum).

Food Categories	*n*	Occurrence of Biogenic Amines (mg/kg)								Reference
		Histamine		Tyramine		Putrescine		Cadaverine		
		Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	
Vegetables and Vegetable Products										
Asparagus (wheat)	5	0.34 (0.62)	nd–1.42	0.69 (0.88)	nd–2.1	13.08 (2.81)	8.58–16.27	0.12 (0.19)	nd–0.43	#
Beans (green)	12	nd	-	2.46 (3.24)	nd–9.86	10.30 (8.61)	2.98–28.81	nd	-	#
	-	nd	-	nd	-	34.9 (6.2)	-	-	-	[46]
Beans (purple)	-	nd	-	nd	-	77.8 (7.7)	-	nd	-	[46]
Beans (yellow)	-	nd	-	nd	-	14.8 (1.4)	-	nd	-	[46]
Broccoli	4	-	-	-	-	9	7–10.5	-	-	[47]
	5	-	-	-	-	6.4 (2.9)	3.4–10.8	-	-	[42]
	10	-	-	-	-	5.7	-	-	-	[48]
	5	-	-	-	-	1.9	1.3–2.6	-	-	[49]
	2	-	-	-	-	-	8.37–20.53	0.1	-	[40]
Cabbage	3	-	-	-	-	-	-	-	-	[47]
	-	-	-	<0.5	-	16	-	-	-	[34]
	2	-	-	-	-	14.18	-	6.03	-	[40]
Cabbage (white)	10	-	-	-	-	6.6	-	-	-	[48]
	8	-	-	-	-	2.7	0.7–6.2	-	-	[49]
Cauliflower	3	nd	-	1.05 (0.91)	nd–1.71	3 (1.49)	1.61–4.58	0.16 (0.27)	nd–0.48	#
	3	-	-	-	-	-	3.1–4.5	-	-	[50]
	7	-	-	-	-	4.9	2.2–7.6	-	-	[47]
	5	-	-	-	-	5.3 (2.1)	3.3–8.9	-	-	[42]
	2	-	-	-	-	3.7	-	-	-	[40]
	-	-	-	<0.5	-	9	-	<0.5		[34]
Carrot	13	nd	-	nd	-	2.27 (2.20)	0.35–8.92	nd	-	#
	3	-	-	-	-	-	1.2–1.8	-	-	[50]
	4	-	-	-	-	2.8	2–3.9	-	-	[47]
	2	-	-	-	-	3.5	-	-	-	[51]
	6	-	-	-	-	1.5 (0.7)	0.7–2.7	-	-	[42]
	10	-	-	-	-	0.7	-	-	-	[48]
	4	-	-	-	-	12.1 (3.9)	7–18	-	-	[52]
	5	-	-	-	-	14.8	8–24.7	-	-	[49]
	-	-	-	1	-	5	-	-	-	[34]
	2	-	-	-	-	-	5.73–14.10	2.55	-	[40]
Celeriac	3	-	-	-	-	6.1	3.7–7.7	-	-	[47]
Chard	8	0.79 (0.41)	nd–1.33	1.90 (0.98)	0.74–3.48	6.38 (3.22)	2.4–11.94	0.13 (0.11)	nd–0.24	#
Courgette	41	nd	-	nd	-	7.94 (4.12)	2.74–24.81	0.32 (0.7)	nd–2.07	#
	-	-	-	2	-	4	-	-	-	[34]
Cucumber	10	nd	-	0.61 (0.89)	nd–2.33	5.42 (3.13)	1.32–10.62	nd	-	#
	3	-	-	-	-	3.2	-	-	-	[50]
	5	-	-	-	-	6.9 (1.4)	5.5–8.7	-	-	[42]
	10	-	-	-	-	8.7	-	-	-	[48]

Table 1. *Cont.*

Food Categories	*n*	Occurrence of Biogenic Amines (mg/kg)								Reference
		Histamine		Tyramine		Putrescine		Cadaverine		
		Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	
Vegetables and Vegetable Products										
	5	-	-	-	-	13.1	2.1–28.8	-	-	[49]
	-	-	-	1	-	25	-	-	-	[34]
	2	-	-	-	-	9.87	-	2.96	-	[40]
Eggplant	23	39.42 (30.66)	4.17–100.6	0.60 (0.90)	nd–2.27	34.30 (6.98)	24.10–48.63	nd	-	#
	-	26	-	-	-	-	-	-	-	[12]
	2	-	-	-	-	18.35	-	5	-	[40]
Lettuce	4	nd	-	nd	-	2.85 (0.75)	2.20–3.90	nd	-	#
	3	-	-	-	-	-	3.3–4.8	-	-	[50]
	3	-	-	-	-	5.6 (1.3)	4.5–7.3	-	-	[42]
	10	-	-	-	-	7.9	-	-	-	[48]
	7	-	-	-	-	20.7	10.2–42.3	-	-	[49]
	2	-	-	-	-	6.87	-	3.27	-	[40]
Mushroom	11	nd	-	nd	-	1.29 (1.20)	0.02–3.65	0.10 (0.4)	nd–1.59	#
	10	-	-	-	-	11.7	-	-	-	[48]
	213	-	-	-	-	-	nd–156	-	-	[53]
Onion	4	nd	-	nd	-	nd	-	nd	-	#
	3	-	-	-	-		5.5–7.2	-	-	[50]
	10	-	-	-	-	0.5	-	-	-	[48]
	6	-	-	-	-	0.6	0.2–1	-	-	[49]
	-	-	-	3	-	2	-		-	[34]
	2	-	-	-	-	3.96	-	29.32	-	[40]
Pepper (green)	9	nd	-	nd	-	90.04 (41.65)	11.7–148.9	0.05 (0.14)	nd–0.41	#
	2	-	-	-	-	-	104–237	5.62	-	[40]
	5	-	-	-	-	70 (31)	13.2–96.9	-	-	[52]
Pepper (red)	8	nd	-	nd	-	2.42 (2.21)	0.59–5.35	nd	-	#
Potato	10	nd	-	0.58 (0.64)	nd–2.2	4.14 (3.06)	1.05–11.68	0.22 (0.54)	nd–1.75	#
	3	-	-	-	-	9.7	-	-	-	[50]
	3	-	-	-	-	17.6	-	-	-	[51]
	6	-	-	-	-	9.7 (2.1)	5.8–12.8	-	-	[42]
	2	-	-	-	-	-	0.1–22.4	-	-	[40]
	10	-	-	-	-	2.8	-	-	-	[48]
	6	-	-	-	-	7.2	1.1–10.5	-	-	[49]
	-	-	-	5	-	8	-	<0.5	-	[34]
Pumpkin	12	0.28 (0.54)	nd–1.90	nd	-	9.87 (6.19)	2.95–24.23	0.54 (0.76)	nd–2.15	#
Spinach	18	31.77 (17.02)	9.46–69.71	2.05 (0.83)	0.785–4.28	4.48 (2.46)	0.14–9.19	nd	-	#
	5	-	-	-	-	4.8	1.8–13.5	-	-	[49]
	-	16	-	6	-	6.0	-	1	-	[34]
	-	-	30–60	-	-	-	-	-	-	[12]
	-	37.5	-	-	-	-	-	-	-	[5]
	2	-	-	-	-	4.41	-	8.48	-	[40]
	-	61 (1.5)	-	nd	-	7.8 (0.1)	-	nd	-	[54]

Table 1. *Cont.*

Food Categories	*n*	Occurrence of Biogenic Amines (mg/kg)								Reference
		Histamine		Tyramine		Putrescine		Cadaverine		
		Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	
Vegetables and Vegetable Products										
	32	-	-	-	-	12.9	nd–119	-	-	[52]
Sweet corn	5	nd	-	nd	-	38.44 (9.50)	30.5–119.2	0.18 (0.25)	nd–0.46	#
Tomato	53	2.51 (4.08)	nd–17.07	0.49 (0.92)	nd–6.38	16.48 (6.93)	6.29–35.55	0.50 (0.48)	nd–2.33	#
	3	-	-	-	-	-	9.3–122	-	-	[50]
	2	-	-	-	-	10.6	-	-	-	[51]
	5	-	-	-	-	10	5.3–20.7	-	-	[49]
	-	6	-	<0.5	-	23	-	<0.5	-	[34]
	-	22	-	-	-	-	-	-	-	[5]
	2	-	-	-	-	23.96	-	1.63	-	[40]
Tomato (concentrated)	19	-	-	-	-	25.9 (8.2)	7.9–41.1	-	-	[38]
Tomato (crushed)	3	1.22 (1.69)	0.24–3.17	0.14 (0.02)	0.12–0.16	9.66 (8.78)	4.5–19.80	0.18 (0.06)	0.12–0.23	#
	-	2	-	2	-	20	-	-	-	[34]
Tomato (ketchup)	3	0.37 (0.64)	nd–1.11	nd	-	1.07 (0.08)	1–1.15	nd	-	#
	24	-	-	-	-	52.5 (54.1)	nd–165	-	-	[38]
	-	22	-	-	-	-	-	-	-	[12]
Legumes and Derivatives										
Beans (white)	6	nd	-	nd	-	0.66 (0.64)	0.35–1.96	nd	-	#
	-	-	-	2	-	3	-	-	-	[34]
Beans (red kidney)	3	-	-	-	-	-	0.3–0.4	-	-	[50]
	5	-	-	-	-	-	nd–4	-	-	[52]
	-	-	-	3	-	1	-	-	-	[34]
Chickpeas	4	nd	-	nd	-	3.63 (2.49)	0.90–6.39	nd	-	#
	-	-	-	<0.50	-	2	-	<0.50	-	[34]
Lentils	7	nd	-	nd	-	8.19 (8.36)	1.96–21.81	nd	-	#
	5	-	-	-	-	-	nd–20.2	-	-	[52]
	-	-	-	-	-	3	-	-	-	[34]
Peanuts	7	nd	-	nd	-	0.87 (1.01)	nd–2.56	nd	-	#
Peas	9	nd	-	nd	-	34.28 (13.50)	8.74–54.44	nd	-	#
	10	-	-	-	-	17.3	-	-	-	[48]
	6	-	-	-	-	32.3	5.5–51.1	-	-	[49]
Peas (frozen)	14	-	-	-	-	46.3 (27)	11.7–107	-	-	[38]
Soybean, dried	3	-	-	-	-	-	1.6–6.5	-	-	[50]
	1	-	-	-	-	17	-	-	-	[47]
	2	-	-	-	-	41	-	-	-	[51]
	13	-	-	-	-	-	3.7–16.8	-	-	[55]
	4	-	-	-	-	30.9 (15.5)	16.3–57	-	-	[52]
	2	-	-	-	-	-	35.2–57.2	-	-	[40]
	5	-	-	-	-	17.1	6.4–24.2	-	-	[49]
Soybean milk	3	nd	-	nd	-	1.02 (0.73)	0.39–1.81	0.28 (0.24)	nd–0.42	[39]
	2	-	-	-	-	2.11	-	13.9	-	[40]

Table 1. *Cont.*

Food Categories	n	Occurrence of Biogenic Amines (mg/kg)								Reference
		Histamine		Tyramine		Putrescine		Cadaverine		
		Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	
Vegetables and Vegetable Products										
Soybean sprouts	3	-	-	-	-	44.71 (3.21)	41.13–47.43	0.21 (0.18)	nd–0.33	[39]
Tofu	6	nd	-	nd	-	0.76 (0.55)	nd–1.49	0.67 (0.49)	nd–1.42	#
	4	-	-	-	-	nd	-	-	-	[49]
	19	-	-	-	-	2.6 (1.4)	nd-5	-	-	[56]
	2	-	-	-	-	1.76	-	18.4	-	[40]

Here, *n*: number of samples; SD: standard deviation; nd: not detected; -: values not reported by the study; #: data on the biogenic amine content from our own database of Spanish market products.

Table 2. Biogenic amine contents (mg/kg fresh weight) found in fruits and nuts. Data are presented as average (standard deviation) and range (minimum–maximum).

Food Categories	n	Occurrence of Biogenic Amines (mg/kg)								Reference
		Histamine		Tyramine		Putrescine		Cadaverine		
		Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	
Fruit and Fruit Products										
Apple	3	-	-	-	-	-	0.4–1.7	-	-	[50]
	2	-	-	-	-	nd	-	-	-	[51]
	2	-	-	-	-	1.5	-	nd	-	[40]
Apple juice	10	nd	-	0.67 (0.50)	nd–1.6	1.02 (0.35)	0.59–1.68	2.30 (1.53)	0.55–4.27	[41]
Avocado	5	nd	-	1.81 (2.06)	0.58–5.44	nd	-	nd	-	#
	-	23	-	-	-	-	-	-	-	[12]
	2	-	-	-	-	nd	-	nd	-	[40]
Banana	8	nd	-	0.53 (0.79)	nd–1.85	37.94 (8.32)	25.50–49.49	nd	-	#
	2	-	-	-	-	-	15.86–41.05	10.83	-	[40]
Cherry	5	nd	-	nd	-	3.08 (0.51)	2.35–3.46	nd	-	#
	2	-	-	-	-	4.67	-	nd	-	[40]
Grape	2	-	-	-	-	9.34	-	5.93	-	[40]
Grapefruit	2	nd	-	nd	-	55.55 (12.8)	46.52–64.57	nd	-	#
	2	-	-	-	-	51.1	-	nd	-	[40]
Grapefruit Juice	3	-	-	-	-	98.6	-	-	-	[50]
	10	0.31 (0.58)	nd–1.74	nd	-	10.08 (4.11)	7.17–20.8	1 (0.64)	0.38–2.28	[41]
Guava	21	-	-	-	-	1	0.4–1.8	-	-	[57]
Kiwi	13	nd	-	nd	-	2.49 (3.96)	0.5–15.57	nd	-	#
	2	-	-	-	-	1.06	-	nd	-	[40]
	-	1.9 (0.1)	-	nd	-	3.1 (0.1)	-	nd	-	[54]
Lemon	3	nd	-	nd	-	2.33 (2.02)	nd–3.67	nd	-	#
Mandarin	21	nd	-	0.94 (1.31)	nd–5.76	90.16 (36.6)	12.29–173.8	nd	-	#
	10	-	-	-	-	122 (44.2)	67.3–200	-	-	[42]

Table 2. *Cont.*

Food Categories	*n*	Occurrence of Biogenic Amines (mg/kg)								Reference
		Histamine		Tyramine		Putrescine		Cadaverine		
		Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	
Fruit and Fruit Products										
Mango	21	-	-	-	-	0.9	nd–2.7	-	-	[57]
Orange	12	nd	-	nd	-	91.24 (41.7)	11.34–151.1	nd	-	#
	3	-	-	-	-	-	95.1–140	-	-	[50]
	2	-	-	-	-	117	-	-	-	[51]
	5	-	-	-	-	137 (11.3)	119–153	-	-	[42]
	2	-	-	-	-		54.62–119.82	2.04	-	[40]
Orange juice	3	nd	-	nd	-	45.51 (10.5)	37.35–57.3	nd	-	#
	3	-	-	-	-	85 (11.4)	76.6–100	-	-	[42]
	11	0.46 (0.41)	nd–1.32	nd	-	45.51 (8.35)	34.70–60.97	nd	-	[41]
Papaya	21	-	-	-	-	11	5.3–19.3	-	-	[57]
	2	-	-	-	-	4.67	-	nd	-	[40]
Passion fruit	21	-	-	-	-	17.9	6.5–40.5	-	-	[57]
Pear	3	-	-	-	-	-	23.6–24.2	-	-	[50]
	2	-	-	-	-	1.5	-	0.41	-	[40]
Peach	2	nd	-	nd	-	1.92 (0.14)	1.82–2.02	nd	-	#
	2	-	-	-	-	0.35	-	<0.10	-	[40]
Pineapple	6	nd	-	nd	-	4.20 (2.17)	1.39–7.96	nd	-	#
	2	-	-	-	-	4.05	-	3.07	-	[40]
	21	-	-	-	-	1.1	nd–2.5	-	-	[57]
Pineapple juice	12	2.44 (1.59)	nd–4.61	0.87 (0.86)	nd–1.93	1.79 (0.16)	1.53–1.98	1.21 (1.22)	nd–3.14	[41]
Plum	2	nd	-	4.02 (4.32)	0.96–7.07	nd	-	nd	-	#
Strawberry	9	nd	-	nd	-	3.77 (1.52)	2.04–6.42	nd	-	#
	2	-	-	-	-	0.97	-	4.29	-	[40]
Nuts										
Almonds	7	nd	-	nd	-	2.47 (1.24)	nd–4.36	nd	-	#
	2	-	-	-	-	4.32	-	5.57	-	[40]
Chestnuts	2	nd	-	nd	-	4.53 (3.40)	2.12–6.93	nd	-	#
	2	-	-	-	-	5.2	-	1.33	-	[40]
Hazelnuts	9	nd	-	0.49 (0.85)	nd–2.63	1.18 (1.09)	nd–3.19	nd	-	#
Nuts	6	nd	-	nd	-	5.64 (4.17)	2.82–13.79	nd	-	#
Pistachios	7	nd	-	nd	-	14.84 (14.0)	4.31–39.51	1.65 (4.37)	nd–11.58	#
	2	-	-	-	-	43	-	3.27	-	[40]
Sunflower seeds	2	nd	-	nd	-	0.50 (0.19)	0.36–0.63	nd	-	#
	2	-	-	-	-	3	-	22.58	-	[40]

Here, *n*: number of samples; SD: standard deviation; nd: not detected; -: values not reported by the study; #: data on the biogenic amine content from our own database of Spanish market products.

Table 3. Biogenic amine contents (mg/kg fresh weight) found in cereals and cereal-based products. Data are presented as average (standard deviation) and range (minimum–maximum).

Food Categories	*n*	Occurrence of Biogenic Amines (mg/kg)								Reference
		Histamine		Tyramine		Putrescine		Cadaverine		
		Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	
Barley	2	nd	-	nd	-	2.19 (1.55)	1.09–3.28	nd	-	#
Bread, white	2	nd	-	nd	-	nd	-	nd	-	#
	3	-	-	-	-	-	1.5–1.8	-	-	[50]
	10	-	-	-	-	1.1	-	-	-	[48]
	2	-	-	-	-	1.32	-	2.35	-	[40]
Bread, wholemeal	6	nd	-	nd	-	1.96 (1.45)	nd–4.32	nd	-	#
Cereal (corn, chocolate)	8	nd	-	nd	-	0.32 (0.44)	nd–0.93	nd	-	#
	10	-	-	-	-	-	2-2.2	-	-	[50]
Oats	2	nd	-	nd	-	0.67 (0.32)	0.44–0.89	nd	-	#
Pasta (wheat)	7	nd	-	nd	-	1.56 (1.65)	0.81–4.52	nd	-	#
Rice	2	nd	-	nd	-	2.4 (0.03)	2.38–2.42	nd	-	#
	2	-	-	-	-	<0.9	-	-	-	[51]
	2	-	-	-	-	1.2	-	-	-	[40]
	6	-	-	-	-	0.2	0.2–0.3	-	-	[49]
	10	-	-	-	-	0.2	-	-	-	[48]
Wheat germ	2	nd	-	nd	-	31.64 (0.35)	31.39–31.9	0.63 (0.08)	0.57–0.69	#
	2	-	-	-	-	62.1	-	-	-	[40]

Here, *n*: number of samples; SD: standard deviation; nd: not detected; -: values not reported by the study; #: data on the biogenic amine content from our own database of Spanish market products.

Putrescine contents in wholemeal bread were slightly higher than in bread made with refined flour. In white bread, low contents of cadaverine have also been reported, although only in one study, and from the analysis of two samples.

4. Evolution of Amine Contents during Storage and Cooking

The variability of amine contents observed among samples of the same product can be attributed mainly to conditions of production, transport, and storage [42].

The storage temperature is one of the most important factors in the formation of biogenic amines [11,29]. Refrigeration delays or reduces the aminogenic potential of microorganisms, although the formation of amines at refrigeration temperatures (4–10 °C) has been reported. The influence of the conservation temperature has been widely studied in foods such as meats, fish, and fermented products [29,30,58], but scarcely in plant-origin foods.

A study conducted by Simon-Sarkadi et al. [59] showed a clear increase in putrescine in different types of green leafy vegetables (lettuce, endives, Chinese cabbage, and radicchio) during six days of storage at 5 °C. The authors concluded that there was a positive correlation between putrescine contents and the hygienic state of these foods (total microorganism counts). Tyramine contents also showed a tendency to increase slightly. Histamine was present only in Chinese cabbage and in very low concentrations, remaining stable throughout the study period. In contrast, when Moret et al. [34] studied the effect of storage temperature on the amine content in various vegetables (parsley, zucchini, broccoli, and cucumber), no significant changes in histamine, tyramine, putrescine, and cadaverine were observed after three weeks of refrigeration.

Lavizzari et al. [32] also reported an increase in histamine in different spinach samples over 12–15 days of storage at 6 °C, noting that the relatively high pH of this vegetable favored the growth of Gram-negative bacteria, which could have been responsible for the formation of this amine during storage. The contents of tyramine and putrescine did not undergo significant changes under these storage conditions. It should be noted that in two of the five trials carried out in this study, histamine levels decreased in the last days of storage. The authors suggested that this histamine degradation could have been due to the action of bacteria with DAO activity, as well as the effect of the pH, which reached values above 8 [32]. Another study also recently reported the complete degradation of histamine in a spinach sample (61 mg/kg) after three weeks of storage at 4 °C [54].

Modified atmosphere packaging, together with low storage temperatures, is commonly used to extend the life of fresh vegetables and fruits. This type of packaging can influence the capacity of microorganisms to form amines [30,58,60]. Esti et al. [43] monitored the contents of amines during the ripening of cherries and apricots packaged in modified atmospheres and stored at 0 °C, and found that after 20 days of storage the contents of amines (mainly putrescine) had decreased by 20% compared to the initial value. Although the authors did not provide an explanation for this reduction, it could have been due to putrescine serving as a substrate for polyamine formation [36].

Another factor that can affect the content of biogenic amines in foods of plant origin, especially vegetables, is the culinary process. Again, the results reported in the literature were variable, depending on the type of cooking and the amine in question.

Latorre-Moratalla et al. [61] evaluated the effect of cooking spinach in water, with or without salt. The cooking process reduced the histamine content in all the samples by an average of 83% with respect to the raw product (after a correction for the dilution effect of the cooking). Analysis confirmed a transfer of histamine to the cooking water, which was not enhanced by the addition of salt. Likewise, Kumar et al. [33] observed the loss of 11–14% histamine in eggplants boiled at 100 °C for 10 min. Veciana et al. [62] also concluded that the putrescine content in certain vegetables (spinach, cauliflower, Swiss chard, potato, and green beans) is reduced by transfer to the cooking water. However, this heat treatment had no effect on the putrescine content in other vegetables such as pepper, pea, and asparagus. Eliassen et al. [42] also found no significant differences in putrescine levels among different types of raw and boiled vegetables (carrot, broccoli, cauliflower, and potato),

although they acknowledged that the low number of samples analyzed (two per food) was a limitation when trying to reach a conclusion.

Conversely, three recent studies have shown an increase in amine levels after a cooking process. According to Lo Scalzo et al. [63], boiling and grilling enhanced the putrescine content in a specific variety of eggplant by 55% and 32%, respectively. In the other two varieties of eggplant tested, the cooking had no effect. Similarly, Preti et al. [46] reported a significant increase in putrescine in green beans after boiling, whereas steaming did not modify the contents. According to the work performed by Chung et al. [64], frying brought about a 2.5- and 4-fold increase in histamine in carrots and seaweed, respectively. The authors attributed this increase to the loss of water caused by the high heat treatment. The same process had no effect on spinach and onions. However, it should be noted that in this study, the contents of histamine in all foods were well below 1 mg/kg, both before and after frying.

Amines are thermostable compounds, so in principle changes in contents can only be due to their transfer to the cooking water or by dilution or concentration effects of the culinary process, in which the food gains or loses water.

5. Plant-Origin Foods in Low-Histamine Diets

At present, the main strategy to prevent the onset of histamine intolerance symptoms is to follow a low-histamine diet. Its efficacy has been demonstrated in different clinical studies, which have always described an improvement or remission of gastrointestinal, dermatological, and neurological symptoms [3,6,18–20,22,24,27,65–67] if the diet was followed.

Current low-histamine diets exclude foods that patients associate with the onset of symptoms [1–8], such as blue fish and their preserves, and all kinds of fermented products (cheeses, sausages, wine, beer, sauerkraut, and fermented soy derivatives), all of which are susceptible to having high contents of histamine and other amines. A high number of nonfermented plant-origin foods are also excluded: The average contents of biogenic amines and polyamines in these foods are shown in Table 4. As can be seen, with the exception of spinach, eggplant, tomatoes, and avocado, for which high amounts of histamine have been described, the rest contained very little or no histamine, so a priori should not be responsible for triggering symptoms. However, some of them had relatively high contents of other biogenic amines and polyamines.

Table 4. Content of histamine and other biogenic amines (mg/kg fresh weight) in plant-origin foods excluded from different low-histamine diets [1–8]. Data obtained from own database and from different scientific studies [5,12,34,38–42,47–55,57].

Food Items	Histamine	Putrescine	Cadaverine	Tyramine	Spermidine	Spermine
Spinach [a]	9–70	nd–119	nd–9	1–10	14–53	nd–9
Eggplant [a]	4–101	24–49	nd–5	nd–2	2–12	nd–6
Tomato [a]	nd–17	5–122	nd–2	nd–6	2–16	nd–2
Ketchup [a]	nd–22	nd–165	nd	nd	nd–33	nd–12
Avocado [a]	nd–23	nd	nd	0.5–5	nd–7	2–8
Citrus (fresh and juices) [b]	nd–2	7–200	nd–2	nd–5	nd–12	nd–5
Mushroom [b]	nd	nd–156	nd	nd	9–155	nd–13
Banana [b]	nd	15–50	nd–10	nd–2	8–16	nd–3
Soybean or soybean sprouts [b]	nd	2–57	nd–0.3	nd	33–389	7–114
Nuts [b]	nd	nd–40	nd–23	nd–3	6–40	2–33
Pears [b]	-	2–25	nd–0.4	-	30–76	8–49
Lentils [b]	nd	nd–21	nd	nd	15–107	5–18
Chickpeas [b]	nd	1–6	nd–0.5	nd–0.5	15–85	4–32
Peanuts [c]	nd	nd–3	nd	nd	23–48	5–13
Kiwi [c]	nd–2	nd–15	nd	nd	3–6	nd–2
Papaya [c]	-	5–20	nd	-	4–8	nd–2
Strawberry [c]	nd	2–6	nd–4	nd	5–10	nd–2
Pineapple [c]	nd	nd–8	nd–3	nd	nd–3	nd–1
Plum [c]	nd	nd	nd	1–7	2–3	nd–4

Here, nd: not detected; -: values not reported by the studies; [a] plant-origin foods with histamine; [b] plant-origin foods without histamine but with high contents of other amines; [c] plant-origin foods with low levels of all amines.

Putrescine, cadaverine, and tyramine are all substrates of the DAO enzyme, so if present in high amounts they may increase the adverse effects of histamine by competing as rival substrates or for binding sites in the intestinal mucosa [1,9,68,69]. The high putrescine contents found in citrus fruits, mushrooms, soybeans, bananas, and nuts could thus explain why patients associate their consumption with the onset of histamine intolerance symptoms. However, it should be noted that some foods with similar or even much higher putrescine contents, such as green pepper, peas, or corn, are permitted in low-histamine diets (Table 1).

The polyamines spermidine and spermine can also be metabolized by DAO, albeit with a lower affinity [68,69], and therefore their presence should also be taken into account in this type of diet (Table 4). Thus, the exclusion of foods such as soybeans, mushrooms, lentils, chickpeas, peanuts, nuts, and pears may be justified by their high polyamine content.

Finally, the levels of biogenic amines and polyamines found in kiwi, papaya, strawberry, pineapple, and plum are too low to justify their exclusion. Some authors consider these foods, along with others such as milk, shellfish, and eggs, as endogenous histamine releasers, although by mechanisms still not well understood [1,11,70].

6. Conclusions

Biogenic amine data in nonfermented plant-origin foods from the different reviewed studies showed a great variability both within the same food item and among them. Putrescine was the most frequent biogenic amine found in fresh vegetables, legumes, fruits, and cereals, and only a limited number of products contained relevant levels of histamine (eggplant, spinach, tomato, and avocado). Tyramine and cadaverine were usually more scarcely found in plant-origin foods. Generally, low levels of histamine and putrescine may have a physiological origin. However, undesirable microbial enzymatic activity during production or storage may lead to the accumulation of high levels of these amines.

No single trend has emerged in the evolution of amine contents during refrigerated storage, which might be at least partly due to the different experimental designs of the studies. In some cases, refrigeration seems to have prevented the formation of certain amines, but this remains a hypothesis, as no study performed a comparative analysis of samples stored under refrigeration and at room temperature. The increase in the biogenic amine content during refrigerated storage reported by other authors may be attributed to bacterial activity. Additionally, some studies have observed an influence of culinary process on the biogenic amine content, mainly derived from the transfer of these compounds to the boiling water or by dilution or concentration effects of the applied treatment.

The exclusion of a high number of plant-origin foods from low-histamine diets cannot be accounted for by their histamine contents, but is more likely due to high levels of putrescine or spermidine. The plant-origin foods consumed by people with histamine intolerance should be of maximum freshness, since histamine and other amines may continue to form during refrigerated storage. The cooking of vegetables in water (boiling) is another relevant strategy for this population, since it can reduce the contents of histamine and other amines in the food.

Author Contributions: Conceptualization, M.T.V.-N., M.L.L.-M., and M.C.V.-C.; Investigation, S.S.-P., O.C.-B., and J.R.-G.; Writing—Original draft preparation, S.S.-P., O.C.-B., and M.L.L.-M.; Writing—Review and editing, M.T.V.-N., M.L.L.-M., and M.C.V.-C.; Supervision, M.C.V.-C.

Funding: This research received no external funding.

Acknowledgments: Sònia Sánchez-Pérez is a recipient of a doctoral fellowship from the University of Barcelona (APIF2018).

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Maintz, L.; Novak, N. Histamine and histamine intolerance. *Am. J. Clin. Nutr.* **2007**, *85*, 1185–1196. [CrossRef] [PubMed]
2. Böhn, L.; Störsrud, S.; Törnblom, H.; Bengtsson, U.; Simrén, M. Self-reported food-related gastrointestinal symptoms in IBS are common and associated with more severe symptoms and reduced quality of life. *Am. J. Gastroenterol.* **2013**, *108*, 634–641. [CrossRef] [PubMed]
3. Rosell-Camps, A.; Zibetti, S.; Pérez-Esteban, G.; Vila-Vidal, M.; Ferrés Ramis, L.; García-Teresa-García, E. Intolerancia a la histamina como causa de síntomas digestivos crónicos en pacientes pediátricos. *Rev. Esp. Enferm. Dig.* **2013**, *105*, 201–207. [CrossRef] [PubMed]
4. Veciana-Nogués, M.T.; Vidal-Carou, M.C. Dieta baja en histamina. In *Nutrición y dietética clínica*, 3rd ed.; Salas-Salvadó, J., Bonada-Sanjaume, A., Trallero-Casaña, R., Saló-Solà, M., Burgos-Peláez, R., Eds.; Ediciones Elsevier España: Barcelona, Spain, 2014; pp. 443–448.
5. Lefèvre, S.; Astier, C.; Kanny, G. Histamine intolerance or false food allergy with histamine mechanism. *Rev. Fr. Allergol.* **2016**, *57*, 24–34. [CrossRef]
6. Wagner, N.; Dirk, D.; Peveling-Oberhag, A.; Reese, I.; Rady-Pizarro, U.; Mitzel, H.; Staubach, P.A. Popular myth—Low-histamine diet improves chronic spontaneous urticaria—Fact or fiction? *J. Eur. Acad. Dermatol. Venereol.* **2017**, *31*, 650–655. [CrossRef] [PubMed]
7. Ede, G. Histamine intolerance: Why freshness matters? *J. Evol. Health* **2017**, *2*, 11. [CrossRef]
8. Swiss Interest Group Histamine Intolerance (SIGHI)—Leaflet Histamine Elimination Diet. Available online: http://www.histaminintoleranz.ch/downloads/SIGHI-Leaflet_HistamineEliminationDiet.pdf (accessed on 25 October 2018).
9. EFSA Panel on Biological Hazards (BIOHAZ). Scientific opinion on risk based control of biogenic amines formation in fermented foods. *EFSA J.* **2011**, *9*, 2393. [CrossRef]
10. World Health Organization and Food and Agriculture Organization of the United Nations. *Joint FAO/WHO Expert Meeting on the Public Health Risks of Histamine and other Biogenic Amines from Fish and Fishery Products: Meeting Report*; World Health Organization: Geneva, Switzerland, 2013.
11. Kovacova-Hanuskova, E.; Buday, T.; Gavliakova, S.; Plevkova, J. Histamine, histamine intoxication and intolerance. *Allergol. Immunopathol.* **2015**, *43*, 498–506. [CrossRef]
12. Jarisch, R.; Wantke, F.; Raithel, M.; Hemmer, W. Histamine and biogenic amines. In *Histamine Intolerance. Histamine and Seasickness*; Jarisch, R., Ed.; Springer: Stuttgart, Germany, 2014; pp. 3–44.
13. Comas-Basté, O.; Latorre-Moratalla, M.L.; Bernacchia, R.; Veciana-Nogués, M.T.; Vidal-Carou, M.C. New approach for the diagnosis of histamine intolerance based on the determination of histamine and methylhistamine in urine. *J. Pharm. Biomed. Anal.* **2017**, *14*, 379–385. [CrossRef]
14. Maintz, L.; Yu, C.F.; Rodríguez, E.; Baurecht, H.; Bieber, T.; Illig, T.; Weidinger, S.; Novak, N. Association of single nucleotide polymorphisms in the diamine oxidase gene with diamine oxidase serum activities. *Allergy* **2011**, *66*, 893–902. [CrossRef]
15. García-Martín, E.; Martínez, C.; Serrador, M.; Alonso-Navarro, H.; Ayuso, P.; Navacerrada, F.; Agúndez, J.A.G.; Jiménez-Jiménez, F.J. Diamine oxidase rs10156191 and rs2052129 variants are associated with the risk for migraine. *Headache* **2015**, *55*, 276–286. [CrossRef] [PubMed]
16. Meza-Velázquez, R.; López-Márquez, F.; Espinosa-Padilla, S.; Rivera-Guillen, M.; Ávila-Hernández, J.; Rosales-González, M. Association of diamine oxidase and histamine N-methyltransferase polymorphisms with presence of migraine in a group of Mexican mothers of children with allergies. *Neulología* **2017**, *32*, 500–507. [CrossRef]
17. Sattler, J.; Häfner, D.; Klotter, H.J.; Lorenz, W.; Wagner, P.K. Food-induced histaminosis as an epidemiological problem: Plasma histamine elevation and haemodynamic alterations after oral histamine administration and blockade of diamine oxidase (DAO). *Agent Action* **1988**, *23*, 361–365. [CrossRef]
18. Steinbrecher, I.; Jarisch, R. Histamine and headache. *Allergologie* **2005**, *28*, 85–91. [CrossRef]
19. Maintz, L.; Benfadal, S.; Allam, J.P.; Hagemann, T.; Fimmers, R.; Novak, N. Evidence for a reduced histamine degradation capacity in a subgroup of patients with atopic eczema. *J. Allergy Clin. Immunol.* **2006**, *117*, 1106–1112. [CrossRef] [PubMed]

20. Worm, M.; Fielder, E.; Döle, A.S.; Schink, T.; Hemmer, W.; Jarisch, R.; Suberbier, T. Exogenous histamine aggravates eczema in a subgroup of patients with atopic dermatitis. *Acta Derm. Venereol.* **2009**, *89*, 52–56. [CrossRef]
21. Honzawa, Y.; Nakase, H.; Matsuura, M.; Chiba, T. Clinical significance of serum diamine oxidase activity in inflammatory bowel disease: Importance of evaluation of small intestinal permeability. *Inflamm. Bowel Dis.* **2011**, *17*, E23–E25. [CrossRef] [PubMed]
22. Mušič, E.; Korošec, P.; Šilar, M.; Adamič, K.; Košnik, M.; Rijavec, M. Serum diamine oxidase activity as a diagnostic test for histamine intolerance. *Wien. Klin. Wochenschr.* **2013**, *12*, 239–243. [CrossRef]
23. Manzotti, G.; Breda, D.; Gioacchino, M.; Burastero, S.E. Serum diamine oxidase activity in patients with histamine intolerance. *Int. J. Immunopath. Pharmacol.* **2016**, *29*, 105–111. [CrossRef]
24. Hoffmann, M.; Gruber, E.; Deutschmann, A.; Jahnel, J.; Hauer, A. Histamine intolerance in children with chronic abdominal pain. *Arch. Dis. Child.* **2016**, *98*, 832–833. [CrossRef]
25. Pinzer, T.C.; Tietz, E.; Waldmann, E.; Schink, M.; Neurath, M.F.; Zopf, Y. Circadian profiling reveals higher histamine plasma levels and lower diamine oxidase serum activities in 24% of patients with suspected histamine intolerance compared to food allergy and controls. *Allergy* **2018**, *73*, 949–957. [CrossRef] [PubMed]
26. Kacik, J.; Wróblewska, B.; Lewicki, S.; Zdanowski, R.; Kalicki, B. Serum diamine oxidase in pseudoallergy in the pediatric population. *Adv. Exp. Med. Biol.* **2018**, *1039*, 35–44. [CrossRef] [PubMed]
27. Son, J.H.; Chung, B.Y.; Kim, H.O.; Park, C.W. A histamine-free diet is helpful for treatment of adult patients with chronic spontaneous urticaria. *Ann. Dermatol.* **2018**, *30*, 164–172. [CrossRef] [PubMed]
28. Izquierdo-Casas, J.; Comas-Basté, O.; Latorre-Moratalla, M.L.; Lorente-Gascón, M.; Duelo, A.; Vidal-Carou, M.C.; Soler-Singla, L. Low serum diamine oxidase (DAO) activity levels in patients with migraine. *J. Physiol. Biochem.* **2018**, *74*, 93–99. [CrossRef] [PubMed]
29. Bover-Cid, S.; Latorre-Moratalla, M.L.; Veciana-Nogués, M.T.; Vidal-Carou, M.C. Processing contaminants: Biogenic amines. In *Encyclopedia of Food Safety*; Motarjemi, Y., Moy, G.G., Todd, E.C.D., Eds.; Elsevier Inc.: Burlington, MA, USA, 2014; Volume 2, pp. 381–391.
30. Gardini, F.; Özogul, Y.; Suzzi, G.; Tabanelli, G.; Özogul, F. Technological factors affecting biogenic amine content in foods: A review. *Front. Microbiol.* **2016**, *7*, 1218. [CrossRef] [PubMed]
31. Vidal-Carou, M.C.; Veciana-Nogués, M.T.; Latorre-Moratalla, M.L.; Bover-Cid, S. Biogenic amines: Risks and control. In *Handbook of Fermented Meat and Poultry*, 2nd ed.; Toldrá, F., Hui, Y.H., Astiasarán, I., Sebranek, J.G., Talon, R., Eds.; Wiley-Blackwell: Hoboken, NJ, USA, 2014; pp. 413–428.
32. Lavizzari, T.; Veciana-Nogués, M.T.; Weingart, O.; Bover-Cid, S.; Mariné-Font, A.; Vidal-Carou, M.C. Occurrence of biogenic amines and polyamines in spinach and changes during storage under refrigeration. *J. Agric. Food Chem.* **2007**, *55*, 9514–9519. [CrossRef] [PubMed]
33. Kumar, M.N.K.; Bbu, B.N.H.; Venkayesh, Y.P. Higher histamine sensitivity in non-atopic subjects by skin prick test may result in misdiagnosis of eggplant allergy. *Immunol. Investig.* **2009**, *38*, 93–103. [CrossRef]
34. Moret, S.; Smela, D.; Populin, T.; Conte, L. A survey on free biogenic amine content of fresh and preserved vegetables. *Food Chem.* **2005**, *89*, 355–361. [CrossRef]
35. Trivedi, K.; Borkovcová, I.; Karpíšková, R. Tyramine production by enterococci from various foodstuffs: A threat to the consumers. *Czech J. Food Sci.* **2009**, *27*, S357–S360. [CrossRef]
36. Kalač, P. Health effects and occurrence of dietary polyamines: A review for the period 2005–mid 2013. *Food Chem.* **2014**, *161*, 27–39. [CrossRef]
37. Bouchereau, A.; Aziz, A.; Larher, F.; Martin-Tanguy, J. Polyamines and environmental challenges: Recent development. *Plant Sci.* **1999**, *140*, 103–125. [CrossRef]
38. Kalač, P.; Svecová, S.; Pelikánová, T. Levels of biogenic amines in typical vegetable products. *Food Chem.* **2002**, *77*, 349–351. [CrossRef]
39. Toro-Funes, N.; Bosch-Fuste, J.; Latorre-Moratalla, M.L.; Veciana-Nogués, M.T.; Vidal-Carou, M.C. Biologically active amines in fermented and non-fermented commercial soybean products from the Spanish market. *Food Chem.* **2015**, *173*, 1119–1124. [CrossRef] [PubMed]
40. Nishimura, K.; Shiina, R.; Kashiwagi, K.; Igarashi, K. Decrease in polyamines with aging and their ingestion from food and drink. *J. Biochem.* **2006**, *139*, 81–90. [CrossRef] [PubMed]
41. Preti, R.; Bernacchia, R.; Vinci, G. Chemometric evaluation of biogenic amines in commercial fruit juices. *Eur. Food Res. Technol.* **2016**, *242*, 2031–2039. [CrossRef]

42. Eliassen, K.A.; Reistad, R.; Risøen, U.; Rønning, H.F. Dietary polyamines. *Food Chem.* **2002**, *78*, 273–280. [CrossRef]
43. Esti, M.; Volpe, G.; Masignan, D.; Compagnone, E.; La Notte, E.; Palleschi, G. Determination of amines in fresh and modified atmosphere packaged fruits using electrochemical biosensors. *J. Agric. Food Chem.* **1998**, *46*, 4233–4237. [CrossRef]
44. Kalač, P.; Křížek, M.; Pelikánová, T.; Langová, M.; Veškrna, O. Contents of polyamines in selected foods. *Food Chem.* **2005**, *90*, 561–564. [CrossRef]
45. González-Aguilar, G.A.; Zacarias, L.; Perez-Amador, M.A.; Carbonell, J.; Lafuente, M.T. Polyamine content and chilling susceptibility are affected by seasonal changes in temperature and by conditioning temperature in cold-stored "Fortune" mandarin fruit. *Physiol. Plant* **2000**, *108*, 140–146. [CrossRef]
46. Preti, R.; Rapa, M.; Vinci, G. Effect of Steaming and boiling on the antioxidant properties and biogenic amines content in Green Bean (*Phaeseolus vulgaris*) varieties of different colours. *J. Food Quality* **2017**. [CrossRef]
47. Ziegler, W.; Hahn, M.; Wallnöfer, P.R. Changes in biogenic amine contents during processing of several plant foods. *Deut. Lebensm. Rundsch.* **1994**, *90*, 108–112.
48. Cipolla, B.G.; Havouis, R.; Moulinoux, J.P. Polyamine contents in current foods: A basis for polyamine reduced diet and a study of its long-term observance and tolerance in prostate carcinoma patients. *Amino Acids* **2007**, *33*, 203–212. [CrossRef] [PubMed]
49. Nishibori, N.; Fujihara, S.; Akatuki, T. Amounts of polyamines in foods in Japan and intake by Japanese. *Food Chem.* **2007**, *100*, 491–497. [CrossRef]
50. Bardócz, S.; Grant, G.; Brown, D.S.; Ralph, A.; Pusztai, A. Polyamines in food—Implications for growth and health. *J. Nutr. Biochem.* **1993**, *4*, 66–71. [CrossRef]
51. Okamoto, A.; Sugi, E.; Koizumi, Y.; Yanadiga, F.; Udaka, S. Polyamine content of ordinary foodstuffs and various fermented foods. *Biosci. Biotechnol. Biochem.* **1997**, *61*, 1582–1584. [CrossRef] [PubMed]
52. Kalač, P.; Krausová, P. A review of dietary polyamines: Formation, implications for growth and health and occurrence in foods. *Food Chem.* **2005**, *90*, 219–230. [CrossRef]
53. Dadáková, E.; Pelikánová, T.; Kalač, P. Content of biogenic amines and polyamines in some species of European wild-growing edible mushrooms. *Eur. Food Res. Technol.* **2009**, *230*, 163–171. [CrossRef]
54. Dionex. Determination of biogenic amines in fruit, vegetables, and chocolate using ion chromatography with suppressed, conductivity and integrated pulsed amperometric detections. *Appl. Update* **2016**, *162*, 1–8.
55. Glória, M.B.A.; Tavares-Neto, J.; Labanca, R.A.; Carvalho, M.S. Influence of cultivar and germination on bioactive amines in soybeans (*Glycine max* L. Merril). *J. Agric. Food Chem.* **2005**, *53*, 7480–7485. [CrossRef]
56. Byun, B.Y.; Bai, X.; Mah, J.H. Occurrence of biogenic amines in Doubanjiang and Tofu. *Food Sci. Biotech.* **2013**, *22*, 55–62. [CrossRef]
57. Santiago-Silva, P.; Labanca, R.; Gloria, B. Functional potential of tropical fruits with respect to free bioactive amines. *Food Res. Int.* **2011**, *44*, 1264–1268. [CrossRef]
58. Naila, A.; Flint, S.; Fletcher, G.; Bremer, P.; Meerdink, G. Control of biogenic amines in food-existing and emerging approaches. *J. Food Sci.* **2010**, *75*, 139–150. [CrossRef] [PubMed]
59. Simon-Sarkadi, L.; Holazapfel, W.H.; Halasz, A. Biogenic amine content and microbial contamination of leafy vegetables during storage at 5C. *J. Food Biochem.* **1994**, *17*, 407–418. [CrossRef]
60. Chong, C.Y.; Abu Bakar, F.; Russly, A.R.; Jamilah, B.; Mahyudin, N.A. The effects of food processing on biogenic amines formation. *Int. Food Res. J.* **2011**, *18*, 867–876.
61. Latorre-Moratalla, M.L.; Comas-Basté, O.; Veciana-Nogués, M.T.; Vidal-Carou, M.C. La cocción reduce el contenido de histamina de las espinacas. In *11a Reunión anual de la Sociedad Española de Seguridad Alimentaria*; Sociedad Española de Seguridad Alimentaria: Pamplona, Spain, 2015.
62. Veciana-Nogués, M.T.; Latorre-Moratalla, M.L.; Toro-Funes, N.; Bosh-Fusté, J.; Vidal-Carou, M.C. Efecto de la cocción con y sin sal en el contenido de poliaminas de las verduras. *Nutr. Hosp.* **2014**, *30*, 53.
63. Lo Scalzo, R.; Fibiani, M.; Francese, G.; D'Alessandro, A.; Rotino, G.L.; Conte, P.; Mennella, G. Cooking influence on physico-chemical fruit characteristics of eggplant (*Solanum melongena* L.). *Food Chem.* **2016**, *194*, 835–842. [CrossRef] [PubMed]
64. Chung, B.Y.; Park, S.Y.; Byun, Y.S.; Son, J.H.; Choi, Y.W.; Cho, Y.S.; Kim, H.O.; Park, C.W. Effect of different cooking methods on histamine levels in selected foods. *Ann. Dermatol.* **2017**, *29*, 706–714. [CrossRef]
65. Wantke, F.; Gotz, M.; Jarisch, R. Histamine-free diet: Treatment of choice for histamine-induced food intolerance and supporting treatment for chronic headaches. *Clin. Exp. Allergy* **1993**, *23*, 982–985. [CrossRef]

66. Guida, B.; De Martino, C.; De Martino, S.; Tritto, G.; Patella, V.; Trio, R.; D'Agostino, C.; Pecoraro, P.; D'Agostino, L. Histamine plasma levels and elimination diet in chronic idiopathic urticaria. *Eur. J. Clin. Nutr.* **2000**, *54*, 155–158. [CrossRef]
67. Siebenhaar, L.; Melde, A.; Magerl, T.; Zuberier, T.; Church, M.K.; Maurer, M. Histamine intolerance in patients with chronic spontaneous urticaria. *J. Eur. Acad. Dermatol. Venereol.* **2016**, *30*, 1774–1777. [CrossRef]
68. Schwelberger, H.G.; Bodner, E. Purification and characterization of diamine oxidase from porcine kidney and intestine. *Biochim. Biophys. Acta* **1997**, *1340*, 152–164. [CrossRef]
69. Finney, J.; Moon, H.J.; Ronnebaum, T.; Lantz, M.; Mure, M. Human copper-dependent amine oxidases. *Arch Biochem. Biophys.* **2014**, *546*, 19–32. [CrossRef] [PubMed]
70. Vlieg-Boerstra, B.J.; Van der Heide, S.; Oude, J.N.G.; Kluin-Nelemans, J.C.; Dubois, A.E. Mastocytosis and adverse reactions to biogènic amines and histamine-releasing foods. What is the evidence? *Neth. J. Med.* **2005**, *63*, 244–249. [PubMed]

© 2018 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

3.2. Revisió crítica de les dietes baixes en histamina en base als continguts d'histamina i altres amines biògenes dels aliments exclosos

PUBLICACIÓ 3

Low-histamine diets: Is the exclusion of foods justified by their histamine content?

Sònia Sánchez-Pérez, Oriol Comas-Basté, M. Teresa Veciana-Nogués, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou.

Nutrients, 2021, 13, 1395.

Índex d'impacte (JCR 2021): 6,706

Posició en l'àrea "Nutrition & Dietetics": Q1 (15/90)

COMUNICACIÓ ESCRITA 3

Oriol Comas-Basté, Sònia Sánchez-Pérez**,** Nelly Carolina Muñoz-Esparza, Salvador Hernández-Macias, M. Teresa Veciana-Nogués, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou. **Dietary management of histamine intolerance: Establishing the biogenic amine profile of foods excluded in low-histamine diets.** *Conferencia de la Federación Iberoamericana de Nutrición (FINUT),* 2020, Ciudad de México.

COMUNICACIÓ ESCRITA 4

Sònia Sánchez-Pérez**,** Oriol Comas-Basté, Salvador Hernández-Macias, M. Luz Latorre-Moratalla, M. Teresa Veciana-Nogués i M. Carmen Vidal-Carou. **Estimación de la ingesta de histamina para validar la exclusión de alimentos en el tratamiento dietético de la intoleràncIa a la histamina.** *13ª Reunión Anual de la Sociedad Española de Seguridad Alimentaria (SESAL),* 2017, Girona.

PLANTEJAMENT I OBJECTIU

Conceptualment, les dietes baixes en histamina haurien d'eliminar aquells aliments susceptibles de contenir histamina. No obstant, realitzar aquest tipus de dietes, o dit d'una altra manera, evitar el consum d'aliments rics en histamina, no resulta tan fàcil com podria semblar. En primer lloc, no existeix un nivell d'histamina consensuat per sota del qual un aliment es considera baix en aquest compost. Hi ha autors que apunten que aliments amb nivells per sota dels 50 mg/kg es poden considerar baixos en histamina, mentre que d'altres autors són molt més exigents i mantenen aquest llindar per sota d'1 mg/kg [120–123]. D'altra banda, no existeix una regulació específica que permeti o obligui a la indústria alimentària a declarar la presència o absència d'histamina en l'etiquetatge. Aquesta informació ajudaria als individus amb intoleràncIa a la histamina a seleccionar de forma conscient aquells aliments més apropiats. A més, la disparitat que existeix en el tipus d'aliments a excloure segons les diferents dietes descrites en la literatura científica dificulta la recomanació de pautes adequades en el marc d'una dieta baixa en histamina.

L'objectiu d'aquest estudi va ser revisar críticament les dietes baixes en histamina descrites en la literatura en base als continguts d'histamina i altres amines biògenes dels aliments exclosos.

METODOLOGIA

Es va realitzar una recerca exhaustiva d'articles científics relacionats amb dietes baixes en histamina a través de les bases de dades *PubMed* i *Web of Science*. Només es van considerar aquelles publicacions que clarament especificaven quins aliments són permesos o exclosos en el marc d'una dieta baixa en histamina. Els continguts d'amines (histamina, putrescina, cadaverina, tiramina, espermidina i espermina) es van obtenir de la base de dades del grup de recerca que inclou més de 1800 mostres d'aliments i begudes del mercat espanyol [7].

RESULTATS

Es van identificar un total de 10 publicacions científiques que especificaven els aliments que es poden o no consumir en el context d'una dieta baixa en histamina. Es va observar una gran heterogeneïtat en els aliments que s'haurien d'excloure en les diferents dietes revisades, essent els productes fermentats els únics que s'eliminaven en tots els casos. És important destacar que la majoria d'aliments tant sols s'excloïen en el 50% o menys de les dietes revisades.

Tots els productes fermentats, els peixos i derivats i certs aliments d'origen vegetal (tomàquet, albergínia i espinacs), aliments que sovint presenten continguts destacats d'histamina, van representar tants sols el 32% del total d'aliments exclosos de les dietes. En general, els diferents tipus de productes fermentats exclosos mostren, segons la base de dades del grup [7], nivells d'histamina relativament baixos (valors mitjans d'entre 22 i 74 mg/kg), encara que, en alguns casos, aquests poden arribar a ser força elevats. En aquest sentit, el 5% de les mostres de derivats carnis fermentats, formatges i derivats de la soja fermentats presentaven valors d'histamina per sobre dels 130 mg/kg, 203 mg/kg i 486 mg/kg, respectivament [7]. Pel que fa als peixos i derivats, generalment no contenen histamina o en valors molt baixos, com correspon al productes frescos, encara que, en algunes mostres de salmó i de conserves de peix es van assolir continguts de fins a 111 mg/kg i 657 mg/kg d'histamina, respectivament [7]. Per contra, segons la nostra base de dades, es troba histamina en pràcticament totes les mostres d'espinacs, albergínia i tomàquet, encara que generalment en continguts més baixos (amb valors mitjans de 32 mg/kg, 39 mg/kg i 3 mg/kg, respectivament) [7].

Pel que fa al 68% d'aliments exclosos restants, i segons les nostres dades, no contenen histamina [7]. En canvi, alguns d'aquests aliments presenten quantitats rellevants de putrescina (cítrics, plàtans, soja, carbassa i fruits secs). Per exemple, en el cas dels cítrics és molt freqüent trobar valors destacables d'aquesta amina amb valors de fins a 170 mg/kg [7].

A part dels aliments que contenen histamina o nivells significatius de putrescina, es va identificar un elevat nombre de productes (41%) que, en principi, no contenen cap amina o en continguts molt baixos [7]. Aquest seria el cas per exemple, de les cireres, les prunes, els fruits vermells, l'alvocat, la xocolata o el té, que no obstant, també són exclosos de les dietes publicades pel tractament de la intoleràncIa a la histamina.

TAKE-HOME MESSAGE

Actualment existeix una clara falta de consens en quant als aliments que han de ser eliminats en el tractament dietètic de la intoleràncIa a la histamina. Únicament el 32% dels aliments exclosos en les dietes publicades contenen histamina. No obstant això, la presència d'altres amines, especialment de putrescina, podria justificar l'eliminació d'alguns dels aliments.

nutrients

MDPI

Article

Low-Histamine Diets: Is the Exclusion of Foods Justified by Their Histamine Content?

Sònia Sánchez-Pérez [1,2,3], Oriol Comas-Basté [1,2,3], M. Teresa Veciana-Nogués [1,2,3], M. Luz Latorre-Moratalla [1,2,3] and M. Carmen Vidal-Carou [1,2,3,*]

[1] Departament de Nutrició, Ciències de l'Alimentació i Gastronomia, Facultat de Farmàcia i Ciències de l'Alimentació, Campus de l'Alimentació de Torribera, Universitat de Barcelona (UB), Av. Prat de la Riba 171, 8921 Santa Coloma de Gramenet, Spain; soniasanchezperez@ub.edu (S.S.-P.); oriolcomas@ub.edu (O.C.-B.); veciana@ub.edu (M.T.V.-N.); mariluzlatorre@ub.edu (M.L.L.-M.)
[2] Institut de Recerca en Nutrició i Seguretat Alimentària (INSA·UB), Universitat de Barcelona (UB), Av. Prat de la Riba 171, 8921 Santa Coloma de Gramenet, Spain
[3] Xarxa d'Innovació Alimentària (XIA), C/Baldiri Reixac 4, 8028 Barcelona, Spain
* Correspondence: mcvidal@ub.edu; Tel.: +34-934-031-984

Abstract: A low-histamine diet is currently the most advised strategy to prevent the symptomatology of histamine intolerance. Conceptually, these diets should be founded on the exclusion of histamine-containing foods, although a certain disparity is found within the list of excluded foods in accordance with the different low-histamine diets available in the literature. This study aimed to critically review low-histamine diets reported in the scientific literature, according to the histamine and other biogenic amine contents of the excluded foods. A total of ten scientific studies that provided specific recommendations on the foods that must be avoided within the framework of a low-histamine diet were found. Overall, the comparative review brought out the great heterogenicity in the type of foods that are advised against for histamine intolerant individuals. Excluded foods were, in most cases, different depending on the considered diet. Only fermented foods were unanimously excluded. The exclusion of 32% of foods could be explained by the occurrence of high contents of histamine. The presence of putrescine, which may interfere with histamine degradation by the DAO enzyme at the intestinal level, could partly explain the reason why certain foods (i.e., citrus fruits and bananas) were also frequently reported in low-histamine diets. Finally, there was a range of excluded foods with an absence or very low levels of biogenic amines. In this case, certain foods have been tagged as histamine-liberators, although the mechanism responsible has not yet been elucidated.

Keywords: histamine; low-histamine diet; histamine-free diet; histamine intolerance; biogenic amines; histamine-releasing foods

check for updates

Citation: Sánchez-Pérez, S.; Comas-Basté, O.; Veciana-Nogués, M.T.; Latorre-Moratalla, M.L.; Vidal-Carou, M.C. Low-Histamine Diets: Is the Exclusion of Foods Justified by Their Histamine Content?. *Nutrients* **2021**, *13*, 1395. https://doi.org/10.3390/nu13051395

Academic Editor: Bahram H. Arjmandi

Received: 26 March 2021
Accepted: 19 April 2021
Published: 21 April 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

In recent years, a significant increase in the frequency of food intolerances (i.e., non-toxic and non-immune mediated reactions to food) has been detected in most developed societies. Food intolerances are disabling disorders that provoke an important decrease in the quality of life of this population [1,2]. Among them, histamine intolerance, also referred to as food histaminosis or hypersensitivity to food histamine, arises from the failure of the diamine oxidase (DAO) enzyme to degrade dietary histamine at the intestinal level. DAO deficit results in an increase in systemic histamine concentrations and the subsequent onset of symptoms [3–5]. This enzyme deficiency may have a genetic or pathological etiology (i.e., secondary to certain inflammatory bowel diseases) [6,7]. Moreover, some widely used pharmacologic drugs have also been described as potential DAO inhibitors, although in a punctual and reversible manner [8,9]. More recently, a potential etiological relationship has been suggested between a dysbiosis in the intestinal microbiota and histamine intolerance, although this hypothesis still needs to be further studied [10].

Plasma histamine accumulation can provoke a wide number of nonspecific gastrointestinal and extraintestinal clinical manifestations (i.e., dermatological, respiratory, neurological and hemodynamic complaints) [3–5]. The most frequent and severe symptoms, according to a recent comprehensive study, were abdominal distension, diarrhea, postprandial fullness, abdominal pain and constipation, followed by headaches, dizziness, and palpitations [2]. Moreover, in 97% of cases the onset of three or more symptoms concerning different organs were reported, thus, underlining the complexity of the clinical picture of histamine intolerance [2].

Currently, the most advised strategies to prevent the onset of symptoms are the follow-up of a low-histamine diet and the supplementation with exogenous DAO enzyme to enhance the intestinal histamine degradation [11–13]. As regards low-histamine diets, several clinical studies are continuously gathering increasing evidence on their efficacy on the improvement or remissions of symptoms [5,14–23]. The vast majority of these studies report efficacy rates higher than 70%, although some of them face certain limitations in terms of the number of patients and/or in the duration of the dietary intervention [3].

Conceptually, low-histamine diets should be based on the exclusion of histamine-containing foods. Histamine in foods is mainly formed by the bacterial decarboxylation of its precursor amino acid, histidine [24]. Therefore, foods susceptible to accumulating high contents of this amine are those that are microbiologically altered by spoilage bacteria; fermented products, due to the histaminogenic capacity of fermentative bacteria [25].

The design of a low-histamine diet is challenging due to different handicaps. One of these is the lack of consensus on the histamine level below which a food is said to be considered low in histamine. Thus, variable histamine levels in food ranging between 5–50 mg/kg have been pointed out as potential thresholds, while other authors are much more demanding and consider foods with low histamine concentrations to be those that contain amounts below 1 mg/kg [12,26–28]. Moreover, there is no specific regulation for the food industry to declare the occurrence or absence of histamine in food labelling, which could help histamine intolerant individuals to make suitable and informed choices.

Overall, providing dietary recommendations and guidelines in the frame of a low-histamine diet is difficult for healthcare professionals. In fact, disparity is found in the list of excluded foods reported by the different available low-histamine diets. Therefore, the aim of this work was to critically review the low-histamine diets from the literature, according to the contents of histamine and other biogenic amines found in the excluded foods.

2. Materials and Methods

2.1. Identification of Low-Histamine Diets

A selective search of scientific articles concerning low-histamine diets was performed through the PubMed and Web of Science bibliographic databases using the following keywords: "low-histamine diet", "histamine-free diet", "histamine elimination diet", "histamine restricted diet", "histamine intolerance treatment" and "dietary management of histamine intolerance". Only studies that clearly specified the foods allowed and/or excluded within a low-histamine diet were considered for further assessment.

2.2. Content of Histamine and Other Biogenic Amines in Foods and Beverages Excluded from Low-Histamine Diets

Data on histamine and other biogenic amines (i.e., putrescine, cadaverine, tyramine, spermidine and spermine) content in foods and beverages from the Spanish market were obtained from the self-produced and updated database [11,29].

3. Results

The selective search performed in this study resulted in a total of ten scientific publications, which provided specific recommendations about the range of foods that may be consumed or must be avoided within the framework of a low-histamine diet [14,16,17,20,23,26,30–33]. Overall, most of these studies based their recommendations on previous studies (mainly

dealing with histamine contents in foods) or on those foods that patients associated with the onset of symptoms. Figure 1 graphically summarizes the extensive list of the avoided foods according to the literature review and the count of low-histamine diets that exclude each foodstuff. This comparative review showed great heterogenicity in the foods that should be excluded by histamine intolerant people. Firstly, all low-histamine diets unanimously advised the elimination of many fermented foods and beverages (i.e., dry-fermented sausages, cured cheese, wine and beer). On the other hand, it is worth highlighting that the majority of foods were only excluded by 50% or less of the revised diets. These results confirm the lack of consensus that currently exists in this type of diet.

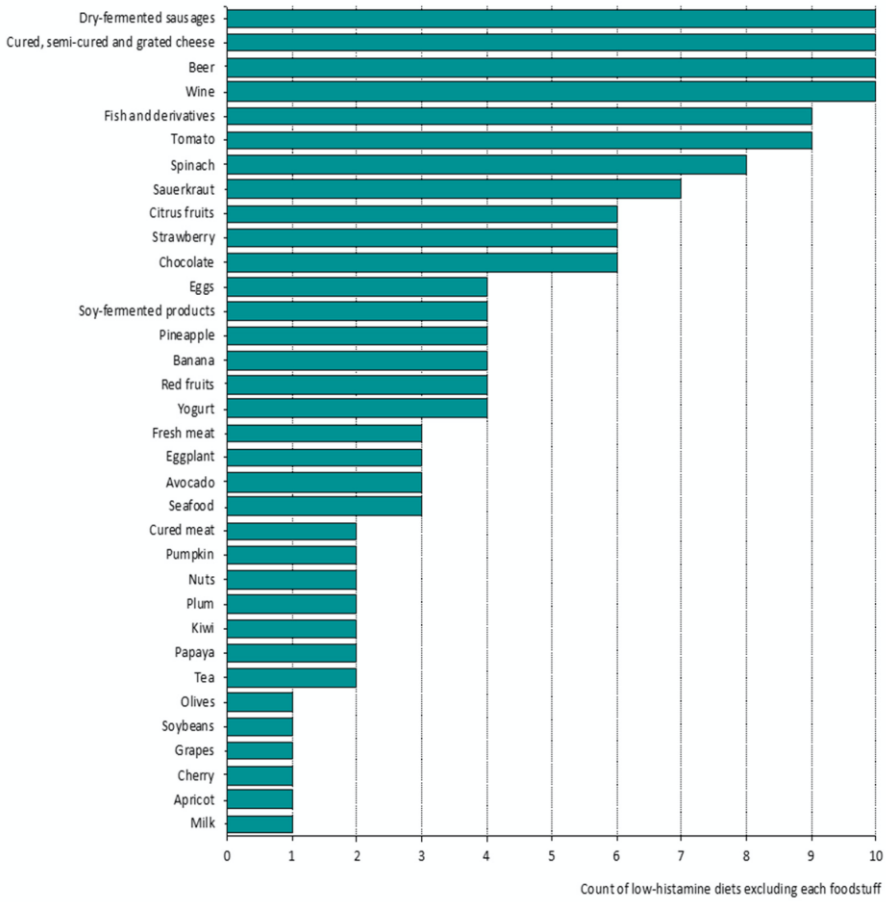


Figure 1. List of the avoided foods according to the literature review on low-histamine diets and count of references that exclude each foodstuff [14,16,17,20,23,26,30–33].

The distribution of histamine levels in all excluded foods is shown in Figure 2. The exclusion of all foods listed above could not be explained by the occurrence of histamine. In fact, most of the foodstuffs retailed in Spain showed histamine levels below 1 mg/kg, considered by some authors as the threshold to define a food low in histamine. Contrarily, fermented foods had a large variability, even within samples of the same production batch due to the essentially microbial origin of histamine. Moreover, the nature of the food, the

bacterial strain and many other factors that influence the growth and metabolic activity of the bacteria can also have an impact on the accumulation of this compound. This variability is precisely one of the causes of the complexity of issuing recommendations and, consequently, as a precautionary measure all the foods, that a priori may contain histamine, are eliminated.

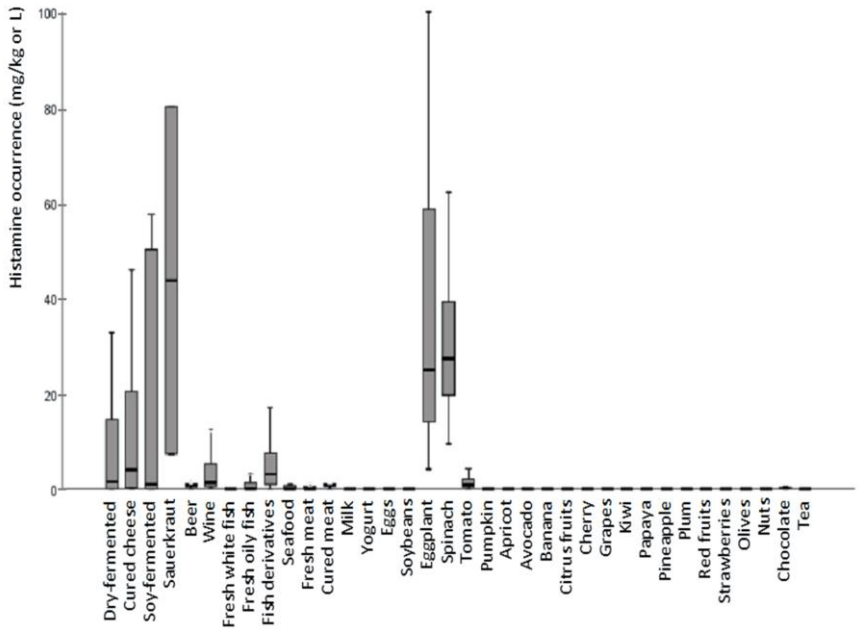


Figure 2. Histamine distribution (mg/kg or L) in foods marketed in Spain excluded from low-histamine diets [11,29].

Fermented foods (i.e., dry-fermented sausages, cured-cheese, sauerkraut and soy-fermented derivatives) are products that can potentially accumulate high histamine contents (Figure 2). In fermented foods, the presence of histamine depends on both the hygienic quality of the raw materials and/or manufacturing processes, and the histaminogenic capacity of technological bacteria [34,35]. As can be seen in Table 1, histamine levels in fermented foods marketed in Spain were relatively low (with mean values ranging between 22 and 74 mg/kg), but in certain cases these types of foods reached high histamine levels, with 5% of samples measuring above 203 mg/kg in cheese, 130 mg/kg in dry-fermented sausages and 486 mg/kg in soy-fermented products. Apart from histamine, in this food category, other biogenic amines could be frequently found, mainly tyramine. The presence of tyramine is strongly associated with the enzymatic activity of many fermentative lactic acid bacteria species. Maximum tyramine levels of 750 mg/kg in dry-fermented products, 1500 mg/kg in cheese and 1700 mg/kg in fermented vegetables were found. The occurrence of putrescine and cadaverine was also frequent, though at lower and more variable levels than tyramine (Table 1).

Table 1. Biogenic amines occurrence (mg/kg or L) found in different food and beverages from the Spanish market. Data are presented as average (standard deviation), P95 and minimum–maximum [11,29].

Foods	*n*	Occurrence of Biogenic Amines (mg/kg or L)					
		Histamine	**Putrescine**	**Cadaverine**	**Tyramine**	**Spermidine**	**Spermine**
Dry-fermented sausages	424	21.57 (52.10) 129.95 ND-474.82	68.23 (101.40) 280.3 ND-537.05	32.45 (72.96) 172.10 ND-658.05	140.9 (119.59) 378.51 ND-742.60	5.39 (5.94) 18.80 3.04–32.65	25.12 (23.85) 59.94 0.34–224.15
Cured, semi-cured and grated cheese	80	33.10 (77.10) 203.30 ND-389.86	68.90 (141.30) 423.00 ND-666.92	87.25 (283.55) 356.52 ND-2036.90	128 (264.41) 613.46 ND-1567.50	8.49 (12.40) 36.38 ND-68.92	1.84 (4.46) 12.58 ND-21.03
Soy-fermented products	21	73.95 (184.51) 486.31 ND-730.06	13.48 (6.71) 22.2 2.73–31.06	6.88 (11.97) 35.08 ND-36.95	187.24 (446.59) 930 ND-1730.17	34.84 (38.23) 105.47 ND-124.03	4.81 (5.24) 11.15 ND-21.89
Sauerkraut	5	43.74 (51.45) 76.48 7.36–80.12	232.66 (148.53) 327.18 127.63–337.68	76.49 (73.85) 123.49 24.27–128.71	43.47 (28.05) 61.32 23.63–63.3	5.29 (3.86) 7.75 2.56–8.02	0.85 (0.40) 1.10 0.56–1.13
Beer	176	1.23 (2.47) 3.28 ND-21.60	3.16 (2.89) 7.61 ND-14.50	1.28 (3.94) 4.66 ND-31.40	6.31 (8.04) 25.44 0.55–46.80	0.48 (0.81) 1.7 ND-6.30	0.19 (0.61) 1.14 ND-3.90
Wine	299	3.63 (5.86) 12.3 0.09–34.25	ND	ND	2.42 (2.47) 7.5 ND-15.85	ND	ND
Fresh white fish	31	1.14 (6.46) ND ND-36.55	1.33 (2.67) 7.43 ND-10.50	1.61 (6.01) 4.8 ND-33.65	1.03 (3.39) 6.51 ND-17.10	2.25 (1.91) 5.32 ND-7.85	6.78 (2.65) 11.27 2.05–13.50
Fresh oily fish	49	3.27 (15.71) 6.66 ND-111.26	2.37 (6.71) 5.02 ND-39.89	13.22 (67.40) 10.45 ND-400.23	1.18 (5.43) 2.19 ND-37.20	6.69 (3.09) 11.39 1.20–11.90	14.47 (9.75) 31.67 1.05 -37.03
Preserved and semi-preserved fish	151	10.03 (53.32) 20.39 ND-657.05	2.79 (3.81) 9.02 ND-21.15	7.41 (10.79) 29.23 ND-55.80	8.23 (14.87) 40.6 ND-88.50	3.61 (2.78) 7.94 0.37–11.80	7.48 (6.05) 17.05 ND-35.20
Seafood	7	ND	3.02 (3.01) 7.62 1.44–9.79	ND	0.15 (0.27) 0.58 ND-0.65	4.03 (3.23) 7.94 0.82–8.37	10.63 (6.92) 19.21 4.93–19.73
Fresh meat	199	ND	1.35 (2.66) 3.04 ND-9.68	5.02 (14.55) 28.71 ND-51.16	4.32 (8.62) 35.89 ND-38.77	1.16 (4.59) 3.4 ND-13.96	17.08 (4.59) 29.55 9.70–25.69
Cured meat	23	4.89 (22.70) 3.54 ND-150	4.65 (5.18) 9.24 ND-17.40	38.03 (92.82) 49.64 ND-305	3.43 (10.56) 42.58 ND-46.50	6.05 (0.92) 6.88 4.5–7.30	37.82 (10.46) 42.58 24.9–62.10
Milk	5	ND	ND	ND	ND	ND	ND
Yogurt	5	ND	2.04 (2.01) 2.23 ND-4.05	ND	ND	1.07 (0.75) 0.69 0.50–1.75	0.28 (0.39) 0.3 ND-0.50
Eggs	14	ND	ND	ND	ND	3.61 (1.54) 4.43 ND-4.47	4.48 (1.72) 5.27 0.32–5.31
Soybeans	5	ND	19.07 (4.39) 21.86 15.96–22.17	9.04 (1.09) 9.74 8.27–9.81	ND	99.55 (3.52) 101.79 97.06–102.04	25.92 (7.34) 30.58 20.73–31.10
Eggplant	23	39.42 (30.66) 98.84 4.17–100.64	34.30 (6.98) 46.29 24.10–48.63	ND	0.60 (0.90) 2.24 ND-2.27	5.06 (1.93) 7.7 2.54–7.97	0.47 (0.48) 1.29 ND-1.38
Spinach	18	31.77 (17.02) 63.37 9.46–69.72	4.48 (2.46) 7.70 0.14–9.20	ND (0.02) 0.01 ND-0.08	2.05 (0.83) 3.10 0.79–4.28	28.22 (9.72) 44.53 15.63–52.98	3.33 (1.89) 6.13 ND-8.85

Table 1. *Cont.*

Foods	*n*	Occurrence of Biogenic Amines (mg/kg or L)					
		Histamine	**Putrescine**	**Cadaverine**	**Tyramine**	**Spermidine**	**Spermine**
Tomato	53	2.51 (4.08) 13.83 ND-17.07	16.48 (6.93) 30.16 6.29–35.55	0.50 (0.48) 1.42 ND-2.33	0.49 (0.92) 1.21 ND-6.38	3.04 (1.41) 5.69 2.91–7.90	0.08 (0.16) 0.36 ND-0.73
Pumpkin	13	ND	9.87 (6.19) 19.17 2.95–24.23	0.58 (0.78) 1.82 ND-2.15	ND	10.32 (2.83) 13.88 6.19–14.98	1.77 (1.99) 5.21 0.5–6.88
Apricot	4	ND	ND	ND	ND	5.86 (1.59) 6.50 4.16–7.68	ND
Avocado	5	ND	ND	ND	1.81 (2.06) 4.65 0.58–5.44	3.15 (3.27) 6.69 0.18–6.72	4.50 (2.52) 7.61 2.02–7.92
Banana	8	ND	37.94 (8.32) 47.37 25.50–49.49	ND	0.53 (0.79) ND ND-1.85	11.91 (2.90) 15.10 7.62–15.79	1.33 (0.97) 2.67 ND- 2.75
Citrus fruits	38	ND	79.75 (44.36) 146.16 1.21–173.81	ND	ND	2.57 (1.28) 4.86 0.18–6.24	0.12 (0.36) 1.02 ND-1.14
Cherry	5	ND	3.42 (0.06) 3.46 3.42–3.46	ND	ND	2.37 (0.16) 2.47 2.26–2.47	ND
Grapes	10	ND	2.69 (0.34) 4.05 1–4.30	ND	ND	5.25 (2.61) 8.6 ND-9.70	2.59 (0.11) 2.56 2.35–2.68
Kiwi	13	ND	1.47 (0.47) 2.07 0.48–2.17	ND	ND	5.35 (1.06) 6.39 2.72–6.39	0.73 (0.56) 1.41 ND-1.50
Papaya	6	ND	7.25 (5.80) 11.86 ND-12.48	ND	ND	14.35 (4.32) 15.45 10.32–19.07	1.16 (1.59) 2.06 ND-2.99
Pineapple	5	ND	2.69 (1.42) 3.89 0.56–3.97	ND	ND	1.92 (1.26) 3.15 0.27–3.18	0.48 (0.21) 0.75 0.32–0.77
Plum	6	ND	ND	ND	4.02 (4.32) 6.76 0.96–7.07	2.68 (0.30) 2.87 2.47–2.89	1.74 (2.47) 3.31 ND- 3.48
Red fruits	7	ND	ND	ND	7.37 (1.03) 9.36 3.34–11.52	5.58 (1.16) 2.54 0.78–3.98	1.97 (1.61) 1.65 ND–3.73
Strawberries	9	ND	3.77 (1.52) 6.09 2.04–6.41	ND	ND	6.00 (1.56) 8.52 4.62–9.86	0.46 (0.69) 1.5 ND-1.62
Olives	5	ND	2.64 (1.58) 4.2 1.54–4.45	ND	1.95 (1.85) 3.7 0.28–3.94	ND	ND
Nuts	47	ND	4.40 (7.11) 12.58 ND-39.51	0.25 (1.69) ND ND-11.58	0.11 (0.41) 0.66 ND-2.63	28.64 (24) 55.23 6.21–140.55	11.14 (8.91) 23.73 ND-50.81
Chocolate	15	ND	0.41 (0.65) 1.89 ND- 1.98	0.42 (0.96) ND ND-2.78	3.70 (1.24) 5.69 2.27–5.81	3.11 (0.70) 4.23 2.17–4.65	2.00 (0.90) 2.65 ND-2.72
Tea	9	ND	2.61 (0.49) 3.12 2.66–3.37	ND	5.07 (3.80) 8.34 ND-10.08	5.86 (1.18) 6.59 3.66–7.64	18.32 (5.31) 22.84 8.23–23.94

In fermented beverages (e.g., wine and beer) histamine and other biogenic amine contents were much lower than those reported for other fermented foods. However, it must

be noted that the presence of alcohol will enhance the toxic effect of histamine [11,36,37]. In fact, alcohol and its metabolite, acetaldehyde, compete with histamine for the enzyme responsible for their metabolization (aldehyde dehydrogenase), thus, resulting in the accumulation of this amine in the organism [35,38].

Fish and fish derivatives are also usually excluded in low-histamine diets. As shown in Table 1, in most of the fresh fish and derivatives retailed in Spain (i.e., semi-preserved and preserved) no histamines or only in low amounts were detected (P95 below 20 mg/kg). The low occurrence of histamine was also reported by EFSA for this same food category. In fact, only 27% of a total of 6329 European fishery products showed histamine, and generally at low levels [38]. Nevertheless, high histamine concentrations could be achieved in the case of inadequate freshness of raw fish and/or hygienic deficiencies during the manufacturing of fish derivatives. An example is the 111 mg/kg found in fresh salmon and the 657 mg/kg of histamine determined in canned sardines (Table 1). For this reason, low histamine diets usually advise the against the consumption of fish, specifically certain scombroid species (e.g., mackerel, tuna, sardines and anchovy), which are susceptible to histamine accumulation due to their high free histidine contents [25]. Moreover, the action of some of spoilage bacteria derived from the lack of freshness could also entail the formation of other amines, especially putrescine and cadaverine (Table 1). The thermostable nature of biogenic amines implies that thermal treatments applied for the obtention of preserved canned fish do not help diminish their occurrence [5].

As regards fresh, cooked and cured meat, as in fish, the absence of histamine or other biogenic amines is expected, so long as freshness and correct hygienic conditions of the products or manufacturing processes are guaranteed (Table 1). In fact, as can be seen in Figure 1, fresh and cured meat were only excluded by three and two low-histamine diets, respectively. However, the presence of these foods in most low-histamine diets could entail a risk for histamine intolerant individuals if freshness is not guaranteed.

Histamine was also found in some plant-origin products, such as tomato, eggplant and spinach (Figure 2). The origin of low histamine levels in these foods may be physiological, but a high accumulation of this amine has been related to bacterial decarboxylase activity which occurs during storage [11,39]. The study performed by Lavizzari et al., showed a significant increase in histamine concentrations in spinach samples during 15 days of refrigerated storage [39]. The relatively high pH of spinach would allow for the implantation and growth of some Gram-negative bacteria (Enterobacteriaceae and Pseudomonadaceae groups), which would ultimately be responsible of histamine formation [39].

On the other hand, 68% of the not-allowed foods in low-histamine diets did not show significant histamine levels in any of the analyzed samples (Figure 2). Among them, citrus fruits, bananas, soybeans, pumpkins and nuts showed relevant amounts of putrescine (Table 1). In the case of citrus fruits, outstanding levels of putrescine are very often found, with mean levels of 79 mg/kg and maximum levels up to 173 mg/kg in the case of mandarines. In fact, despite the absence of histamine in citrus fruits, 60% of the revised low-histamine diets advised for their exclusion (Figure 1). Paradoxically, there are also certain foods with relevant amounts of putrescine, the exclusion of which is not listed in low-histamine diets [11,36,37]. This is the case for zucchini, peas, green peppers and sweet corn, among others, which could achieve maximum levels of putrescine ranging 25–150 mg/kg, depending on the product.

It has been reported that certain biogenic amines, mainly putrescine and cadaverine, could interfere with histamine degradation by the DAO enzyme at the intestinal level, being responsible for the major absorption and subsequent toxic potential enhancement of histamine. However, there is still scarce experimental evidence supporting this working hypothesis. The studies carried out years ago by Arunlakshana et al. (1954), Mongar (1957) and Hui and Taylor (1985) pointed out this potentially inhibitory effect of other biogenic amines on histamine metabolism both through in vitro assays and in animal models [40–42]. Concretely, Mongar (1957) observed that different aliphatic diamines, such as putrescine

and cadaverine, could potentiate histamine-induced contractions of guinea pig ileum due to the fact that they could competitively inhibit the DAO enzyme [41].

Apart from the foods containing histamine or significant levels of putrescine, there was a wide range (53%) of excluded foods without histamine and with no detected or a low/very low occurrence of putrescine, cadaverine, tyramine, spermidine and/or spermine. Thus far, the available evidence does not help to explain to what extent the presence of low levels of these amines (alone or combined) may interfere with histamine degradation by the DAO enzyme and be responsible for the triggering of the symptoms of histamine intolerance. According to our knowledge, there is only one study on this topic, which was performed back in 1985 by Hui and Taylor, demonstrating the inhibitory effect of putrescine and cadaverine on histamine degradation in rats when these amines were present at levels four to five-fold higher [42]. Curiously, as may be seen in Figure 1, the vast majority of these foods are only excluded in one to three of the low-histamine diets, with the exception of chocolate, strawberry, eggs, pineapple and yogurt.

Moreover, certain foods have been tagged as histamine-liberators, as they could trigger the release of endogenous histamine. The list of foods with suggested histamine-releasing capacity, that may be found in various scientific articles, includes citrus fruits, seafood, papaya, tomato, nuts, pineapple, spinach, chocolate and strawberries, among others [4,5,43]. However, the mechanism responsible for this potential effect has not yet been elucidated. In fact, the only available extensive review as regards the putative histamine-releasing capacity of certain foods performed by Vlieg-Boerstra et al. (2005), clearly stated that there is a lack of evidence supporting this mechanism. There are no clinical studies in humans supporting the widely held belief that foods could have the ability to release histamine and this hypothesis is only based on few and no conclusive in vitro or animal studies [43].

In summary, while the evidence supporting the clinical efficacy of low-histamine diets is progressively growing, there is still a lack of consensus on the foods that must be avoided in the dietary management of histamine intolerance. The critical review performed herein demonstrates that the exclusion of only 32% of foods could be justified by their histamine content. The presence of other biogenic amines could partly help to explain the relationship that some patients have established between the consumption of certain histamine-free foods and the onset of symptoms. However, low-histamine diets continue to require the attention of researchers, both to clarify the specific interaction of other amines in histamine metabolism and to elucidate the potential mechanisms of the so-called histamine-releasing foods.

Author Contributions: Conceptualization, M.T.V.-N., M.L.L.-M., M.C.V.-C.; investigation, S.S.-P., O.C.-B., M.L.L.-M.; writing—original draft preparation, S.S.-P., O.C.-B., M.L.L.-M.; writing—review and editing, S.S.-P., O.C.-B., M.T.V.-N., M.L.L.-M., M.C.V.-C.; supervision, M.C.V.-C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: Sònia Sánchez-Pérez is a recipient of a doctoral fellowship from the University of Barcelona (APIF2018).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Tuck, C.J.; Biesiekierski, J.R.; Schmid-Grendelmeier, P.; Pohl, D. Food Intolerances. *Nutrients* **2019**, *11*, 1684. [CrossRef]
2. Schnedl, W.J.; Lackner, S.; Enko, D.; Schenk, M.; Holasek, S.J.; Mangge, H. Evaluation of Symptoms and Symptom Combinations in Histamine Intolerance. *Intest. Res.* **2019**, *17*, 427–433. [CrossRef]
3. Comas-Basté, O.; Sánchez-Pérez, S.; Veciana-Nogués, M.T.; Latorre-Moratalla, M.; Vidal-Carou, M.C. Histamine Intolerance: The Current State of the Art. *Biomolecules* **2020**, *10*, 1181. [CrossRef]
4. Kovacova-Hanuskova, E.; Buday, T.; Gavliakova, S.; Plevkova, J. Histamine, Histamine Intoxication and Intolerance. *Allergol. Imunopathol.* **2015**, *43*, 498–506. [CrossRef]
5. Maintz, L.; Novak, N. Histamine and Histamine Intolerance. *Am. J. Clin. Nutr.* **2007**, *85*, 1185–1196. [CrossRef]

6. García-Martín, E.; García-Menaya, J.; Sánchez, B.; Martínez, C.; Rosendo, R.; Agúndez, J.A.G. Polymorphisms of Histamine-Metabolizing Enzymes and Clinical Manifestations of Asthma and Allergic Rhinitis. *Clin. Exp. Allergy* **2007**, *37*, 1175–1182. [CrossRef] [PubMed]
7. Enko, D.; Meinitzer, A.; Mangge, H.; Kriegshaüser, G.; Halwachs-Baumann, G.; Reininghaus, E.Z.; Bengesser, S.A.; Schnedl, W.J. Concomitant Prevalence of Low Serum Diamine Oxidase Activity and Carbohydrate Malabsorption. *Can. J. Gastroenterol. Hepatol.* **2016**, *2016*, 1–4. [CrossRef] [PubMed]
8. Sattler, J.; Häfner, D.; Klotter, H.-J.; Lorenz, W.; Wagner, P.K. Food-Induced Histaminosis as an Epidemiological Problem: Plasma Histamine Elevation and Haemodynamic Alterations after Oral Histamine Administration and Blockade of Diamine Oxidase (DAO). *Agents Actions* **1988**, *23*, 361–365. [CrossRef] [PubMed]
9. Leitner, R.; Zoernpfenning, E.; Missbichler, A. Evaluation of the Inhibitory Effect of Various Drugs / Active Ingredients on the Activity of Human Diamine Oxidase in Vitro. *Clin. Transl. Allergy* **2014**, *4*, 23. [CrossRef]
10. Schink, M.; Konturek, P.C.; Tietz, E.; Dieterich, W.; Pinzer, T.C.; Wirtz, S.; Neurath, M.F.; Zopf, Y. Microbial Patterns in Patients with Histamine Intolerance. *J. Physiol. Pharmaco.* **2018**, *69*, 579–593. [CrossRef]
11. Sánchez-Pérez, S.; Comas-Basté, O.; Rabell-González, J.; Veciana-Nogués, M.T.; Latorre-Moratalla, M.L.; Vidal-Carou, M.C. Biogenic Amines in Plant-Origin Foods: Are They Frequently Underestimated in Low-Histamine Diets? *Foods* **2018**, *7*, 205. [CrossRef]
12. San Mauro Martin, I.; Brachero, S.; Garicano Vilar, E. Histamine Intolerance and Dietary Management: A Complete Review. *Allergol. Immunopathol.* **2016**, *44*, 475–483. [CrossRef]
13. Comas-Basté, O.; Latorre-Moratalla, M.L.; Rabell-González, J.; Veciana-Nogués, M.T.; Vidal-Carou, M.C. Lyophilised Legume Sprouts as a Functional Ingredient for Diamine Oxidase Enzyme Supplementation in Histamine Intolerance. *LWT Food Sci. Technol.* **2020**, *125*, 109–201. [CrossRef]
14. Wagner, N.; Dirk, D.; Peveling-Oberhag, A.; Reese, I.; Rady-Pizarro, U.; Mitzel, H.; Staubach, P. A Popular Myth–Low-Histamine Diet Improves Chronic Spontaneous Urticari–Fact or Fiction? *J. Eur. Acad. Dermatol. Venereol.* **2017**, *31*, 650–655. [CrossRef]
15. Worm, M.; Fiedler, E.M.; Dölle, S.; Schink, T.; Hemmer, W.; Jarisch, R.; Zuberbier, T. Exogenous Histamine Aggravates Eczema in a Subgroup of Patients with Atopic Dermatitis. *Acta Derm. Venereol.* **2009**, *89*, 52–56. [CrossRef]
16. Rosell-Camps, A.; Zibetti, S.; Pérez-Esteban, G.; Vila-Vidal, M.; Ferrés-Ramis, L.; García-Teresa-García, E. Histamine Intolerance as a Cause of Chronic Digestive complaints in Pediatric Patients. *Rev. Esp. Enferm. Dig.* **2013**, *105*, 201–207. [CrossRef] [PubMed]
17. Mušič, E.; Korošec, P.; Šilar, M.; Adamič, K.; Košnik, M.; Rijavec, M. Serum Diamine Oxidase Activity as a Diagnostic Test for Histamine Intolerance. *Wien. Klin. Wochenschr.* **2013**, *125*, 239–243. [CrossRef] [PubMed]
18. Steinbrecher, I.; Jarisch, R. Histamin Und Kopfschmerz. *Allergologie* **2005**, *28*, 85–91. [CrossRef]
19. Siebenhaar, F.; Melde, A.; Magerl, M.; Zuberbier, T.; Church, M.K.; Maurer, M. Histamine Intolerance in Patients with Chronic Spontaneous Urticaria. *J. Eur. Acad. Dermatol. Venereol.* **2016**, *30*, 1774–1777. [CrossRef]
20. Wantke, F.; Gotz, M.; Jarisch, R. Histamine-Free Diet: Treatment of Choice for Histamine-Induced Food Intolerance and Supporting Treatment for Chronical Headaches. *Clin. Exp. Allergy* **1993**, *23*, 982–985. [CrossRef] [PubMed]
21. Lackner, S.; Malcher, V.; Enko, D.; Mangge, H.; Holasek, S.J.; Schnedl, W.J. Histamine-Reduced Diet and Increase of Serum Diamine Oxidase Correlating to Diet Compliance in Histamine Intolerance. *Eur. J. Clin. Nutr.* **2019**, *73*, 102–104. [CrossRef]
22. Guida, B.; de Martino, C.; de Martino, S.; Tritto, G.; Patella, V.; Trio, R.; D'agostino, C.; Pecoraro, P.; Agostino, L.D. Histamine Plasma Levels and Elimination Diet in Chronic Idiopathic Urticaria. *Eur. J. Clin. Nutr.* **2000**, *54*, 155–158. [CrossRef] [PubMed]
23. Son, J.H.; Chung, B.Y.; Kim, H.O.; Park, C.W. A Histamine-Free Diet Is Helpful for Treatment of Adult Patients with Chronic Spontaneous Urticaria. *Ann. Dermatol.* **2018**, *30*, 164–172. [CrossRef] [PubMed]
24. Latorre-Moratalla, M.L.; Comas-Basté, O.; Bover-Cid, S.; Vidal-Carou, M.C. Tyramine and Histamine Risk Assessment Related to Consumption of Dry Fermented Sausages by the Spanish Population. *Food Chem. Toxicol.* **2017**, *99*, 78–85. [CrossRef] [PubMed]
25. Comas-Basté, O.; Latorre-Moratalla, M.L.; Sánchez-Pérez, S.; Veciana-Nogués, M.T.; Vidal-Carou, M.C. Histamine and Other Biogenic Amines in Food. From Scombroid Poisoning to Histamine Intolerance. In *Biogenic Amines*; Proestos, C., Ed.; IntechOpen: London, UK, 2019.
26. Joneja, J.M.; Carmona-Silva, C. Outcome of a Histamine-Restricted Diet Based on Chart Audit. *J. Nutr. Environ. Med.* **2001**, *11*, 249–262. [CrossRef]
27. Lehane, L.; Olley, J. Histamine Fish Poisoning Revisited. *Int. J. Food Microbiol.* **2000**, *58*, 1–37. [CrossRef]
28. Veciana-Nogués, M.T.; Vidal-Carou, M.C. Dieta baja en histamina. In *Dieta y Nutrición Clínica*; Salas-Salvadó, J., Bonada Sanjaume, A., Trallero Casaña, R., Trallero Casaña, R., Burgos Peláez, R., Eds.; Elsevier: Barcelona, Spain, 2008; pp. 443–448.
29. Bover-Cid, S.; Latorre-Moratalla, M.L.; Veciana-Nogués, M.T.; Vidal-Carou, M.C. Processing Contaminants: Biogenic Amines. In *Encyclopedia of Food Safety*; Motarjemi, Y., Moy, G.G., Todd, E.C.D., Eds.; Elsevier Inc.: Waltham, MA, USA, 2014; Volume 2, pp. 381–391. [CrossRef]
30. King, W.; McCargar, L.; Joneja, J.M.; Barr, S.I. Benefits of a Histamine-Reducing Diet for Some Patients with Chronic Urticaria and Angioedema. *Can. J. Diet. Pract. Res.* **2000**, *61*, 24–26. [PubMed]
31. Maintz, L.; Benfadal, S.; Allam, J.P.; Hagemann, T.; Fimmers, R.; Novak, N. Evidence for a Reduced Histamine Degradation Capacity in a Subgroup of Patients with Atopic Eczema. *J. Allergy Clin. Immunol.* **2006**, *117*, 1106–1112. [CrossRef]

32. Böhn, L.; Störsrud, S.; Törnblom, H.; Bengtsson, U.; Simrén, M. Self-Reported Food-Related Gastrointestinal Symptoms in IBS Are Common and Associated with More Severe Symptoms and Reduced Quality of Life. *Am. J. Gastroenterol.* **2013**, *108*, 634–641. [CrossRef] [PubMed]
33. Lefèvre, S.; Astier, C.; Kanny, G. Intolérance à l'histamine Ou Fausses Allergies Alimentaires de Mécanisme Histaminique. *Rev. Fr. Allergol.* **2017**, *57*, 24–34. [CrossRef]
34. Naila, A.; Flint, S.; Fletcher, G.; Bremer, P.; Meerdink, G. Control of Biogenic Amines in Food–Existing and Emerging Approaches. *J. Food Sci.* **2010**, *75*, 139–150. [CrossRef]
35. Vidal-Carou, M.C.; Veciana-Nogués, M.T.; Latorre-Moratalla, M.L.; Bover-Cid, S. Biogenic amines: Risks and control. In *HandBook of Fermented Meat and Poultry*; Toldrá, F., Hui, Y., Astiasarán, I., Sebranek, J., Talon, R., Eds.; John Wiley & Sons, Ltd.: Oxford, UK, 2015; pp. 413–428.
36. Kalač, P.; Švecová, S.; Pelikánová, T. Levels of Biogenic Amines in Typical Vegetable Products. *Food Chem.* **2002**, *77*, 349–351. [CrossRef]
37. Moret, S.; Smela, D.; Populin, T.; Conte, L.S. A Survey on Free Biogenic Amine Content of Fresh and Preserved Vegetables. *Food Chem.* **2005**, *89*, 355–361. [CrossRef]
38. EFSA Panel on Biological Hazards (BIOHAZ). Scientific Opinion on Risk Based Control of Biogenic Amine Formation in Fermented Foods. *EFSA J.* **2011**, *9*, 1–93. [CrossRef]
39. Lavizzari, T.; Veciana-Nogués, M.T.; Weingart, O.; Bover-Cid, S.; Mariné-Font, A.; Vidal-Carou, M.C. Occurrence of Biogenic Amines and Polyamines in Spinach and Changes during Storage under Refrigeration. *J. Agric. Food Chem.* **2007**, *55*, 9514–9519. [CrossRef]
40. Arunlakshana, O.; Mongar, J.L.; Schild, H.O. Potentiation of Pharmacological Effects of Histamine by Histaminase Inhibitors. *J. Physiol.* **1954**, *123*, 32–54. [CrossRef] [PubMed]
41. Mongar, J.L. Effect of Chain Length of Aliphatic Amines on Histamine Potentiation and Release. *Br. J. Pharmacol.* **1957**, *12*, 140–148. [CrossRef]
42. Hui, J.Y.; Taylor, S.L. Inhibition of in Vivo Histamine Metabolism in Rats by Foodborne and Pharmacologic Inhibitors of Diamine Oxidase, Histamine N-Methyltransferase, and Monoamine Oxidase. *Toxicol. Appl. Pharmacol.* **1985**, *81*, 241–249. [CrossRef]
43. Vlieg-Boerstra, B.J.; van der Heide, S.; Oude Elberink, J.N.G.; Kluin-Nelemans, J.C.; Dubois, A.E.J. Mastocytosis and Adverse Reactions to Biogenic Amines and Histamine-Releasing Foods: What Is the Evidence? *Neth. J. Med.* **2005**, *63*, 244–249.

3.3. Influència d'altres amines biògenes en la degradació de la histamina per part de l'enzim DAO

PUBLICACIÓ 4

The rate of histamine degradation by diamine oxidase is compromised by other biogenic amines.

Sònia Sánchez-Pérez, Oriol Comas-Basté, Judit Costa-Catala, Irache Iduriaga-Platero, M. Teresa Veciana-Nogués, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou. *Frontiers in Nutrition*, 2022, 9, 897028.

Índex d'impacte (JCR 2021): 6,590
Posició en l'àrea "Nutrition & Dietetics": Q1 (16/90)

COMUNICACIÓ ORAL 1 | Premi a la millor comunicació oral

Sònia Sánchez-Pérez, Oriol Comas-Basté, Judit Costa-Catala, M. Teresa Veciana-Nogués, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou. **Dietary diamines significantly reduce the degradation rate of histamine by DAO enzyme.** *VI Workshop de l'Institut de Recerca en Nutrició i Seguretat Alimentària (INSA),* 2022, Barcelona.

COMUNICACIÓ ORAL 2

Sònia Sánchez-Pérez, Oriol Comas-Basté, Judit Rabell-González, M. Teresa Veciana-Nogués, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou. **Incertidumbres de la intoxicación histamínica.** *14ª Reunión anual de la Sociedad Española de Seguridad y Calidad Alimentaria (SESAL)*, 2018, Salamanca.

COMUNICACIÓ ESCRITA 5

Sònia Sánchez-Pérez, Giulietta Solari, Max Carreras-Leal de Ibarra, Judit Costa-Catala, Oriol Comas-Basté, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou. **Dietes bajas en histamina: el porqué de la exclusión de algunos alimentos sin histamina.** *XIX Congreso de la Sociedad Latinoamericana de Nutrición (SLAN)*, 2021, virtual, Paraguai.

PLANTEJAMENT I OBJECTIU

Segons la revisió crítica de les dietes baixes en histamina de l'apartat anterior, alguns aliments sense histamina però que contenen alts nivells d'altres amines biògenes, com per exemple putrescina, podrien ser també responsables de l'aparició de símptomes clínics en individus intolerants a la histamina. Així, sembla factible que la presència d'altres amines, especialment les diamines, puguin influir en la degradació de la histamina a nivell intestinal per competència amb l'enzim DAO. Tot i això, les dades experimentals disponibles sobre l'efecte d'aquestes altres amines en la degradació de la histamina procedeixen d'escassos estudis realitzats a finals del segle XX [81–84]. Per tant, l'objectiu d'aquest estudi va ser avaluar *in vitro* si la presència de diferents amines pot influir en la degradació de la histamina per part de l'enzim DAO.

METODOLOGIA

En primer lloc, es va estudiar l'activitat catabòlica *in vitro* de l'enzim DAO d'origen porcí en sis substrats amino diferents: histamina, putrescina, cadaverina, tiramina, espermidina i espermina, cadascuna a una concentració de 25 mg/L. Seguidament, es van realitzar diferents assajos per avaluar la velocitat de degradació d'una concentració coneguda d'histamina (25 mg/L) en presència de diferents quantitats d'aquests altres substrat amino. Les proporcions assajades van ser les següents (histamina:substrat amino) 1:0,25; 1:1; 1:4; 1:20. Addicionalment, també es van realitzar assajos considerant una combinació de putrescina i cadaverina, a les mateixes proporcions abans mencionades.

L'activitat DAO *in vitro* es va determinar segons la metodologia prèviament descrita per Comas-Basté i col. (2019), basada en la determinació de la taxa de degradació dels diferents substrats amino per cromatografia líquida d'alta eficàcia i detecció fluorimètrica (UHPLC-FL).

RESULTATS

Es va confirmar que totes les amines assajades eren substrat de l'enzim DAO. La histamina va ser el substrat que es va degradar a una major velocitat (28,16 ± 1,00 mU), essent significativament diferent a l'observada per les altres amines ($p<0,001$). L'activitat DAO per la putrescina i la cadaverina va ser aproximadament un 50% més baixa que l'observada per la histamina i molt menor en el cas de la tiramina, l'espermidina i l'espermina.

Quant a la interferència d'altres amines en la degradació de la histamina per la DAO, la presència de putrescina o cadaverina va reduir significativament l'activitat enzimàtica a totes les proporcions estudiades ($p<0,05$). El major efecte es va observar quan la putrescina o la cadaverina es trobaven a concentracions 20 vegades superiors a la de la histamina, reduint-se aquesta velocitat fins a un 70% i un 80%, respectivament. Segons l'estudi cinètic, quan la histamina es trobava sola al medi, als 90 minuts d'assaig la histamina va ser totalment degradada, mentre que a aquest mateix temps, la histamina degradada en presència d'alts nivells de putrescina i/o cadaverina va ser 5 vegades menor. Cal destacar que la presència concomitant de les dues diamines al medi de reacció no va provocar cap efecte addicional sobre la degradació de la histamina.

En el cas de la tiramina, l'espermidina i l'espermina només concentracions molt elevades reduirien significativament la velocitat de degradació de la histamina, observant-se percentatges de reducció d'entre un 32% i un 45%, en funció de l'amina present.

TAKE-HOME MESSAGE

La presència de putrescina, cadaverina, tiramina, espermidina i espermina provoquen una reducció de la velocitat de degradació *in vitro* de la histamina per l'enzim DAO. Aquesta inhibició depèn tant del substrat amino com de la seva concentració.

frontiers | Frontiers in Nutrition

ORIGINAL RESEARCH
published: 25 May 2022
doi: 10.3389/fnut.2022.897028

The Rate of Histamine Degradation by Diamine Oxidase Is Compromised by Other Biogenic Amines

Sònia Sánchez-Pérez[1,2,3†], Oriol Comas-Basté[1,2,3†], Judit Costa-Catala[1,2,3], Irache Iduriaga-Platero[1,2,3], M. Teresa Veciana-Nogués[1,2,3], M. Carmen Vidal-Carou[1,2,3‡] and M. Luz Latorre-Moratalla[1,2,3‡]*

[1] Departament de Nutrició, Ciències de l'Alimentació i Gastronomia, Facultat de Farmàcia i Ciències de l'Alimentació, Campus de l'Alimentació de Torribera, Universitat de Barcelona (UB), Santa Coloma de Gramenet, Spain, [2] Institut de Recerca en Nutrició i Seguretat Alimentària (INSA·UB), Universitat de Barcelona (UB), Santa Coloma de Gramenet, Spain, [3] Xarxa d'Innovació Alimentària (XIA), Barcelona, Spain

OPEN ACCESS

Edited by:
Jun-Hu Cheng,
South China University of Technology, China

Reviewed by:
Giulia Tabanelli,
University of Bologna, Italy
Chiara Montanari,
University of Bologna, Italy

****Correspondence:***
M. Luz Latorre-Moratalla
mariluzlatorre@ub.edu

[†] These authors have contributed equally to this work and share first authorship

[‡] These authors have contributed equally to this work and share last authorship

Specialty section:
This article was submitted to Nutrition and Food Science Technology, a section of the journal Frontiers in Nutrition

Received: *15 March 2022*
Accepted: *27 April 2022*
Published: *25 May 2022*

Citation:
Sánchez-Pérez S, Comas-Basté O, Costa-Catala J, Iduriaga-Platero I, Veciana-Nogués MT, Vidal-Carou MC and Latorre-Moratalla ML (2022) The Rate of Histamine Degradation by Diamine Oxidase Is Compromised by Other Biogenic Amines. Front. Nutr. 9:897028. doi: 10.3389/fnut.2022.897028

Nowadays, certain uncertainties related to the onset of histamine adverse effects remain unsolved and still require further research. Questions still to be resolved include the wide range of doses at which dietary histamine may trigger symptoms of intoxication (100–10,000 mg/kg) or the appearance of symptoms of histamine intolerance after the consumption of foods presumable without histamine. It seems feasible that other amines, by acting as competitive substrates, could interfere with histamine degradation by the intestinal enzyme diamine oxidase (DAO). Therefore, the aim of this study was to elucidate the interference of different amines on the rate of histamine degradation by DAO. A series of *in vitro* enzymatic assays were performed using histamine as the reaction substrate combined with different proportions of putrescine, cadaverine, tyramine, spermidine, and spermine (1:0.25, 1:1, 1:4, 1:20). Putrescine and cadaverine significantly delayed histamine degradation at all tested concentrations ($p < 0.001$). The greatest effect was observed when putrescine or cadaverine concentrations were 20-fold higher than that of histamine, its degradation being reduced by 70 and 80%, respectively, compared to histamine alone (28.16 ± 1.0 mU). In contrast, tyramine, spermidine and spermine significantly inhibited the histamine degradation rate only at the highest concentration (1:20), reducing it by 32–45%. These results demonstrate that other biogenic amines interfere with histamine metabolization by DAO *in vitro*, the extent depending on the substrate. These findings could explain why susceptibility to dietary histamine is so variable and account for the discrepancies in the scientific databases regarding the amount of histamine that triggers adverse health effects.

Keywords: histamine, putrescine, cadaverine, biogenic amines, diamine oxidase (DAO), histamine toxicity, histamine intolerance, histamine degradation

INTRODUCTION

According to data from the European Food Safety Authority (EFSA) and the European Center for Disease Prevention and Control (ECDC), histamine is one of the main causal agents of adverse health effects associated with food consumption (1). Histamine intoxication occurs after the ingestion of foods with unusually high amounts of histamine, which overwhelm the intestinal enzymatic capacity for its degradation (2–4). In the last decades, numerous studies have been

carried out in this field, but certain uncertainties remain to be clarified, such as why symptoms of intoxication are triggered by extremely variable amounts of histamine in the ingested foods, the reported values ranging from 100 to 10,000 mg/kg (5–11).

The possibility that individuals differ in their histamine-metabolizing capacity has attracted interest in recent years, as it could account for the variable reactions to dietary histamine (2, 3, 12–14). Deficient activity of the intestinal enzyme diamine oxidase (DAO) can explain why some individuals are sensitive to doses of histamine that are safe for the healthy population, the severity of symptoms being correlated with the degree of deficit (12, 13, 15). The inability (or impaired ability) to degrade histamine from food is a clinical disorder known as histamine intolerance. Histamine intolerance, beyond helping to resolve the aforementioned uncertainties, includes a wide range of non-specific gastrointestinal and extra-intestinal symptoms (dermatological, respiratory, neurological, and hemodynamic), due to the ubiquitous distribution of the four histamine receptors in organs and tissues (4, 15, 16). Despite the similarity of certain symptoms, histamine intolerance differs from allergy in that no immune mechanism is involved. DAO deficiency may have a genetic, pathological, or pharmacological etiology. Another explanation, proposed recently, is that it is caused by dysbiosis of the intestinal microbiota, although this hypothesis still needs further study (17–21).

On the other hand, there is evidence that the copresence of other biogenic amines could enhance the adverse effects of histamine (14, 22–24). For example, dose-response studies have found that pure histamine triggers symptoms of intoxication at far higher doses than the histamine ingested from spoiled fish, which may contain other amines, such as putrescine and cadaverine (2, 14, 22). Moreover, according to a recent critical review of different low-histamine diets, certain foods without histamine but containing high levels of other biogenic amines could be responsible of the onset of clinical symptoms in histamine-intolerant individuals (16). Thus, it seems feasible that these other biogenic amines, especially diamines, could compete for deamination by DAO in the intestines, and thus impede histamine degradation, with more noticeable effects in susceptible individuals. However, the available experimental data about the effect of other biogenic amines on histamine degradation are scarce and outdated (25–28). Therefore, the aim of the present study was to elucidate if different amines interfere with the degradation rate of histamine by DAO. For this purpose, a series of *in vitro* enzymatic assays were performed using histamine as the reaction substrate combined with different proportions of putrescine, cadaverine, tyramine, spermidine, and spermine.

MATERIALS AND METHODS

Reagents and Chemicals

Histamine dihydrochloride, putrescine dihydrochloride, cadaverine dihydrochloride, tyramine hydrochloride, spermidine trihydrochloride and spermine tetrahydrochloride, and purified DAO from porcine kidney were purchased from Sigma-Aldrich (St. Louis, MO, United States). UHPLC-grade methanol and acetonitrile, hydrochloric acid 0.1 M, perchloric acid 70%, sodium di-hydrogen phosphate anhydrous, and di-sodium hydrogen phosphate anhydrous were obtained from PanReac Química (Castellar del Vallès, Spain). Acetic acid, boric acid, 1-octanesulfonic acid sodium salt, ammonium formate, phthaldialdehyde (OPA), and brij® L23 solution were acquired from Sigma-Aldrich (St. Louis, MO, United States), and formic acid, sodium acetate anhydrous, potassium hydroxide and 2-mercaptoethanol from Merck (Darmstadt, Germany). A LaboStar System from Evoqua Water Technologies (Warrendale, PA, United States) was used to produce ultrapure water (18.2 MΩ cm).

Experimental Design

The catabolic activity of porcine DAO was tested on six different amino substrates: histamine, putrescine, cadaverine, tyramine, spermidine and spermine, each at a concentration of 25 mg/L. Additionally, several assays were performed to test the degradation rate of a specific concentration of histamine (25 mg/L) by DAO in the presence of different amounts of the other individual amino substrates. Thus, the resulting substrate proportions assayed in this study were the following (histamine:amino substrate): 1:0.25, 1:1, 1:4, and 1:20. Moreover, additional assays considering a mixture of putrescine and cadaverine were also performed at the same four substrate proportions (the proportion refers to the sum of both diamines in equivalent amounts). Three independent experiments were performed in duplicate for each condition.

In vitro Determination of Diamine Oxidase Activity

DAO activity *in vitro* was determined following the procedure previously described by Comas-Basté et al. (29). This method is based on the measurement of the degradation rate of amino substrates during the oxidative deamination reaction catalyzed by DAO. Briefly, purified DAO (0.14 U/mL) was homogenized in 20 mL of 0.05 M phosphate buffer solution (pH 7.2) and placed in a shaker incubator (Ivymen 100-D, JP SELECTA S.A., Abrera, Spain). The addition of a standard solution of the corresponding amino substrate to the working mixture marked the start of the enzymatic reaction. The mixture was kept at 37°C under constant stirring (200 rpm). Aliquots of 500 μL were progressively subtracted every 15 min until 180 min of the assay or until the complete disappearance of the substrate. To stop the enzymatic reaction, 15 μL of 2N perchloric acid solution was added to the working mixture and centrifuged at 27,216 × g for 5 min. The supernatant was filtered through a 0.22 μm GHP filter and stored at 4°C until UHPLC-FL analysis. The aliquots underwent chromatographic analysis to monitor the degradation rate of the amino substrates and calculate DAO activity, which was expressed as nmol of degraded amine per minute (mU).

Statistical Analysis

Statistical analysis of data was performed using IBM SPSS Statistics 23.0 software package (IBM Corporation, Armonk,

NY, United States). One-way ANOVA followed by a Tukey's *post hoc* test was applied to investigate the statistical significance of changes in the enzymatic activity in the different studied conditions. Differences with $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Degradation Rate of Different Amino Substrates by Diamine Oxidase

All the assayed amino compounds acted as substrates of the enzyme DAO of porcine origin (**Table 1**). The highest rate of degradation by DAO was observed in histamine (28.16 ± 1.00 mU), with significant differences compared to the other tested amines ($p < 0.001$). DAO activity was approximately 50% lower on the diamines putrescine and cadaverine than on histamine, whereas on tyramine, spermidine and spermine it was considerably lower than on histamine, putrescine or cadaverine. These results agree with those of Ignesti et al. who demonstrated that porcine kidney DAO had a higher affinity for histamine than other assayed amines; it was 9 and 12-fold lower for putrescine and cadaverine, respectively, and almost 300-fold lower for spermidine (30).

The affinity of DAO seems to be influenced by the chemical nature of each compound, especially the molecule length and presence of aliphatic or aromatic moieties (31). According to Blaschko et al. (32), during the enzymatic oxidation of a diamine, the amino substrate is anchored to the enzyme through the two amino groups, forming an intermediate ring compound. It has been suggested that the longer the chain separating the two amino

TABLE 1 | Enzymatic activity (mU) of porcine diamine oxidase (DAO) (mean ± *SD*) on different amino substrates.

Substrates			DAO activity (mU)
Common name	**Chemical name**	**Chemical structure**	
Histamine	2-(1H-imidazol-4-yl) ethanamine		28.16 ± 1.00^{a}
Putrescine	Butane-1,4-diamine		14.11 ± 0.99^{b}
Cadaverine	Pentane-1,5-diamine		16.20 ± 1.20^{b}
Tyramine	4-(2-Aminoethyl) phenol		1.77 ± 0.61^{c}
Spermidine	N1-(3-Aminopropyl) butane-1,4-diamine		0.65 ± 0.24^{c}
Spermine	N1,N4-Bis (3-aminopropyl) butane-1,4-diamine		0.58 ± 0.10^{c}

Different letters denote statistically significant differences ($p < 0.001$).

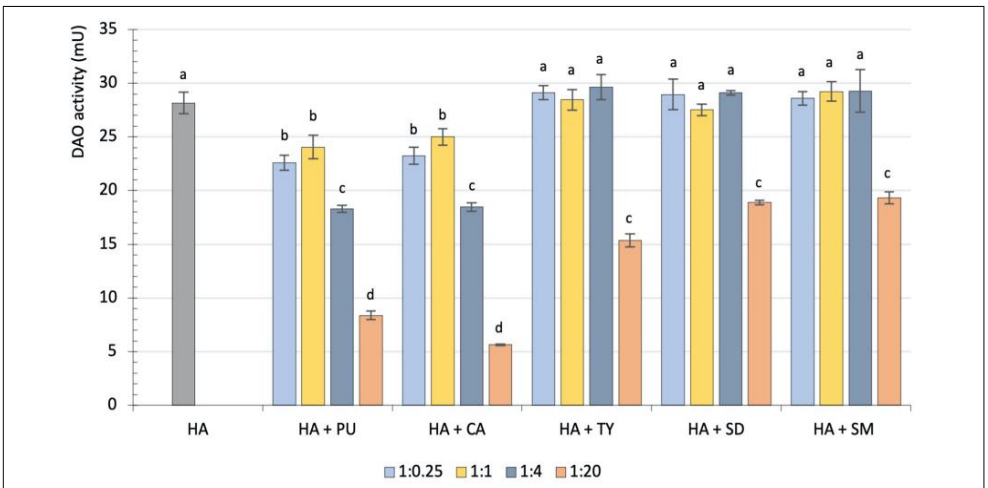


FIGURE 1 | Comparison of diamine oxidase (DAO) activity (mU) on histamine by itself (gray) and on histamine together with different proportions of putrescine, cadaverine, tyramine, spermidine or spermine: 1:0.25 (light blue), 1:1 (yellow), 1:4 (dark blue) and 1:20 (orange). Different letters denote statistically significant differences ($p < 0.05$). HA, Histamine; PU, Putrescine; CA, Cadaverine; TY, Tyramine; SD, Spermidine; SM, Spermine.

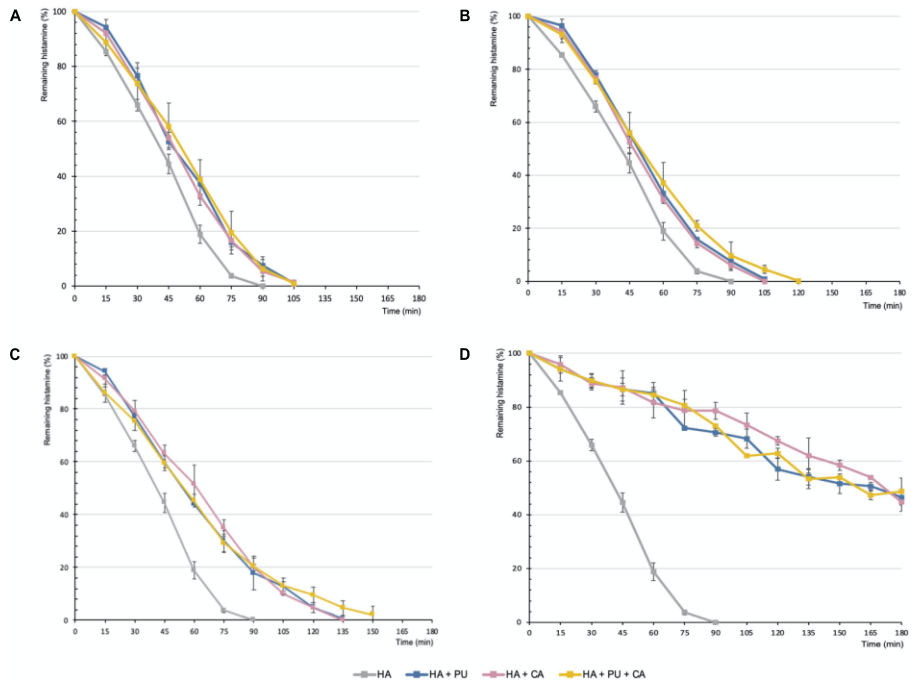


FIGURE 2 | Degradation of histamine (25 mg/L) by itself or with different amounts of putrescine, cadaverine or a mixture of both diamines (sum of equivalent amounts of putrescine and cadaverine). The resulting substrate proportions assayed were the following (histamine:amino substrate): 1:0.25 **(A)**, 1:1 **(B)**, 1:4 **(C)**, and 1:20 **(D)**. HA, Histamine; PU, Putrescine; CA, Cadaverine.

groups, the weaker the bond of the molecule with DAO of animal origin (32).

Substrate preference also seems to depend on the origin of DAO. When extracted from mammals, such as pig, rat or human (30, 33, 34), DAO is reported to have a greater affinity for histamine than for other amines, whereas DAO of plant origin prefers putrescine and cadaverine. Pietrangeli et al. found that DAO from red pea (*Lathyrus cicera* L.) degraded histamine at a slightly lower rate than putrescine and cadaverine (31).

Interference of Other Amines With Histamine Degradation by Diamine Oxidase

Having demonstrated that other amines frequently found in foods are also substrates of DAO, a quantitative assessment was performed to determine the extent of their interference with DAO degradation of histamine. The competition of these rival substrates for deamination in the intestine would lead to an increase of histamine in plasma, with potentially toxic effects.

The influence of different amounts of putrescine, cadaverine, tyramine, spermidine and spermine on the rate of histamine degradation by porcine kidney DAO is shown in **Figure 1**. When histamine was assayed together with putrescine or cadaverine, its degradation rate was significantly reduced, regardless of the added amount of these other diamines ($p < 0.05$). When the proportions of putrescine or cadaverine were fourfold higher than histamine, the reduction was approximately 35%, and when 20-fold higher, it increased significantly to 70 and 80%, respectively ($p < 0.001$). Regarding tyramine, spermidine and spermine, no inhibitory effect was observed when these amino substrates were present in concentrations lower than, equal to or fourfold higher than histamine (**Figure 1**). Only high amounts of tyramine, spermidine or spermine (1:20) significantly inhibited the histamine degradation rate, reducing it by 32–45%. These results confirm that the interference of other biogenic amines with histamine degradation by DAO is directly related to their specific enzymatic activity.

To date, only a few studies carried out in the 1950s and 1980s have assessed the potential influence of competing amino substrates on histamine metabolization by DAO, examining the physiological effects provoked by histamine in animal models (25–28). For example, in 1954 Arunlakshana et al. reported that cadaverine potentiated the contractile effects of histamine

in the ileum, trachea and uterus in guinea pigs (26), whereas other compounds added that were not DAO substrates had no impact on histamine metabolization. Lyons et al. reported a 45% increase in histamine absorption in rat intestinal segments when coperfused with cadaverine, accompanied by a 19.6% reduction of imidiazoleacetic acid, the product generated by DAO activity on histamine (28). A few years later, Hui and Taylor similarly reported an increase of unmetabolized histamine in the urinary excretion of rats when putrescine and/or cadaverine were also administered (27), whereas tyramine had no significant effect. Therefore, the current findings confirm these previously reported results, although to the best of our knowledge, the present study is the first to demonstrate the inhibitory effect of other amines (mainly putrescine and cadaverine) on histamine degradation by DAO by directly measuring the enzymatic activity *in vitro*.

A kinetic study of histamine degradation in the presence of different amounts of putrescine, cadaverine, or a mixture of both diamines (sum of equivalent amounts of putrescine and cadaverine) was performed (**Figure 2**), as these were the diamines that most compromised the degradation rate of histamine by DAO. Moreover, both substrates are found in food and their formation is frequently linked to the same type of bacteria as histamine.

As can be seen in **Figure 2**, putrescine and cadaverine had similar inhibitory effects throughout the oxidative deamination of histamine by DAO ($p > 0.05$). Moreover, the concomitant presence of both diamines in the reaction mixture had no significant additional effect ($p > 0.05$). These results can be attributed to both substrates having very similar affinity values for DAO (30).

When histamine by itself (25 mg/L) was exposed to DAO enzyme, it disappeared completely after 90 min (**Figure 2**). In contrast, in the presence of increasing proportions of putrescine and/or cadaverine, the percentage of histamine remaining at 90 min also increased. Thus, 10% of undegraded histamine was found when assayed with lower or equal amounts of other diamines (**Figures 2A,B**). This amount increased to 20% when the proportion of putrescine and/or cadaverine was fourfold higher than that of histamine (**Figure 2C**). Notably, when these diamines were added in the highest proportion (1:20, taking into account the sum of both diamines), histamine degradation was strongly delayed and almost 80% remained at 90 min (**Figure 2D**). Correspondingly, Mongar reported that the capacity of cadaverine to potentiate the effects of histamine progressively increased with concentration (25). On the other hand, in 1985, Hui and Taylor observed that putrescine or cadaverine had to be administered at levels 4–5-fold higher than histamine to inhibit its degradation by DAO (27), and had no inhibitory effect at an equal dose.

Overall, these results demonstrate that other biogenic amines interfere with histamine degradation by DAO, the extent varying according to the substrate. These findings could help explain the variable susceptibility to dietary histamine and the discrepancies in the literature and scientific databases regarding the amount of histamine responsible for triggering adverse health effects. In fact, certain fermented products (i.e., dry-fermented sausages, cheeses, and plant-fermented products) and semi-preserved fish derivatives frequently contain higher levels of putrescine and/or cadaverine than histamine (16, 35). For example, 71% of samples of dry-fermented sausages and 55% of ripened cheeses were found to have concentrations of putrescine and/or cadaverine at least fourfold higher than histamine. In fermented foods, high amounts of biogenic amines are related to poor hygienic conditions of the raw materials and/or the manufacturing processes, as well as to the aminogenic capacity of the fermentative bacteria (4). In the case of fresh fish, the food category more frequently associated with histamine intoxication outbreaks (In 2020, 100% of these outbreaks occurred at European level were caused by the consumption of fish according to data provided in the joint Zoonoses Report issued by EFSA and ECDC), the occurrence of other biogenic amines is less common than in fermented foods (only 28% of samples had concentrations of putrescine and/or cadaverine at least fourfold higher than histamine) (36). The larger serving size of fish products, together with the extremely high histamine content arising from hygienic defects in their manufacturing or conservation processes, could explain their stronger association with histamine intoxication outbreaks in comparison with fermented products (that often also contain other diamines together with histamine). Furthermore, it is also important to highlight that, in the context of the whole diet, many histamine-free food items are a potential source of significant amounts of other diamines, which could interfere with the intestinal degradation of histamine and enhance its toxicity.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

MV-N, MV-C, and ML-M: conceptualization. SS-P, OC-B, JC-C, and II-P: investigation. SS-P, OC-B, and ML-M: writing—original draft preparation. SS-P, OC-B, JC-C, II-P, MV-N, MV-C, and ML-M: writing—review and editing. MV-C and ML-M: supervision. All authors have read and agreed to the published version of the manuscript.

FUNDING

This research was funded by Grant PID2020-117670RB-C21 funded by MICIN/AEI/10.13039/501100011033 and "ERDF A way of making Europe."

ACKNOWLEDGMENTS

SS-P was a recipient of a doctoral fellowship from the University of Barcelona (APIF2018).

REFERENCES

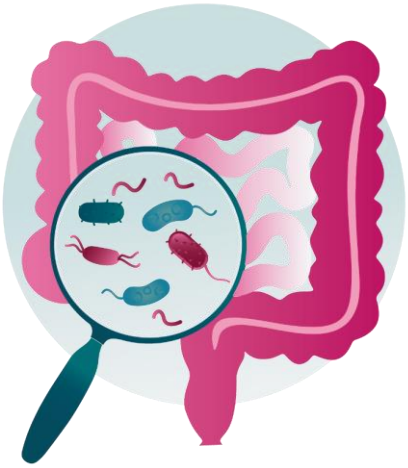
1. European Food Safety Authority [EFSA], European Centre for Disease Prevention and Control [ECDC]. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. *EFSA J.* (2018) 15:5077. doi: 10.2903/j.efsa.2017.5077
2. Food and Agriculture Organization of the United Nations [FAO], World Health Organization [WHO]. *Public Health Risks of Histamine and Other Biogenic Amines from Fish and Fishery Products. Meeting Report*. Rome: Food and Agriculture Organization (2013).
3. EFSA Panel on Biological Hazard [BIOHAZ]. Scientific opinion on risk based control of biogenic amine formation in fermented foods. *EFSA J.* (2011) 9:2393. doi: 10.2903/j.efsa.2011.2393
4. Comas-Basté O, Latorre-Moratalla ML, Sánchez-Pérez S, Veciana-Nogués MT, Vidal-Carou MC. Histamine and other biogenic amines in food. From scombroid poisoning to histamine intolerance. In: Proestos C editor. *Biogenic Amines*. London: IntechOpen (2019).
5. Colombo FM, Cattaneo P, Confalonieri E, Bernardi C. Histamine food poisonings: a systematic review and meta-analysis. *Crit Rev Food Sci Nutr.* (2018) 58:1131–51. doi: 10.1080/10408398.2016.1242476
6. Lee YC, Kung HF, Wu CH, Hsu HM, Chen HC, Huang TC, et al. Determination of histamine in milkfish stick implicated in food-borne poisoning. *J Food Drug Anal.* (2016) 24:63–71. doi: 10.1016/j.jfda.2015.06.009
7. Petrovic J, Babić J, Jaksic S, Kartalovic B, Ljubojevic D, Cirkovic M. Fish product-borne histamine intoxication outbreak and survey of imported fish and fish products in Serbia. *J Food Prot.* (2016) 79:90–4. doi: 10.4315/0362-028X.JFP-15-190
8. Fariñas-Cabrero MA, Berbel-Hernández C, Allué-Tango M, Díez-Hillera M, Herrero-Marcos JA. Brote Epidémico Por Consumo de Pez Mantequilla: kerriorea e Intoxicación Histamínica. *Rev Esp Salud Pública.* (2015) 89:99–105. doi: 10.4321/s1135-57272015000100011
9. Tortorella V, Masciari P, Pezzi M, Mola A, Tiburzi SP, Zinzi MC, et al. Histamine poisoning from ingestion of fish or scombroid syndrome. *J Emerg Med Case Rep.* (2014) 2014:482531. doi: 10.1155/2014/482531
10. Demoncheaux JP, Michel R, Mazenot C, Duflos G, Iacini C, Delaval F, et al. A large outbreak of scombroid fish poisoning associated with eating yellowfin Tuna (Thunnus Albacares) at a military mass catering in Dakar, Senegal. *Epidemiol Infect.* (2012) 140:1008–12. doi: 10.1017/S0950268811001701
11. Dalgaard P, Emborg J, Kjølby A, Sørensen ND, Ballin NZ. Histamine and biogenic amines: formation and importance in seafood. In: Børresen T editor. *Improving Seafood Products for the Consumer*. Cambridge: British Welding Research Association (2008). p. 292–324. doi: 10.1533/9781845694586.3.292
12. Kovacova-Hanuskova E, Buday T, Gavliakova S, Plevkova J. Histamine, histamine intoxication and intolerance. *Allergol Immunopathol.* (2015) 43:498–506. doi: 10.1016/j.aller.2015.05.001
13. Maintz L, Novak N. Histamine and histamine intolerance. *Am J Clin Nutr.* (2007) 85:1185–96. doi: 10.1093/ajcn/85.5.1185
14. Lehane L, Olley J. Histamine fish poisoning revisited. *Int J Food Microbiol.* (2000) 5:1–37. doi: 10.1016/s0168-1605(00)00296-8
15. Comas-Basté O, Sánchez-Pérez S, Veciana-Nogués MT, Latorre-Moratalla ML, Vidal-Carou MC. Histamine intolerance: the current state of the art. *Biomolecules.* (2020) 10:1–26. doi: 10.3390/biom10081181
16. Sánchez-Pérez S, Comas-Basté O, Veciana-Nogués MT, Latorre-Moratalla ML, Vidal-Carou MC. Low-Histamine diets: is the exclusion of foods justified by their histamine content? *Nutrients.* (2021) 13:1395. doi: 10.3390/nu13051395
17. García-Martín E, García-Menaya J, Sánchez B, Martínez C, Rosendo R, Agúndez JAG. Polymorphisms of histamine-metabolizing enzymes and clinical manifestations of asthma and allergic rhinitis. *Clin Exp Allergy.* (2007) 37:1175–82. doi: 10.1111/j.1365-2222.2007.02769.x
18. Enko D, Meinitzer A, Mangge H, Kriegshaüser G, Halwachs-Baumann G, Reininghaus EZ, et al. Concomitant prevalence of low serum diamine oxidase activity and carbohydrate malabsorption. *Can J Gastroenterol Hepatol.* (2016) 2016:4893501. doi: 10.1155/2016/4893501
19. Sattler J, Häfner D, Klotter HJ, Lorenz W, Wagner PK. Food-induced histaminosis as an epidemiological problem: plasma histamine elevation and haemodynamic alterations after oral histamine administration and blockade of Diamine Oxidase (DAO). *Agents Actions.* (1988) 23:361–5. doi: 10.1007/BF02142588
20. Leitner R, Zoernpfenning E, Missbichler A. Evaluation of the inhibitory effect of various Drugs / active ingredients on the activity of human diamine oxidase in vitro. *Clin Transl Allergy.* (2014) 4:23. doi: 10.1186/2045-7022-4-s3-p23
21. Schink M, Konturek PC, Tietz E, Dieterich W, Pinzer TC, Wirtz S, et al. Microbial patterns in patients with histamine intolerance. *J Physiol Pharmacol.* (2018) 69:579–93. doi: 10.26402/jpp.2018.4.09
22. Hungerford JM. Scombroid poisoning: a review. *Toxicon.* (2010) 56:231–43. doi: 10.1016/j.toxicon.2010.02.006
23. Visciano P, Schirone M, Tofalo R, Suzzi G. Histamine poisoning and control measures in fish and fishery products. *Front Microbiol.* (2014) 5:500. doi: 10.3389/fmicb.2014.00500
24. Taylor SL, Eitenmiller RR. Histamine food poisoning: toxicology and clinical aspects. *Crit Rev Toxicol.* (1986) 17:91–128. doi: 10.3109/10408448609023767
25. Mongar JL. Effect of chain length of aliphatic amines on histamine potentiation and release. *Br J Pharmacol.* (1957) 12:140–8. doi: 10.1111/j.1476-5381.1957.tb00112.x
26. Arunlakshana O, Mongar JL, Schild HO. Potentiation of pharmacological effects of histamine by histaminase inhibitors. *Physiol J.* (1954) 123:32–54. doi: 10.1113/jphysiol.1954.sp005032
27. Hui JY, Taylor SL. Inhibition of in vivo histamine metabolism in rats by foodborne and pharmacologic inhibitors of diamine oxidase, histamine N-Methyltransferase, and Monoamine Oxidase. *Toxicol Appl Pharmacol.* (1985) 81:241–9. doi: 10.1016/0041-008x(85)90160-7
28. Lyons DE, Beery JT, Lyons SA, Taylor SL, Lyons DE, Lyons JT. Cadaverine and aminoguanidine potentiate the uptake of histamine in vitro in perfused intestinal segments of rats. *Toxicol Appl Pharmacol.* (1983) 70:445–58. doi: 10.1016/0041-008x(83)90162-x
29. Comas-Basté O, Latorre-Moratalla ML, Sánchez-Pérez S, Veciana-Nogués MT, Latorre-Moratalla ML, Vidal-Carou MC. In vitro determination of diamine oxidase activity in food matrices by an enzymatic assay coupled to UHPLC-FL. *Anal Bioanal Chem.* (2019) 411:7595–602. doi: 10.1007/s00216-019-02178-2
30. Ignesti G. Equations of substrate-inhibition kinetics applied to pig kidney diamine oxidase (DAO, E.C. 1.4.3.6). *J Enzyme Inhib Med Chem.* (2003) 18:463–73. doi: 10.1080/14756360310001605543
31. Pietrangeli P, Federico R, Mondovì B, Morpurgo L. Substrate specificity of copper-containing plant amine oxidases. *J Inorg Biochem.* (2007) 101:997–1004. doi:10.1016/j.jinorgbio.2007.03.014
32. Blaschko H, Hawkins J. Enzymic oxidation of aliphatic diamines. *Br J Pharmacol Chemother.* (1950) 5:625–32. doi: 10.1111/j.1476-5381.1950.tb00612.x
33. Lortie MJ, Novotny WF, Peterson OW, Vallon V, Malvey K, Mendonca M, et al. Agmatine, a bioactive metabolite of arginine: production, degradation, and functional effects in the kidney of the rat. *J Clin Investig.* (1996) 97:413–20. doi: 10.1172/JCI118430

34. Elmore BO, Bollinger JA, Dooley DM. Human kidney diamine oxidase: heterologous expression, purification, and characterization. *J Biol Inorg Chem.* (2002) 7:565–79. doi: 10.1007/s00775-001-0331-1
35. Bover-Cid S, Latorre-Moratalla ML, Veciana-Nogués MT, Vidal-Carou MC. Processing contaminants: biogenic amines. In: Motarjemi Y editor. *Encyclopedia of Food Safety*. Vol. 2, Amsterdam: Elsevier (2014). p. 381–91. doi: 10.1016/b978-0-12-378612-8.00216-x
36. European Food Safety Authority [EFSA], European Centre for Disease Prevention and Control [ECDC]. The European Union one health 2020 zoonoses report. *EFSA J.* (2021) 19:6971. doi: 10.2903/j.efsa.2021.6971

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Sánchez-Pérez, Comas-Basté, Costa-Catala, Iduriaga-Platero, Veciana-Nogués, Vidal-Carou and Latorre-Moratalla. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



ÀMBIT 2

Intolerància a la histamina i microbiota intestinal

Objectiu 4: Estudiar el perfil i la composició de la microbiota intestinal d'individus amb símptomes d'intolerància a la histamina i comparar-los amb els d'una població sana.

Objectiu 5: Avaluar potencials canvis en el perfil i composició de la microbiota intestinal en un petit subgrup de pacients al llarg de nou mesos del tractament dietètic de la intolerància a la histamina.

3.4. Estudi del perfil i composició de la microbiota intestinal d'individus amb símptomes d'intolerància a la histamina i la seva comparació amb els d'una població sana

PUBLICACIÓ 5

Intestinal dysbiosis in patients with histamine intolerance

Sònia Sánchez-Pérez, Oriol Comas-Basté, Adriana Duelo, M. Teresa Veciana-Nogués, Mercedes Berlanga, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou. *Nutrients*, 2022, 14, 1774.

Índex d'impacte (JCR 2021): 6,706
Posició en l'àrea "Nutrition & Dietetics": Q1 (15/90)

COMUNICACIÓ ORAL 3

Sònia Sánchez-Pérez, Oriol Comas-Basté, Adriana Duelo, Judit Costa-Catala, M. Teresa Veciana-Nogués, Mercedes Berlanga, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou. **Is there a different intestinal microbial pattern for histamine intolerants?** *IX Reunión de Jóvenes Investigadores de la Sociedad Española de Nutrición,* 2022, Cartagena.

COMUNICACIÓ ORAL 4

Sònia Sánchez-Pérez, Ricard Celorio-Sardà, Oriol Comas-Basté, Salvador Hernández-Macias, Judit Costa-Catala, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou. **Alteración en la diversidad de la microbiota intestinal en individuos intolerantes a la histamina.** *16ª Reunión anual de la Sociedad Española de Seguridad y Calidad Alimentaria (SESAL),* 2020, virtual, Barcelona.

COMUNICACIÓ ESCRITA 6

Sònia Sánchez-Pérez, Oriol Comas-Basté, Adriana Duelo, Judit Costa-Catala, M. Teresa Veciana-Nogués, Mercedes Berlanga, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou. **Intestinal dysbiosis in patients with histamine intolerance.** *XIII Conferència Internacional de Dieta Mediterrània*, 2022, Barcelona.

PLANTEJAMENT I OBJECTIU

Segons la majoria d'estudis, la intolerància a la histamina per dèficit de DAO té un origen genètic o adquirit [32]. S'han identificat diferents mutacions en el gen que codifica l'enzim DAO que es relacionen amb una menor activitat enzimàtica. Igualment, aquest dèficit pot ser degut a malalties inflamatòries intestinals que limiten la secreció de la DAO, o bé, a l'acció de certs fàrmacs que poden inhibir-la.

Un altre possible origen, del qual encara no hi ha prou evidències, seria l'existència d'una disbiosi o alteració de la composició de la microbiota intestinal en els individus intolerants a la histamina. Segons l'única referencia que hem trobat a la literatura científica, una disbiosi i una menor diversitat bacteriana estarien contribuint a una inflamació de la mucosa intestinal i, conseqüentment, afavoririen una menor activitat DAO a nivell intestinal [36]. A més, també s'ha descrit la capacitat de formar histamina i altres amines per certs bacteris de la microbiota [37]. Per tant, un desequilibri en la microbiota a favor d'una major presència de bacteris formadors d'histamina podria estar associat també a aquesta intolerància.

En aquest context, l'objectiu d'aquest estudi va ser estudiar el perfil i la composició de la microbiota intestinal en pacients amb intolerància a la histamina, comparant-los amb els d'una població sana.

METODOLOGIA

L'estudi es va dur a terme amb 26 voluntaris, 12 d'ells diagnosticats d'intolerància a la histamina i reclutats en un centre de nutrició i dietètica especialitzat en el tractament dietètic del dèficit de DAO (Institut Clínic del Dèficit de DAO, Barcelona, Espanya). Els criteris d'inclusió per aquests pacients van ser: tenir entre 18 i 65 anys, un diagnòstic d'intolerància a la histamina basat en la presència de símptomes associats a aquesta intolerància en dos o més sistemes o òrgans i resultats negatius d'al·lèrgia alimentària per IgE. Es van excloure de l'estudi aquelles pacients embarassades o en període de lactància i aquells individus que havien començat

una dieta baixa en histamina o que havien pres antibiòtics i/o probiòtics durant el mes anterior a l'inici de l'estudi. El grup control va incloure 14 individus sans sense símptomes d'intolerància a la histamina que van ser reclutats en el Campus de l'Alimentació de la Universitat de Barcelona.

Per tots els participants es va recollir informació sobre les seves dades demogràfiques i els símptomes clínics. La composició de la microbiota intestinal es va analitzar a partir de l'ADN aïllat de mostres de femta. Es van seqüenciar les regions V3-V4 d'ARNr 16S bacterià mitjançant la plataforma Illumina MiSeq (Servei Genòmic i Bioinformàtic de la Universitat Autònoma de Barcelona). L'anàlisi bioinformàtic de les dades es van dur a terme amb la base de dades EzBioCloud (ChunLab, Inc., Seul, Corea). L'alfa-diversitat es va mesurar utilitzant l'índex de Shannon i l'índex de Simpson. La determinació de la beta-diversitat es va realitzar mitjançant l'índex de dissimilitud de Bray-Curtis representat en un anàlisi de coordinades principals (PCoA).

La concentració d'histamina en les mostres fecals es va determinar per un test ELISA (Histamine ELISA kit, Immunodiagnostik AG, Bensheim, Alemany) i, a més, en el grup de pacients intolerants, l'activitat DAO sanguínia es va analitzar mitjançant un radio-immuno assaig (Sciotec Diagnostic Technologies, Tulln, Austria).

L'estudi va ser aprovat pel Comitè de Bioètica de la Universitat de Barcelona (IRB00003099).

RESULTATS

El 83% dels individus diagnosticats d'intolerància a la histamina va mostrar símptomes gastrointestinals i/o neurològics, la meitat va mostrar també manifestacions dermatològiques i un 33% símptomes respiratoris. El valor mitjà de símptomes va ser de 4,3 manifestacions per individu, encara que dos pacients van arribar a reportar 7 i 8 símptomes. En general, les manifestacions més freqüents van ser inflor i dolor abdominal, mal de cap, flatulències, diarrea, acidesa i dolor

muscular i articular. Deu dels dotze pacients van presentar una activitat DAO sanguínia reduïda (<10 U/mL o <80 HDU/mL).

Pel que fa a l'anàlisi de la microbiota intestinal, no es van observar diferències significatives a nivell de fílums. Sí que es van trobar diferències significatives entre els dos grups en l'abundància relativa de quatre famílies bacterianes, destacant la baixa proporció de *Prevotellaceae* en el grup d'intolerants a la histamina, el que es podria associar a un increment de la permeabilitat intestinal.

En general, es van trobar diferències significatives en l'abundància relativa de 21 gèneres i 30 espècies entre el grup control i el d'intolerants a la histamina. Concretament, en el grup d'intolerants es va trobar una proporció significativament inferior del gèneres *Ruminococcus* i *Faecalibacterium,* així com també, de l'espècie *Faecalibacterium prausnitzii*, els quals es troben relacionats amb efectes beneficiosos per la salut intestinal. Contràriament, no es van trobar diferències en l'abundància de *Bifidobacterium* i *Lactobacillus*, gèneres bacterians estretament associats a una bona salut intestinal.

D'altra banda, el percentatge d'alguns gèneres i espècies descrits com a formadors d'histamina va ser significativament més alt en el grup d'individus intolerants a la histamina ($p<0,05$), com per exemple, els gèneres *Staphylococcus* i *Proteus*, diferents gèneres no identificats pertanyents a la família *Enterobacteriaceae* i les espècies *Clostridium perfringens* i *Enterococcus faecalis*.

Quant a la diversitat bacteriana, no es van observar diferències significatives entre ambdós grups en relació a l'alfa-diversitat, és a dir, quant a la riquesa d'espècies i l'abundància de cada una d'elles que presenta cada individu (índex de Shannon, $p=0,411$ i índex de Simpson, $p=0,681$). En canvi, el perfil microbià d'ambdós grups sí que va ser significativament diferent a nivell de gèneres i espècies (beta-diversitat, $p<0,05$). A més, els individus intolerants a la histamina van mostrar una major heterogeneïtat en aquest perfil en comparació al grup d'individus sans.

Els valors d'histamina en femta no van ser significativament diferents entre els dos grups estudiats. La majoria dels individus, tant els sans (71%) com els intolerants (92%), van presentar nivells d'histamina fecals dins dels valors considerats normals (<959 ng/g femta).

Les principals limitacions del present estudi són la mida de la mostra, la falta de representació masculina en el grup d'intolerants a la histamina i, en alguns casos, la impossibilitat de classificar els bacteris més enllà del nivell de família o gènere. Aquestes limitacions han de tenir-se en compte en futurs estudis destinats a dilucidar la relació entre la disbiosi intestinal i la intolerància a la histamina.

TAKE-HOME MESSAGE

Els individus amb intolerància a la histamina mostren una disbiosi intestinal principalment caracteritzada per una menor abundància d'alguns grups bacterians beneficiosos per la salut intestinal i una major proporció d'aquells freqüentment descrits com a formadors d'histamina. A més, el perfil microbià en aquests individus és significativament diferent i més heterogeni (beta-diversitat) que el d'una població sana.

nutrients

MDPI

Article

Intestinal Dysbiosis in Patients with Histamine Intolerance

Sònia Sánchez-Pérez [1,2,3], Oriol Comas-Basté [1,2,3], Adriana Duelo [1,2,3], M. Teresa Veciana-Nogués [1,2,3], Mercedes Berlanga [4], M. Luz Latorre-Moratalla [1,2,3,†] and M. Carmen Vidal-Carou [1,2,3,*,†]

1 Departament de Nutrició, Ciències de l'Alimentació i Gastronomía, Facultat de Farmàcia i Ciències de l'Alimentació, Campus de l'Alimentació de Torribera, Universitat de Barcelona (UB), Av. Prat de la Riba 171, 08921 Santa Coloma de Gramenet, Spain; soniasanchezperez@ub.edu (S.S.-P.); oriolcomas@ub.edu (O.C.-B.); aduelo@ub.edu (A.D.); veciana@ub.edu (M.T.V.-N.); mariluzlatorre@ub.edu (M.L.L.-M.)
2 Institut de Recerca en Nutrició i Seguretat Alimentària (INSA·UB), Universitat de Barcelona (UB), Av. Prat de la Riba 171, 08921 Santa Coloma de Gramenet, Spain
3 Xarxa d'Innovació Alimentària (XIA), C/Baldiri Reixac 4, 08028 Barcelona, Spain
4 Departament de Biologia, Sanitat i Mediambient, Secció de Microbiologia, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona (UB), Av. Joan XXIII 27-31, 08028 Barcelona, Spain; mberlanga@ub.edu
* Correspondence: mcvidal@ub.edu
† These authors contributed equally to this work.

Abstract: An underlying cause of histamine intolerance is diamine oxidase (DAO) deficiency, which leads to defective homeostasis and a higher systemic absorption of histamine. Impaired DAO activity may have a genetic, pharmacological or pathological origin. A recent proposal also suggests it can arise from an alteration in the gut microbiota, although only one study has explored this hypothesis to date. A greater abundance of histamine-secreting bacteria in the gut could lead to the development of histamine intolerance. Thus, the aim of this study was to characterize the composition of the intestinal microbiota of patients with histamine intolerance symptoms and compare it with that of healthy individuals. The study was performed by sequencing bacterial 16S rRNA genes (V3-V4 region) and analyzing the data using the EzBioCloud Database. Dysbiosis of the gut microbiota was observed in the histamine intolerance group who, in comparison with the healthy individuals, had a significantly lower proportion of *Prevotellaceae*, *Ruminococcus*, *Faecalibacterium* and *Faecablibacterium prausnitzii*, which are bacteria related to gut health. They also had a significantly higher abundance of histamine-secreting bacteria, including the genera *Staphylococcus* and *Proteus*, several unidentified genera belonging to the family *Enterobacteriaceae* and the species *Clostridium perfringens* and *Enterococcus faecalis*. A greater abundance of histaminogenic bacteria would favor the accumulation of high levels of histamine in the gut, its subsequent absorption in plasma and the appearance of adverse effects, even in individuals without DAO deficiency.

Keywords: histamine; histamine intolerance; gut microbiota; intestinal dysbiosis; histamine-secreting bacteria; diamine oxidase (DAO) enzyme

check for updates

Citation: Sánchez-Pérez, S.; Comas-Basté, O.; Duelo, A.; Veciana-Nogués, M.T.; Berlanga, M.; Latorre-Moratalla, M.L.; Vidal-Carou, M.C. Intestinal Dysbiosis in Patients with Histamine Intolerance. *Nutrients* **2022**, *14*, 1774. https://doi.org/10.3390/nu14091774

Academic Editor: Eva Untersmayr

Received: 22 March 2022
Accepted: 21 April 2022
Published: 23 April 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

In the last several years, there has been growing interest in characterizing the gut microbiota, both in healthy and unhealthy individuals. It is well known that the eubiotic gut microbiota has an impact on human health and well-being [1]. Although its composition is age-related and becomes more stable in adulthood, it can be altered by a wide range of factors, such as dietary habits, lifestyle, stress, antibiotic use and diseases [1,2]. While the connection between the gut microbiota and certain noncommunicable diseases, such as obesity, diabetes, cancer, gastrointestinal and neurological disorders, is being extensively studied, its role in food intolerance, including that of histamine, is still under explored [3–9].

Histamine intolerance is an adverse reaction to dietary histamine that appears in susceptible individuals [10,11]. This disorder is mainly provoked by a deficiency in the key enzyme responsible for histamine degradation at the intestinal level, diamine oxidase

(DAO), which leads to higher absorption [12–14]. The accumulation of histamine in plasma can affect numerous organs and tissues due to the wide distribution of the four histamine receptors in the organism, resulting in a plethora of gastrointestinal and extra-intestinal symptoms (i.e., dermatological, respiratory, neurological and hemodynamic complaints). According to the retrospective study by Schnedl et al. (2019), the most common symptoms in histamine-intolerant patients are gastrointestinal in nature, above all abdominal distension, postprandial fullness, diarrhea, abdominal pain and constipation [15].

DAO deficiency may have a genetic origin and has been associated with single-nucleotide polymorphisms encoding a protein with reduced histamine degradation capacity [16,17]. On the other hand, impaired DAO activity can also be temporary and reversible, arising as a side effect of some widely used pharmacological drugs, such as clavulanic acid or acetylcysteine, or a secondary symptom of gastrointestinal disorders [18]. In fact, evidence supporting an intestinal origin of histamine intolerance is growing [3]. A group of Austrian researchers found that the mucosal damage caused by gastroenteritis, irritable bowel syndrome (IBS), short bowel syndrome or gastrointestinal surgery led to a concomitant decrease in DAO and lactase activities [19]. Moreover, recent studies have suggested that reduced DAO activity may be linked to nonceliac gluten sensitivity [20–22]. Another cause of DAO deficiency could be an alteration in the composition of the gut microbiota, although to date only one study has explored this hypothesis [23]. Schink et al. (2018) reported that the intestinal dysbiosis in patients diagnosed with histamine intolerance could contribute to mucosal inflammation, a condition that impairs DAO activity [23]. Additionally, the fact that a range of bacterial strains in the human gut are able to produce (*Enterococcus faecalis*, *Bifidobacterium pseudocatenulatum*, *Lactobacillus gasseri*, *Escherichia coli*, *Morganella morganii* and *Proteus mirabillis*) and degrade (*Escherichia coli* and *Klebsiella pneumoniae*) histamine suggests that dysbiosis could influence histamine levels in the intestine [24]. In this context, the aim of this work was to characterize the composition of the gut microbiota of patients with symptoms of histamine intolerance and compare it with that of healthy individuals.

2. Materials and Methods

2.1. Study Design

The study was carried out with 26 volunteers, including 12 patients diagnosed with histamine intolerance (HIT group), who were recruited from a nutrition and dietetic centre specialized in the dietary management of DAO deficiency (DAO Deficiency Clinical Institute, Barcelona, Spain). The inclusion criteria for the histamine-intolerant patients were as follows: age between 18 and 65 years; diagnosis of histamine intolerance based on two or more symptoms described by Maintz and Novak (2007) [25]; and negative results for food allergen-specific IgE. The exclusion criteria were pregnancy, lactation, having started a low-histamine diet and having taken antibiotics and/or probiotics the month before the study. The 14 healthy individuals in the control group, who were free of histamine intolerance symptoms, were recruited from the Food and Nutrition Campus of the University of Barcelona.

Demographic characteristics and clinical symptoms of all study participants were recorded. For the intestinal microbiota and histamine concentration analysis, walnut-sized stool samples were self-collected in sterile vials and stored at −80 °C until their analyses. For the HIT group, plasma DAO activity was also analyzed using a Radio Extraction Assay (REA) according to the manufacturer's instructions (Sciotec Diagnostic Technologies, Tulln, Austria).

All participants were informed in detail about the aim and procedure of the study and gave their written informed consent prior to study inclusion. The study was approved by the Bioethics Committee of the University of Barcelona (IRB00003099).

2.2. Intestinal Microbiota and Histamine Concentration Analysis in Stool Samples

Bacterial DNA was isolated from stool samples using a QIAamp Power Fecal Pro DNA kit (QIAGEN, Germantown, MD, USA) following the manufacturer's instructions. DNA concentration was measured by BioDrop µLite (Biotech, Madrid, Spain). To analyze the composition of the gut microbiota, sequencing of the V3-V4 region of bacterial 16S rRNA was performed on the Illumina MiSeq platform by the Genomic and Bioinformatic Service of the Autonomous University of Barcelona. Then, bioinformatics analysis of the microbiota composition was performed with EzBiocloud Database (ChunLab, Inc., Seoul, Korea). For the 16S rRNA amplicons, sequence data were deposited on the NCBI database by the Bioproject PRJNA811749.

Stool histamine was determined by a competitive enzyme linked immunoassay using the Histamine ELISA kit from Immunodiagnostik AG (Bensheim, Germany) according to the instructions provided by the manufacturer.

2.3. Statistical Analysis

Statistical analyses of participant characteristics and the histamine concentrations in stool samples were performed using the IBM SPSS Statistics 25.0 statistical software package (IBM Corporation, Armonk, NY, USA), applying Student's t or Mann–Whitney tests after the Kolmogorov–Smirnov test for normal distribution. Differences in the microbiota composition between groups were analyzed by the Kruskal–Wallis test for non-parametric data. Alpha diversity was measured using the Shannon index and Simpson's index, and, for beta diversity, Bray–Curtis dissimilarity analysis was performed and visualized using principal coordinates analysis (PCoA). p-values of $p < 0.05$ were considered statistically significant.

3. Results and Discussion

3.1. Participant Characteristics

All participants in the HIT group were female and aged between 21 and 65 years (mean 40.4 ± 12.4). In the control group, the volunteers were 71.4% female and 24–55 years old (40.4 ± 12.4), and no significant differences were observed between the sexes in any of the study parameters (Table 1).

Table 1. Characteristics of the participants from the control and HIT groups.

Participants' Characteristics	Groups	
	Control	HIT
n (%)	14 (53.9%)	12 (46.2%)
Age (average years ± SD)	40.4 ± 12.5	40.4 ± 12.4
Male [*n* (%)]	4 (28.6%)	0 (0%)
Female [*n* (%)]	10 (71.4%)	12 (100%)
Body Mass Index (BMI) [average ± SD]	23.7 ± 3.2	22.2 ± 6.0

The symptoms described by histamine-intolerant patients are summarized in Table 2. Gastrointestinal and neurological disorders were reported by 83% of patients with histamine intolerance, followed by dermatological (50%) and respiratory complaints (33%). The mean number of symptoms per patient was 4.3, although it was striking that two patients reported 7 and 8 symptoms, respectively. Overall, the most frequently reported symptoms were bloating and headache, followed by flatulence; diarrhoea; heart burn; and abdominal, muscular and articular pain. These were also the most common symptoms in histamine-intolerant patients identified by Schendl et al. (2019) in a cohort of 133 individuals [15]. It is worth mentioning that approximately half of the patients in the present study were underweight, with body mass index (BMI) values below 18.5.

Table 2. Clinical manifestations reported by the HIT group (n = 12).

Symptoms	Frequency (%) *
Gastrointestinal tract	
Bloating	75
Flatulencies	33
Abdominal pain	25
Diarrhoea	25
Heartburn	25
Constipation	17
Nausea	17
Skin	
Urticaria	17
Atopic skin	17
Pruritus	8
Eczema	8
Neurologic system	
Headache	75
Dizziness	8
Respiratory apparatus	
Asthma	17
Rhinitis	8
Shortness of breath	8
Other symptoms	
Muscular/articular pain	25
Fatigue	17
Insomnia	8

* The frequency (%) refers to the number of patients suffering these symptoms within the HIT group.

DAO plasmatic activity was deficient in 10 out of the 12 patients with symptoms of histamine intolerance (<10 U/mL). Although DAO plasmatic activity has been proposed as a potential marker of histamine intolerance, its reported prevalence varies greatly (values ranging from 8% to 88%), depending on the study and the symptoms [26]. Discrepancies in the data could be explained by the variable etiology of DAO deficiency, which may be genetic in origin, a secondary symptom of gastrointestinal pathologies, or arise from the consumption of DAO-inhibitor drugs.

3.2. Intestinal Microbiota Composition and Stool Histamine Concentration

The intestinal microbiota of HIT and control groups was analyzed and compared in terms of phylum, family, genus and species. The two study groups shared a similar profile of phyla (Figure 1a), with Firmicutes and Bacteroidetes being the most dominant (approximately 90% of the total gut microbiota). Although without statistical significance, the HIT group showed a slightly higher relative abundance of the phylum Proteobacteria (3.52%) in comparison with the control group (1.88%). Similarly, Schink et al. (2018) reported higher levels of Proteobacteria in patients with histamine intolerance symptoms. According to this work, the intestinal overgrowth with Proteobacteria could result in a low-grade intestinal inflammation that could lead to epithelial dysfunction. High proportions of this phylum have also been found in patients with different intestinal disorders, such as Crohn's disease, ulcerative colitis, colorectal cancer and IBS [27–30]. Intestinal inflammation may increase the amount of oxygen available in the intestinal lumen, resulting in a shift from obligate anaerobic bacteria towards facultative anaerobic bacteria, such as Proteobacteria. Consequently, an increase in its abundance has been postulated as a hallmark of dysbiosis [31,32].

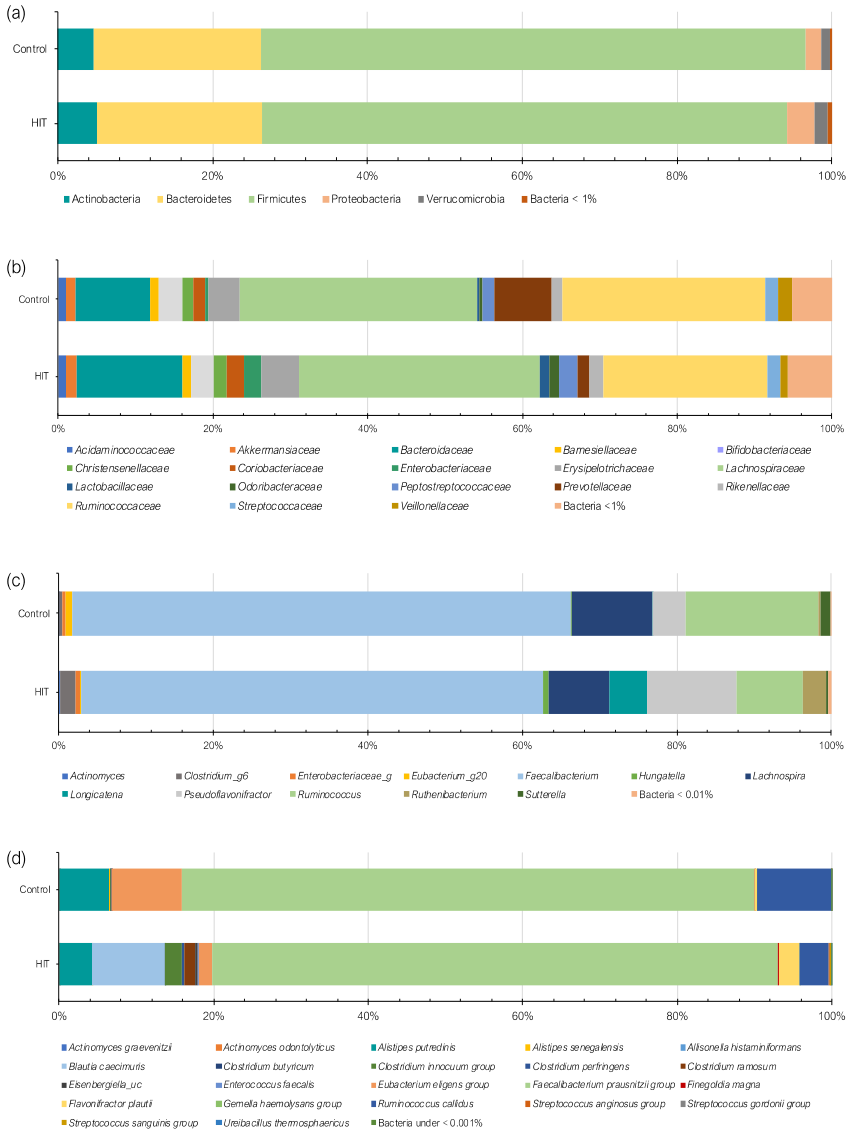


Figure 1. Relative abundance (%) of bacteria at the level of (**a**) phylum, (**b**) family, (**c**) genus and (**d**) species in control and histamine intolerance (HIT) groups. The genera and species are only represented if differences between the study groups were significant.

Regarding bacterial families, *Lachnospiraceae* (Firmicutes), *Ruminococcaceae* (Firmicutes) and *Bacteroidaceae* (Bacteroidetes) represented more than 50% in both the control and HIT groups (Figure 1b). Statistically significant differences between groups were observed in four bacterial families ($p < 0.05$) (Table 3). For example, a lower abundance of *Prevotellaceae* (Bacteroidetes) was found in the HIT group. An under-representation of several members

of this family group may indicate reduced mucin synthesis, which has been associated with increased gut permeability [33]. Schink et al. (2018) reported higher mean values of a marker of intestinal permeability (zonulin) in histamine intolerance patients in comparison with those recommended for the healthy population, suggesting a mild alteration of gut permeability in these patients [23]. According to these authors, an increased gut permeability facilitates the penetration of microbial metabolites, such as histamine, and, in turn, could lead to histamine-associated symptoms. Additionally, bacteria belonging to the *Prevotellaceae* family have been associated with a range of functions in the organism, such as interaction with the immune system and the synthesis of thiamine, folate and short-chain fatty acids [33].

Table 3. Differences in the relative abundance (%) of bacterial families between the control and HIT groups. Data are presented as average ± SD.

Family	Phylum	Control	HIT	*p*-Value
Acholeplasmataceae	Tenericutes	0.001 ± 0.002	0.002 ± 0.001	0.03
Actinomycetaceae	Actinobacteria	0.012 ± 0.011	0.027 ± 0.024	0.02
Prevotellaceae	Bacteroidetes	7.422 ± 7.996	1.548 ± 2.408	0.04
Staphylococcaceae	Firmicutes	0.002 ± 0.007	0.014 ± 0.029	0.03

All the genera and species identified in the microbiome of both study groups are shown in the Supplementary Material (Supplementary Tables S1 and S2). Overall, statistically significant differences were found in the relative abundance of 21 genera and 30 species between control and HIT groups ($p < 0.05$) (Figure 1c,d). In their study with histamine-intolerant patients, Schink et al. (2018) reported significant differences in five bacterial genera, including only those with an abundance greater than 0.01% [23]. Applying the same criterion, in the present study, more differences were identified at the genus level (up to nine).

Although the relative abundance of *Ruminococcus* in the control group was highly variable, it was statistically significantly lower in individuals with histamine intolerance ($p < 0.05$) (Figure 2). *Ruminococcus* is thought to play a role in maintaining a healthy human gut [34]. Members of this genus can degrade complex polysaccharides into a variety of simple sugars, making them more available for the epithelium cells of the large intestine [34,35]. The relative abundance of the genus *Faecalibacterium* (Figure 2), especially the species *Faecalibacterium prausnitzii* (Figure 3), was also significantly lower in the HIT group ($p < 0.05$). Proposed as a marker of gut health, *F. prausnitzii* is one of the most prevalent and abundant producers of butyrate in the human gut, a short-chain fatty acid that represents the main energy source for colonocytes, and it displays protective properties against colorectal cancer and inflammatory bowel diseases [36–38]. Regarding *Bifidobacterium* and *Lactobacillus* (Figure 2), two other bacterial genera frequently associated with good intestinal health, no significant differences were found between the two groups. Only two species displayed a lower mean relative abundance in the HIT group (*Bifidobacterium adolescentis*, $p = 0.034$ and *Lactobacillus rogosae*, $p = 0.017$) (Supplementary Table S2).

Conversely, the genera *Staphylococcus* and *Proteus* were significantly more abundant in the HIT group ($p < 0.05$), with mean values 7- and 1.8-fold higher than in the control group, respectively (Figure 2). Several bacteria from these genera have shown an important capacity to form histamine [39–41]. Moreover, members of the family *Enterobacteriaceae*, known to be among the most prolific histamine-producing bacteria, were also significantly more abundant in the HIT group (Figure 2) [39–41], although they could not be identified at the genus level. It should be mentioned that the ability to form histamine is reported to be strain-dependent [42].

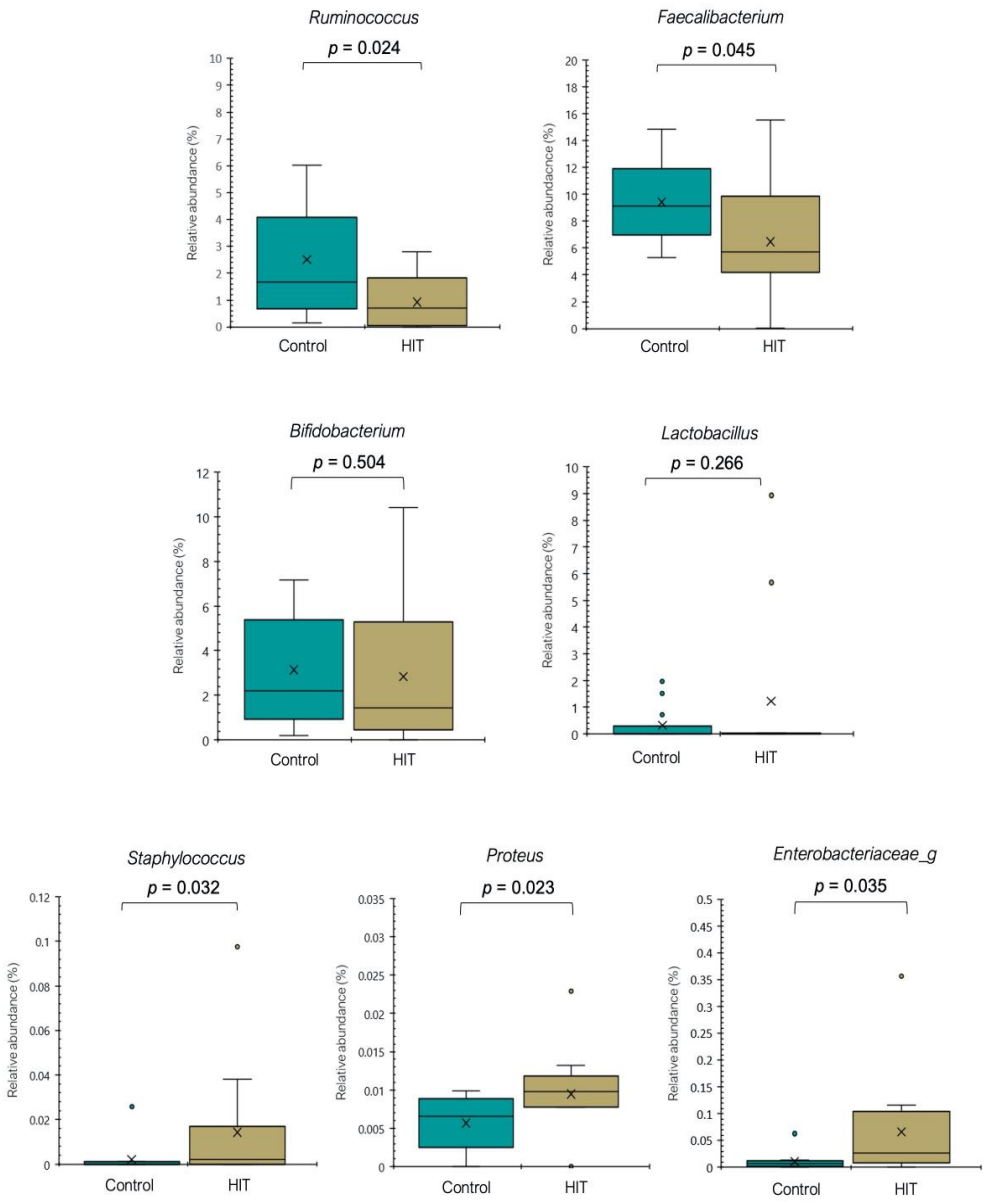


Figure 2. Relative abundance (%) of different genera in the control and HIT groups. Mean values are represented with an × and values statistically considered as outliers (atypical values) are plotted as circles.

To date, studies on histamine-producing bacteria have been mainly focused on strains isolated from food samples. However, the histaminogenic capacity of the gut microbiota has

been studied only recently, and data are still limited [32,43,44]. A systematic in silico search published in 2021 identified 117 species with a putative histamine-secreting capacity within the human gut microbiome [32], many of them belonging to genera extensively reported as histaminogenic, such as *Morganella*, *Lactobacillus*, *Staphylococcus*, *Photobacterium* and *Clostridium* [24,41,42]. For example, according to Mou et al. (2021), *Clostridium perfringens* is one of the species most frequently associated with the enzyme histidine decarboxylase, regardless of strain [32]. In the present study, the occurrence of *C. perfringens* (Figure 3), a bacterium responsible for several gastrointestinal disorders, was more frequently identified in the HIT group and only in two healthy individuals. Similarly, the abundance of *Enterococcus faecalis*, *Proteus mirabilis* and *Escherichia coli* tended to be higher in the HIT group (Figure 3). These species were isolated from the human gut by Pugin et al. (2017) and identified as producers of histamine as well as other biogenic amines, such as putrescine, cadaverine and tyramine [24].

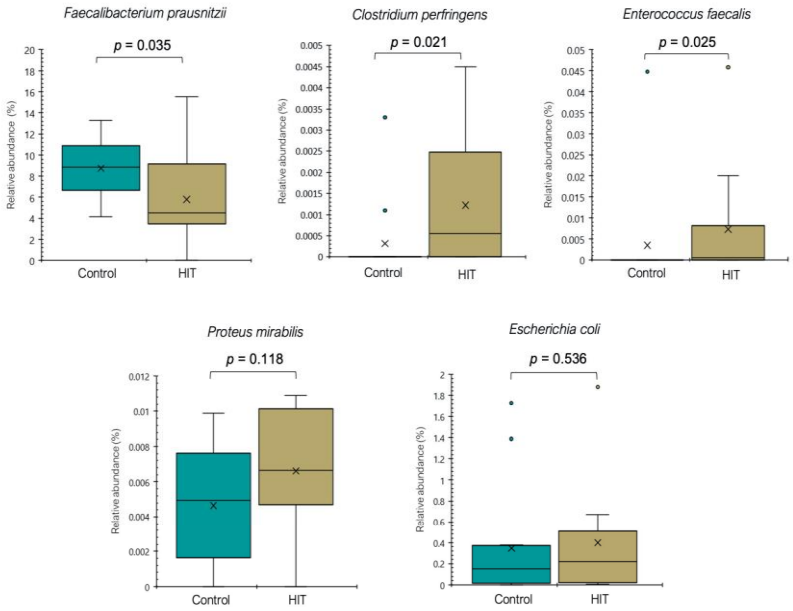


Figure 3. Relative abundance (%) of different species in the control and HIT groups. Mean values are represented with an × and values statistically considered as outliers (atypical values) are plotted as circles.

It has been suggested that histamine secreted by the gut microbiota could have an impact on the health or disease status of the host. In the present study, the higher abundance of intestinal histaminogenic bacteria found in histamine-intolerant patients could have resulted in an excess accumulation and systemic absorption of histamine. Notably, patients with histamine intolerance frequently suffer from DAO deficiency, which could also enhance the toxicity of intestinal histamine. Additionally, an excess of histamine could negatively affect the inflammatory state of the intestinal mucosa. High amounts of histamine secreted by gut bacteria were linked to a proinflammatory response and signs of deteriorating health in murine specimens [43,45,46]. Mishima et al. (2020) suggested that intestinal dysbiosis, involving an over-representation of histamine-secreting bacteria and higher intestinal histamine levels, was potentially associated with the development and aggravation of IBS [47]. After analyzing 2451 stool metagenomes, Mou et al. (2021) also

found that putative histamine-secreting bacteria were significantly enriched in patients with ulcerative colitis and Crohn's disease [32]. According to these authors, the enrichment of histamine-secreting species in IBD patients was not attributed to a single taxon but highly dependent to the cohort characteristics. The involved bacterial taxons included Actinobacteriota, Firmicutes, Proteobacteria and Bacteroidiota, depending on the study; some of them were also found to be increased in the HIT patients from the current study [32]. Another study observed a higher abundance of histamine-producing bacteria in adults diagnosed with asthma [48]. These previous studies, together with the current results, support the potential association between histamine-secreting bacteria and the inflammatory status occurring in this kind of disorder. In contrast, some studies, both in vitro and in murine models, have demonstrated that intestinal histamine exerts immunomodulatory effects by suppressing the production of proinflammatory interleukines [49,50].

Concerning the histamine concentration in stools, no significant differences were found between study groups ($p = 0.681$). As shown in Figure 4, the majority of both healthy and histamine-intolerant individuals (71% and 92%, respectively) displayed fecal histamine levels within the normal range (<959 ng/ g stool). The obtained results are in accordance with those of Schink et al. (2018), who also found very similar histamine levels among stool samples [23]. Therefore, the increased presence of histamine-secreting bacteria found in the HIT group was not associated with a higher histamine excretion in feces.

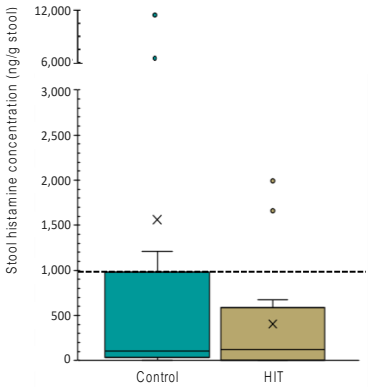


Figure 4. Occurrence of histamine (ng/ g stool) in fecal samples of control and HIT groups. Samples above the dotted line are above the normal range (<959 ng/ g stool). Mean values are represented with an ⇥and values statistically considered as outliers (atypical values) are plotted as circles.

3.3. Bacterial Diversity

Bacterial species diversity was evaluated through indices of alpha diversity (Shannon and Simpson indices) and beta diversity (multidimensional scaling by PCoA and Bray–Curtis dissimilarity). Regarding alpha diversity, which is a measurement of the mean species diversity within the human gut, no significant differences were observed between the HIT and control groups for any of the evaluated indices (Shannon index, $p = 0.411$ and Simpson index, $p = 0.681$). Figure 5 shows the number of identified species belonging to the main genera that differed significantly in abundance between the two groups. Although the HIT group showed a significantly different proportion of genera with the capacity to form histamine (*Staphylococcus* and *Proteus*) and genera considered as a biomarker of a healthy gut (*Ruminococcus* and *Faecalibacterium*), these differences were not observed in terms of species number. However, a lower diversity in *Bifidobacterium* and *Lactobacillus* species was observed in the HIT group, with individuals showing only 69% and 59% of the species found in the control group, respectively (Figure 5).

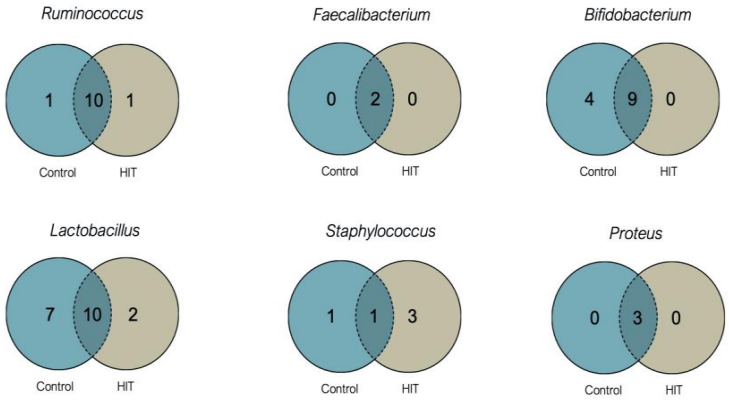


Figure 5. Venn diagrams of specific and shared Operational Taxonomic Units (OTUs) detected for *Ruminococcus*, *Faecalibacterium*, *Bifidobacterium*, *Lactobacillus*, *Staphylococcus* and *Proteus* genera in the control and HIT groups.

In disagreement with our results, Schink et al. (2018) found a lower alpha diversity in a group of 8 histamine-intolerant individuals in comparison with 10 healthy subjects [23]. Similar discrepancies exist in studies on other types of food intolerance or gastrointestinal disorders, some observing a reduced alpha diversity in patient groups [51–53] and others reporting no differences in this parameter [54,55].

Beta diversity refers to the interindividual differences in the distribution pattern of genera and species. In this case, beta diversity determined by the Bray–Curtis index showed statistically significant differences between the two groups, both for genera ($p = 0.024$) and species ($p = 0.029$). As shown in Figure 6, the samples of the HIT group are more scattered compared to those of healthy individuals, which denotes a higher degree of heterogeneity in their intestinal microbiota.

(a)

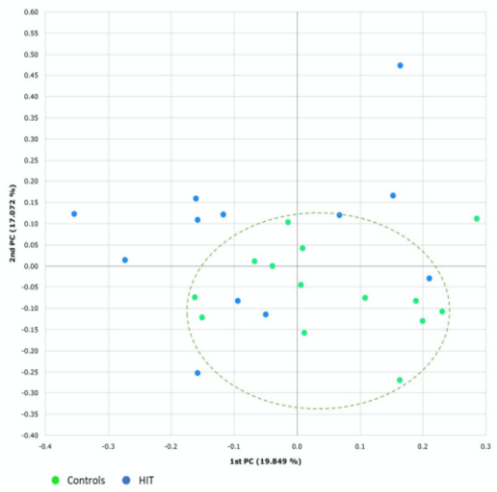


Figure 6. *Cont.*

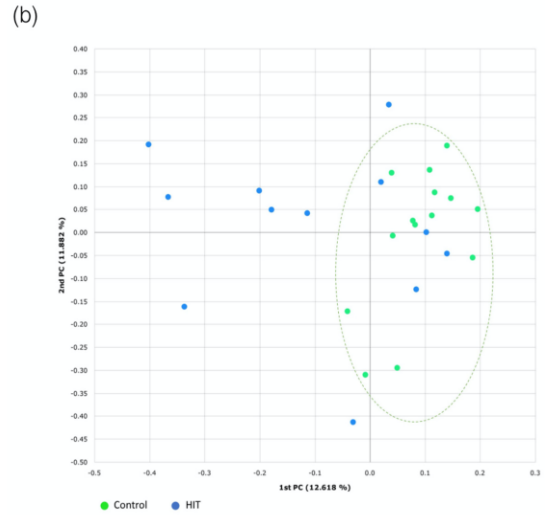


Figure 6. Beta diversity at the (**a**) genus and (**b**) species level determined by the Bray–Curtis index and principal coordinates analysis (PCoA). PC in both axes means "principal component".

4. Conclusions

An imbalance or dysbiosis of the gut microbiota was observed in patients with histamine intolerance in comparison with healthy individuals. In the HIT group, the relative abundance of bacteria associated with gut health, namely *Prevotellaceae*, *Ruminococcus*, *Faecalibacterium* and *Faecablibacterium prausnitzii*, was significantly lower, whereas that of histamine-secreting bacteria was significantly higher, including the genera *Staphylococcus* and *Proteus*, several unidentified genera belonging to the family *Enterobacteriaceae*, and the species *Clostridium perfringens* and *Enterococcus faecalis*. A greater abundance of histaminogenic bacteria may favor the accumulation of high levels of histamine in the gut and its subsequent absorption in plasma, which can trigger adverse health effects. The ability to degrade histamine derived from an intestinal dysbiosis would be easily overwhelmed in individuals with DAO deficiency. This dysbiosis could also provoke mucosal inflammation, a condition that impairs DAO functionality. Therefore, an over-representation of histamine-forming bacteria in the gut could be another possible origin of histamine intolerance.

The main limitations of the present study are the small size of each sample group, the lack of male representation in the HIT group, and in some cases, the impossibility of classifying bacteria beyond the family or genus level. Moreover, the fact that approximately half of the participants from the HIT group showed a reduced BMI may also be considered as a drawback of this study as it could be another factor influencing the gut microbiota composition. These limitations should be born in mind in further studies aimed at elucidating the relationship between intestinal dysbiosis and histamine intolerance. Next steps should focus on the development of studies with a more ambitious design considering a higher number of participants to better understand until what extent an imbalance in the presence of histamine-secreting bacteria or gut health-related bacteria would be etiologically linked with the symptomatology of histamine intolerance. Moreover, it would also be of interest to assess the potential influence of the follow-up of a low-histamine diet in the intestinal microbial pattern of histamine-intolerant individuals.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nu14091774/s1, Table S1: Bacterial genera obtained in the characterization of the intestinal microbiota in control and HIT groups; Table S2: Bacterial species obtained in the characterization of the intestinal microbiota in control and HIT groups.

Author Contributions: Conceptualization, M.T.V.-N., M.L.L.-M. and M.C.V.-C.; investigation, S.S.-P., O.C.-B. and A.D.; data curation, S.S.-P., O.C.-B., M.B. and M.L.L.-M.; writing—original draft preparation, S.S.-P., O.C.-B., M.B. and M.L.L.-M.; writing—review and editing, S.S.-P., O.C.-B., A.D., M.T.V.-N., M.B., M.L.L.-M. and M.C.V.-C.; supervision, M.L.L.-M. and M.C.V.-C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Grant PID2020-117670RB-C21, funded by MICIN/AEI/10.13039/501100011033 and by "ERDF A way of making Europe".

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Bioethics Committee of the University of Barcelona (IRB00003099).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: For the 16S rRNA amplicons, sequence data were deposited on the NCBI database by the Bioproject PRJNA811749.

Acknowledgments: Sònia Sánchez-Pérez was a recipient of a doctoral fellowship from the University of Barcelona (APIF2018).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Power, S.E.; O'Toole, P.W.; Stanton, C.; Ross, R.P.; Fitzgerald, G.F. Intestinal Microbiota, Diet and Health. *Br. J. Nutr.* **2014**, *111*, 387–402. [CrossRef] [PubMed]
2. Rinninella, E.; Cintoni, M.; Raoul, P.; Lopetuso, L.R.; Scaldaferri, F.; Pulcini, G.; Miggiano, G.A.D.; Gasbarrini, A.; Mele, M.C. Food Components and Dietary Habits: Keys for a Healthy Gut Microbiota Composition. *Nutrients* **2019**, *11*, 2393. [CrossRef] [PubMed]
3. Schnedl, W.J.; Enko, D. Histamine Intolerance Originates in the Gut. *Nutrients* **2021**, *13*, 1262. [CrossRef]
4. Ley, R.E.; Bäckhed, F.; Turnbaugh, P.; Lozupone, C.A.; Knight, R.D.; Gordon, J.I. Obesity Alters Gut Microbial Ecology. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 11070–11075. [CrossRef] [PubMed]
5. Pascale, A.; Marchesi, N.; Govoni, S.; Coppola, A.; Gazzaruso, C. The Role of Gut Microbiota in Obesity, Diabetes Mellitus, and Effect of Metformin: New Insights into Old Diseases. *Curr. Opin. Pharmacol.* **2019**, *49*, 1–5. [CrossRef] [PubMed]
6. Raza, M.H.; Gul, K.; Arshad, A.; Riaz, N.; Waheed, U.; Rauf, A.; Aldakheel, F.; Alduraywish, S.; Rehman, M.U.; Abdullah, M.; et al. Microbiota in Cancer Development and Treatment. *J. Cancer Res. Clin. Oncol.* **2019**, *145*, 49–63. [CrossRef] [PubMed]
7. Bhattarai, Y.; Muniz Pedrogo, D.A.; Kashyap, P.C. Irritable Bowel Syndrome: A Gut Microbiota-Related Disorder? *Am. J. Physiol. Gastrointest. Liver Physiol.* **2016**, *312*, G52–G62. [CrossRef]
8. Collins, S.M.; Surette, M.; Bercik, P. The Interplay between the Intestinal Microbiota and the Brain. *Nat. Rev. Microbiol.* **2012**, *10*, 735–742. [CrossRef]
9. Finegold, S.M. Desulfovibrio Species Are Potentially Important in Regressive Autism. *Med. Hypotheses.* **2011**, *77*, 270–274. [CrossRef]
10. Comas-Basté, O.; Sánchez-Pérez, S.; Veciana-Nogués, M.T.; Latorre-Moratalla, M.; Vidal-Carou, M.C. Histamine Intolerance: The Current State of the Art. *Biomolecules* **2020**, *10*, 1181. [CrossRef]
11. EFSA Panel on Biological Hazards (BIOHAZ). Scientific Opinion on Risk Based Control of Biogenic Amine Formation in Fermented Foods. *EFSA J.* **2011**, *9*, 2393. [CrossRef]
12. Hrubisko, M.; Danis, R.; Huorka, M.; Wawruch, M. Histamine Intolerance—The More We Know the Less We Know. *Nutrients* **2021**, *13*, 2228. [CrossRef] [PubMed]
13. Nazar, W.; Plata-Nazar, K.; Sznurkowska, K.; Szlagatys-Sidorkiewicz, A. Histamine Intolerance in Children: A Narrative Review. *Nutrients* **2021**, *13*, 1486. [CrossRef] [PubMed]
14. Shulpekova, Y.O.; Nechaev, V.M.; Popova, I.R.; Deeva, T.A.; Kopylov, A.T.; Malsagova, K.A.; Kaysheva, A.L.; Ivashkin, V.T. Food Intolerance: The Role of Histamine. *Nutrients* **2021**, *13*, 3207. [CrossRef]
15. Schnedl, W.J.; Lackner, S.; Enko, D.; Schenk, M.; Holasek, S.J.; Mangge, H. Evaluation of Symptoms and Symptom Combinations in Histamine Intolerance. *Intest. Res.* **2019**, *17*, 427–433. [CrossRef]
16. García-Martín, E.; García-Menaya, J.; Sánchez, B.; Martínez, C.; Rosendo, R.; Agúndez, J.A.G. Polymorphisms of Histamine-Metabolizing Enzymes and Clinical Manifestations of Asthma and Allergic Rhinitis. *Clin. Exp. Allergy* **2007**, *37*, 1175–1182. [CrossRef]
17. Maintz, L.; Yu, C.F.; Rodríguez, E.; Baurecht, H.; Bieber, T.; Illig, T.; Weidinger, S.; Novak, N. Association of Single Nucleotide Polymorphisms in the Diamine Oxidase Gene with Diamine Oxidase Serum Activities. *Allergy* **2011**, *66*, 893–902. [CrossRef]

18. Leitner, R.; Zoernpfenning, E.; Missbichler, A. Evaluation of the Inhibitory Effect of Various Drugs/Active Ingredients on the Activity of Human Diamine Oxidase in Vitro. *Clin. Transl. Allergy* **2014**, *4*, 23. [CrossRef]
19. Enko, D.; Kriegshäuser, G.; Halwachs-Baumann, G.; Mangge, H.; Schnedl, W.J. Serum Diamine Oxidase Activity Is Associated with Lactose Malabsorption Phenotypic Variation. *Clin. Biochem.* **2017**, *50*, 50–53. [CrossRef]
20. Enko, D.; Meinitzer, A.; Mangge, H.; Kriegshaüser, G.; Halwachs-Baumann, G.; Reininghaus, E.Z.; Bengesser, S.A.; Schnedl, W.J. Concomitant Prevalence of Low Serum Diamine Oxidase Activity and Carbohydrate Malabsorption. *Can. J. Gastroenterol. Hepatol.* **2016**, *2016*, 4893501. [CrossRef]
21. Griauzdaitė, K.; Maselis, K.; Žvirblienė, A.; Vaitkus, A.; Jančiauskas, D.; Banaitytė-Baleišienė, I.; Kupčinskas, L.; Rastenytė, D. Associations between Migraine, Celiac Disease, Non-Celiac Gluten Sensitivity and Activity of Diamine Oxidase. *Med. Hypotheses* **2020**, *142*, 3–6. [CrossRef] [PubMed]
22. Schnedl, W.J.; Enko, D. Considering Histamine in Functional Gastrointestinal Disorders. *Crit. Rev. Food Sci. Nutr.* **2020**, *61*, 2960–2967. [CrossRef] [PubMed]
23. Schink, M.; Konturek, P.C.; Tietz, E.; Dieterich, W.; Pinzer, T.C.; Wirtz, S.; Neurath, M.F.; Zopf, Y. Microbial Patterns in Patients with Histamine Intolerance. *J. Physiol. Pharmacol.* **2018**, *69*, 579–593. [CrossRef]
24. Pugin, B.; Barcik, W.; Westermann, P.; Heider, A.; Wawrzyniak, M.; Hellings, P.; Akdis, C.A.; O'Mahony, L. A Wide Diversity of Bacteria from the Human Gut Produces and Degrades Biogenic Amines. *Microb. Ecol. Health Dis.* **2017**, *28*, 1353881. [CrossRef] [PubMed]
25. Maintz, L.; Novak, N. Histamine and Histamine Intolerance. *Am. J. Clin. Nutr.* **2007**, *85*, 1185–1196. [CrossRef] [PubMed]
26. Izquierdo-Casas, J.; Comas-Basté, O.; Latorre-Moratalla, M.L.; Lorente-Gascón, M.; Duelo, A.; Vidal-Carou, M.C.; Soler-Singla, L. Low Serum Diamine Oxidase (DAO) Activity Levels in Patients with Migraine. *J. Physiol. Biochem.* **2018**, *74*, 93–99. [CrossRef]
27. Gophna, U.; Sommerfeld, K.; Gophna, S.; Doolittle, W.F.; Veldhuyzen Van Zanten, S.J.O. Differences between Tissue-Associated Intestinal Microfloras of Patients with Crohn's Disease and Ulcerative Colitis. *J. Clin. Microbiol.* **2006**, *44*, 4136–4141. [CrossRef]
28. Wang, T.; Cai, G.; Qiu, Y.; Fei, N.; Zhang, M.; Pang, X.; Jia, W.; Cai, S.; Zhao, L. Structural Segregation of Gut Microbiota between Colorectal Cancer Patients and Healthy Volunteers. *ISME J.* **2012**, *6*, 320–329. [CrossRef]
29. Krogius-Kurikka, L.; Lyra, A.; Malinen, E.; Aarnikunnas, J.; Tuimala, J.; Paulin, L.; Mäkivuokko, H.; Kajander, K.; Palva, A. Microbial Community Analysis Reveals High Level Phylogenetic Alterations in the Overall Gastrointestinal Microbiota of Diarrhoea-Predominant Irritable Bowel Syndrome Sufferers. *BMC Gastroenterol.* **2009**, *9*, 1–11. [CrossRef]
30. Morgan, X.C.; Tickle, T.L.; Sokol, H.; Gevers, D.; Devaney, K.L.; Ward, D.V.; Reyes, J.A.; Shah, S.A.; LeLeiko, N.; Snapper, S.B.; et al. Dysfunction of the Intestinal Microbiome in Inflammatory Bowel Disease and Treatment. *Genome Biol.* **2012**, *13*, R79. [CrossRef]
31. Litvak, Y.; Byndloss, M.X.; Tsolis, R.M.; Bäumler, A.J. Dysbiotic Proteobacteria Expansion: A Microbial Signature of Epithelial Dysfunction. *Curr. Opin. Microbiol.* **2017**, *39*, 1–6. [CrossRef] [PubMed]
32. Mou, Z.; Yang, Y.; Hall, A.B.; Jiang, X. The Taxonomic Distribution of Histamine-Secreting Bacteria in the Human Gut Microbiome. *BMC Genom.* **2021**, *22*, 695. [CrossRef] [PubMed]
33. Scheperjans, F.; Aho, V.; Pereira, P.A.B.; Koskinen, K.; Paulin, L.; Pekkonen, E.; Haapaniemi, E.; Kaakkola, S.; Eerola-Rautio, J.; Pohja, M.; et al. Gut Microbiota Are Related to Parkinson's Disease and Clinical Phenotype. *Mov. Disord.* **2015**, *30*, 350–358. [CrossRef] [PubMed]
34. La Reau, A.J.; Suen, G. The Ruminococci: Key Symbionts of the Gut Ecosystem. *J. Microbiol.* **2018**, *56*, 199–208. [CrossRef] [PubMed]
35. Cockburn, D.W.; Koropatkin, N.M. Polysaccharide Degradation by the Intestinal Microbiota and Its Influence on Human Health and Disease. *J. Mol. Biol.* **2016**, *428*, 3230–3252. [CrossRef]
36. Lopez-Siles, M.; Duncan, S.H.; Garcia-Gil, L.J.; Martinez-Medina, M. Faecalibacterium Prausnitzii: From Microbiology to Diagnostics and Prognostics. *ISME J.* **2017**, *11*, 841–852. [CrossRef] [PubMed]
37. Martín, R.; Miquel, S.; Benevides, L.; Bridonneau, C.; Robert, V.; Hudault, S.; Chain, F.; Berteau, O.; Azevedo, V.; Chatel, J.M.; et al. Functional Characterization of Novel Faecalibacterium Prausnitzii Strains Isolated from Healthy Volunteers: A Step Forward in the Use of F. Prausnitzii as a next-Generation Probiotic. *Front. Microbiol.* **2017**, *8*, 1–13. [CrossRef] [PubMed]
38. Laursen, M.F.; Laursen, R.P.; Larnkjær, A.; Mølgaard, C.; Michaelsen, K.F.; Frøkiær, H.; Bahl, M.I.; Licht, T.R. Faecalibacterium Gut Colonization Is Accelerated by Presence of Older Siblings. *mSphere* **2017**, *2*, 2–7. [CrossRef]
39. Durak-Dados, A.; Michalski, M.; Osek, J. Histamine and Other Biogenic Amines in Food. *J. Vet. Res.* **2020**, *64*, 281–288. [CrossRef]
40. Landete, J.M.; de Las Rivas, B.; Marcobal, A.; Muñoz, R. Updated Molecular Knowledge about Histamine Biosynthesis by Bacteria. *Crit. Rev. Food Sci. Nutr.* **2008**, *48*, 697–714. [CrossRef]
41. Refai, M.A.; El-Hariri, M.; Ahmed, Z.A.M.; Jakee, J. el Histamine Producing Bacteria in Fish. *Egypt. J. Aquat. Biol. Fish.* **2020**, *24*, 1–11. [CrossRef]
42. Comas-Basté, O.; Latorre-Moratalla, M.L.; Sánchez-Pérez, S.; Veciana-Nogués, M.T.; Vidal-Carou, M.C. Histamine and Other Biogenic Amines in Food. From Scombroid Poisoning to Histamine Intolerance. In *Biogenic Amines*; Proestos, C., Ed.; IntechOpen: London, UK, 2019. [CrossRef]
43. Krell, T.; Gavira, J.A.; Velando, F.; Fernández, M.; Roca, A.; Monteagudo-Cascales, E.; Matilla, M.A. Histamine: A Bacterial Signal Molecule. *Int. J. Mol. Sci.* **2021**, *22*, 6312. [CrossRef] [PubMed]
44. Sudo, N. Biogenic Amines: Signals Between Commensal Microbiota and Gut Physiology. *Front. Endocrinol.* **2019**, *10*, 504. [CrossRef] [PubMed]

45. Ferstl, R.; Frei, R.; Schiavi, E.; Konieczna, P.; Barcik, P.; Ziegler, M.; Lauener, R.P.; Chassard, C.; Lacroix, C.; Akdis, C.A. Histamine Receptor 2 Is a Key Influence in Immune Responses to Microbial Ligands. *J. Allergy Clin. Immunol.* **2014**, *134*, 741–744. [CrossRef]
46. O'Mahony, L.; Akdis, M.; Akdis, C.A. Regulation of the Immune Response and Inflammation by Histamine and Histamine Receptors. *J. Allergy Clin. Immunol.* **2011**, *128*, 1153–1162. [CrossRef]
47. Mishima, Y.; Ishihara, S. Molecular Mechanisms of Microbiota-Mediated Pathology in Irritable Bowel Syndrome. *Int. J. Mol. Sci.* **2020**, *21*, 8664. [CrossRef]
48. Barcik, W.; Pugin, B.; Westermann, P.; Rodriguez-Pérez, N.; Ferstl, R.; Wawrzyniak, M.; Smolinska, S.; Jutel, M.; Hessel, E.M.; Michalovich, D.; et al. Histamine-Secreting Microbes Are Increased in the Gut of Adult Asthma Patients. *J. Allergy Clin. Immunol.* **2016**, *138*, 1489–1491. [CrossRef]
49. Frei, R.; Ferstl, R.; Konieczna, P.; Ziegler, M.; Simon, T.; Rugeles, T.M.; Mailand, S.; Watanabe, T.; Lauener, R.; Akdis, C.A.; et al. Histamine Receptor 2 Modifies Dendritic Cell Responses to Microbial Ligands. *J. Allergy Clin. Immunol.* **2013**, *132*, 194–204. [CrossRef]
50. Levy, M.; Thaiss, C.A.; Zeevi, D.; Dohnalová, L.; Zilberman-Schapira, G.; Mahdi, J.A.; David, E.; Savidor, A.; Korem, T.; Herzig, Y.; et al. Microbiota-Modulated Metabolites Shape the Intestinal Microenvironment by Regulating NLRP6 Inflammasome Signaling. *Cell* **2015**, *163*, 1428–1443. [CrossRef]
51. Sanchis-Artero, L.; Martínez-Blanch, J.F.; Manresa-Vera, S.; Cortés-Castell, E.; Valls-Gandia, M.; Iborra, M.; Paredes-Arquiola, J.M.; Boscá-Watts, M.; Huguet, J.M.; Gil-Borrás, R.; et al. Evaluation of Changes in Intestinal Microbiota in Crohn's Disease Patients after Anti-TNF Alpha Treatment. *Cells* **2021**, *10*, 3188. [CrossRef]
52. Chen, C.C.; Chen, K.J.; Kong, M.S.; Chang, H.J.; Huang, J.L. Alterations in the Gut Microbiotas of Children with Food Sensitization in Early Life. *Pediatr. Allergy Immunol.* **2016**, *27*, 254–262. [CrossRef] [PubMed]
53. Azcarate-Peril, M.A.; Ritter, A.J.; Savaiano, D.; Monteagudo-Mera, A.; Anderson, C.; Magness, S.T.; Klaenhammer, T.R. Impact of Short-Chain Galactooligosaccharides on the Gut Microbiome of Lactose-Intolerant Individuals. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, E367–E375. [CrossRef] [PubMed]
54. Das, A.; O'Herlihy, E.; Shanahan, F.; O'Toole, P.W.; Jeffery, I.B. The Fecal Mycobiome in Patients with Irritable Bowel Syndrome. *Sci. Rep.* **2021**, *11*, 124. [CrossRef] [PubMed]
55. Wacklin, P.; Laurikka, P.; Lindfors, K.; Collin, P.; Salmi, T.; Lähdeaho, M.L.; Saavalainen, P.; Mäki, M.; Mättö, J.; Kurppa, K.; et al. Altered Duodenal Microbiota Composition in Celiac Disease Patients Suffering from Persistent Symptoms on a Long-Term Gluten-Free Diet. *Am. J. Gastroenterol.* **2014**, *109*, 1933–1941. [CrossRef] [PubMed]

3.5. Avaluació de potencials canvis en el perfil i composició de la microbiota intestinal en un petit subgrup de pacients al llarg del tractament dietètic de la intolerància a la histamina

PUBLICACIÓ 6

The dietary treatment of histamine intolerance reduces the abundance of some histamine-secreting bacteria of the gut microbiota in histamine intolerant women. A pilot study

Sònia Sánchez-Pérez, Oriol Comas-Basté, Adriana Duelo, M. Teresa Veciana-Nogués, Mercedes Berlanga, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou. Acceptada en la revista *Frontiers in Nutrition.*

Índex d'impacte (JCR 2021): 6,590
Posició en l'àrea "Nutrition & Dietetics": Q1 (16/90)

PLANTEJAMENT I OBJECTIU

Diferents estudis han avaluat l'efecte del seguiment de dietes restrictives, com per exemple, dietes baixes en gluten, en FODMAPS o en lactosa, en el perfil i la composició de la microbiota intestinal dels individus que les segueixen, observant-se una reducció en l'abundància de certs bacteris beneficiosos per a la salut intestinal, especialment dels gèneres *Bifidobacterium* i *Lactobacillus*. Com s'ha assenyalat anteriorment en l'apartat 3.4, els individus diagnosticats d'intolerància a la histamina van presentar una disbiosi intestinal, caracteritzada per un increment de certs grups bacterians relacionats amb la formació d'histamina (*Staphylococcus*, *Proteus*, gèneres pertanyents a la família *Enterobacteriaceae*, *Clostridium perfringens* i *Enterococcus faecalis*), així com per una pèrdua de bacteris amb potencials efectes beneficiosos per a la salut intestinal. No obstant, segons el nostre coneixement, no es disposa de cap informació sobre què succeeix amb la microbiota intestinal quan aquests pacients són tractats amb una dieta baixa en histamina, juntament amb una suplementació amb enzim DAO exogen. Per aquest

motiu, es va realitzar un primer estudi preliminar amb l'objectiu d'avaluar potencials canvis en la composició de la microbiota intestinal en un grup de cinc dones que segueixen el tractament dietètic per la intoleràncIa a la histamina.

METODOLOGIA

Aquest estudi pilot es va dur a terme amb un subgrup de cinc dones procedents de l'estudi anterior sobre la caracterització de la microbiota intestinal d'individus amb intoleràncIa a la histamina (apartat 3.4.). Aquestes dones eren pacients del centre de nutrició i dietètica especialitzat en la gestió dietètica del dèficit de DAO (Institut Clínic del Dèficit de DAO, Barcelona, Espanya), el qual va monitoritzar el seguiment del tractament dietètic de la intoleràncIa a la histamina (dieta baixa en histamina i suplementació amb DAO), així com l'evolució dels símptomes. A temps inicial (abans de l'inici del tractament dietètic) totes les pacients van mostrar una activitat DAO reduïda (<10 U/mL). En aquest mateix punt de l'estudi, i segons la informació aportada pel centre de nutrició, les manifestacions clíniques van ser principalment gastrointestinals (inflor abdominal, acidesa, flatulències, diarrea i dolor abdominal) seguit de cefalees i símptomes dermatològics.

El nostre estudi ha avaluat la composició de la microbiota intestinal d'aquestes pacients al llarg de nou mesos del seu tractament dietètic. Les mostres de femta van ser recollides a temps inicial i al final de cada fase de la dieta baixa en histamina (2, 6 i 9 mesos). En la primera fase o fase restrictiva d'aquesta dieta (dos mesos de durada), el centre de nutrició i dietètica va excloure els aliments susceptibles de contenir histamina o altres amines biògenes. En la segona fase (de quatre mesos) es va procedir a la reintroducció gradual d'alguns aliments exclosos, principalment aquells sense o amb baixos nivells d'histamina però amb continguts d'altres amines. Finalment, en la última fase, es van reintroduir la resta d'aliments segons la tolerància interindividual als continguts d'histamina per tal d'assolir una dieta el més equilibrada possible (en el nostre estudi, aquesta fase es va seguir durant tres

mesos). Al llarg de totes les fases, es va administrar una suplementació amb DAO exogen d'origen porcí 20 minuts abans dels principals àpats.

La determinació de la composició de la microbiota intestinal es va dur a terme mitjançant la seqüenciació de les regions V3-V4 d'ARNr 16S bacterià a través de la plataforma Illumina MiSeq (Servei Genòmic i Bioinformàtic de la Universitat Autònoma de Barcelona). Els anàlisis bioinformàtics es van realitzar amb la base de dades EzBioCloud (ChunLab, Inc., Seul, Corea). L'alfa-diversitat es va mesurar utilitzant l'índex de Shannon i l'índex de Simpson. La determinació de la beta-diversitat es va realitzar mitjançant l'índex de dissimilitud de Bray-Curtis representat en un anàlisi de coordinades principals (PCoA).

L'estudi va ser aprovat pel Comitè de Bioètica de la Universitat de Barcelona (IRB00003099).

RESULTATS

En base a la informació aportada pel centre de nutrició, després del tractament dietètic tots les pacients van presentar una reducció en el nombre de símptomes, encara que en cap cas es va arribar a una remissió total de la simptomatologia.

Pel que fa a la composició de la microbiota intestinal, totes les pacients amb intolerància a la histamina considerades en aquest estudi presentaven una disbiosi intestinal abans de començar el tractament dietètic en comparació amb el grup control de l'anterior estudi (apartat 3.4.). Al llarg del tractament dietètic, a nivell de fílums es va observar que, 4 de les 5 pacients van mostrar un perfil similar ($p>0,05$), essent Firmicutes i Bacteroides els fílums amb una major abundància. Excepcionalment, una pacient va mostrar una elevada proporció del fílum Proteobacteria a temps inicial (24%) en comparació amb la resta de les pacients (1% - 2,5%). Després de dos mesos de tractament dietètic, es va observar una reducció de fins al 90% en l'abundància relativa de Proteobacteria en aquesta pacient.

Pel que fa a les famílies bacterianes, es van detectar diferències significatives en un total de 21 grups, tots ells amb una abundància relativa per sota de l'1%. A més, es va trobar una reducció en el nombre total de famílies, que van disminuir des de les 79 a l'inici de l'estudi fins a les 58 als nou mesos de tractament dietètic. La majoria de les famílies bacterianes que es van reduir amb el temps de tractament, estaven relacionades amb efectes no desitjables, com per exemple, *Morganellaceae* i *Pseudomanadaceae*, ambdós descrites per contenir bacteris amb una elevada capacitat histaminogènica.

Als nou mesos de gestió dietètica es van trobar canvis estadísticament significatius en 44 gèneres i 64 espècies. Concretament, es va observar una reducció en l'abundància relativa de bacteris amb reconeguda capacitat per formar histamina. Per exemple, la presència del gènere *Proteus* i de l'espècie *Proteus mirabilis* va reduir-se dràsticament a partir del segon mes de tractament, trobant-se absents en totes les pacients a partir del sisè mes. *Raoutella*, un altre gènere bacterià descrit com a histaminogènic, va mostrar també una reducció estadísticament significativa. D'altra banda, totes les pacients d'aquest estudi van mostrar un increment en la proporció de *Roseburia spp.,* espècies amb capacitat de formar àcids grassos de cadena curta (AGCC) i, per tant, amb propietats beneficioses per l'organisme.

Pel que fa a la diversitat bacteriana, no es van observar canvis en cap de les pacients al llarg del tractament dietètic en termes d'alfa-diversitat (índex de Shannon i índex de Simpson, $p>0,05$) i de beta-diversitat (dissimilitud Bray-Curtis $p>0,05$). No obstant, i encara que sense significació estadística, als nous mesos d'estudi, tres de les cinc pacients van mostrar canvis en el perfil microbià, ressemblant-se més als perfils observats en el grup d'individus sans de l'anterior estudi (apartat 3.4.).

Aquest estudi preliminar presenta certes limitacions entre les quals destaquen la reduïda mida mostral i la falta d'un grup control sense intoleràncIa a la histamina que seguís el mateix tractament dietètic. També han de tenir-se en compte altres limitacions, com la falta de representació masculina, de dades sobre el consum d'aliments i sobre els canvis en la intensitat dels símptomes.

TAKE-HOME MESSAGE

El perfil i composició de la microbiota intestinal en dones amb intolerància a la histamina s'ha vist modificat durant el tractament dietètic d'aquesta intolerància, principalment en una reducció de la proporció de bacteris descrits com a formadors d'histamina. Aquests resultats corresponen a un petit estudi pilot i necessiten ser confirmats amb més treballs que incloguin un major nombre de pacients.

Frontiers: Your manuscript is accepted - 1018463

Frontiers Nutrition Editorial Office <nutrition.editorial.office@frontiersin.org>
Mon 10/3/2022 17:23

To: Sònia Sánchez Pérez <soniasanchezperez@ub.edu>

Dear Dr Sánchez Pérez,

Frontiers Nutrition Editorial Office has sent you a message. Please click 'Reply' to send a direct response

I am pleased to inform you that your manuscript ""The dietary treatment of histamine intolerance reduces the abundance of some histamine-secreting bacteria of the gut microbiota in histamine intolerant women. A pilot study"" has been approved for production and accepted for publication in Frontiers in Nutrition, section Nutrition and Microbes. Proofs are being prepared for you to verify before publication. We will also perform final checks to ensure your manuscript meets our criteria for publication (https://www.frontiersin.org/about/review-system#ManuscriptQualityStandards). Please do not communicate any changes until you receive your proofs.

Any questions? Please visit our Production Help Center page for more information: https://zendesk.frontiersin.org/hc/en-us/categories/200397292-Article-Production-

You can click here to access the final review reports and manuscript: http://www.frontiersin.org/Review/EnterReviewForum.aspx?activationno=96c0c828-96ce-4c01-84bb-66b34a009da8

Kind Regards,

Your Frontiers in Nutrition team

Frontiers | Editorial Office - Collaborative Peer Review Team
www.frontiersin.org
Avenue du Tribunal Fédéral 34, 1005 Lausanne, Switzerland
Office T 41 21 510 1725

For technical issues, please contact our IT Helpdesk (support@frontiersin.org) or visit our Frontiers Help Center (zendesk.frontiersin.org/hc/en-us)

Manuscript title: The dietary treatment of histamine intolerance reduces the abundance of some histamine-secreting bacteria of the gut microbiota in histamine intolerant women. A pilot study

Journal: Frontiers in Nutrition, section Nutrition and Microbes

Article type: Original Research

Authors: Sònia Sánchez Pérez, Oriol Comas-Basté, Adriana Duelo, Teresa Veciana-Nogues, Mercedes Berlanga, M Carmen Vidal-Carou, M. Luz Latorre-Moratalla

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED xx xx 2022
DOI 10.3389/fnut.2022.1018463

Check for updates

OPEN ACCESS

EDITED BY
Balamurugan Ramadass,
All India Institute of Medical Sciences Bhubaneswar, India

REVIEWED BY
Mario Di Gioacchino,
University of Studies G. d'Annunzio Chieti and Pescara, Italy
Brantley Hall,
University of Maryland, College Park, United States

*CORRESPONDENCE
Oriol Comas-Basté
oriolcomas@ub.edu
M. Teresa Veciana-Nogués
veciana@ub.edu
M. Carmen Vidal-Carou
mcvidal@ub.edu
M. Luz Latorre-Moratalla
mariluzlatorre@ub.edu

SPECIALTY SECTION
This article was submitted to Nutrition and Microbes, a section of the journal Frontiers in Nutrition

RECEIVED 13 August 2022
ACCEPTED 03 October 2022
PUBLISHED xx xx 2022

CITATION
Sánchez-Pérez S, Comas-Basté O, Duelo A, Veciana-Nogués MT, Berlanga M, Vidal-Carou MC and Latorre-Moratalla ML (2022) The dietary treatment of histamine intolerance reduces the abundance of some histamine-secreting bacteria of the gut microbiota in histamine intolerant women. A pilot study. *Front. Nutr.* 9:1018463. doi: 10.3389/fnut.2022.1018463

COPYRIGHT
© 2022 Sánchez-Pérez, Comas-Basté, Duelo, Veciana-Nogués, Berlanga, Vidal-Carou and Latorre-Moratalla. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

The dietary treatment of histamine intolerance reduces the abundance of some histamine-secreting bacteria of the gut microbiota in histamine intolerant women. A pilot study

Sònia Sánchez-Pérez[1,2,3], Oriol Comas-Basté[1,2,3]*, Adriana Duelo[1,2,3], M. Teresa Veciana-Nogués[1,2,3]*, Mercedes Berlanga[4], M. Carmen Vidal-Carou[1,2,3]* and M. Luz Latorre-Moratalla[1,2,3]*

[1]Departament de Nutrició, Ciències de l'Alimentació i Gastronomia, Facultat de Farmàcia i Ciències de l'Alimentació, Campus de l'Alimentació de Torribera, Universitat de Barcelona (UB), Santa Coloma de Gramenet, Spain, [2]Institut de Recerca en Nutrició i Seguretat Alimentària (INSA-UB), Universitat de Barcelona (UB), Santa Coloma de Gramenet, Spain, [3]Xarxa d'Innovació Alimentària (XIA), Barcelona, Spain, [4]Departament de Biologia, Sanitat i Mediambient, Secció de Microbiologia, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona (UB), Barcelona, Spain

Restrictive diets for the treatment of different gastrointestinal disorders are reported to change the composition of intestinal microbiota. Recently, it has been proposed that individuals with histamine intolerance suffer from intestinal dysbiosis, having an overabundance of histamine-secreting bacteria, but how it is still unknown this state is affected by the usual dietary treatment of histamine intolerance [i.e., low-histamine diet and the supplementation with diamine oxidase (DAO) enzyme]. Thus, a preliminary study was carried out aiming to evaluate the potential changes on the composition of the intestinal microbiota in a group of five women diagnosed with histamine intolerance undergoing 9 months of the dietary treatment of histamine intolerance. After sequencing bacterial 16S rRNA genes (V3-V4 region) and analyzing the data using the EzBioCloud Database, we observed a reduction in certain histamine-secreting bacteria, including the genera *Proteus* and *Raoultella* and the specie *Proteus mirabilis*. Moreover, it was also observed an increase in *Roseburia* spp., a bacterial group frequently related to gut health. These changes could help to explain the clinical improvement experienced by histamine intolerant women underwent a dietary treatment.

KEYWORDS

histamine intolerance, intestinal microbiota, gut dysbiosis, low-histamine diet, DAO supplementation, histamine-secreting bacteria

Introduction

Dietary habits are among the external factors that exert the most influence on the composition of the intestinal microbiota (1–3). To date, several studies have evaluated the impact of different macro and micronutrients on intestinal microbial patterns (4–6). It has been demonstrated that a high consumption of animal proteins, saturated fat, sugar, and salt could weaken the intestinal barrier due to a higher growth of certain pathogenic bacteria (1). On the contrary, a diet rich in complex polysaccharides and plant protein could be related to an increase of certain health-related bacteria capable of stimulating the production of short-chain fatty acids (SCFA) in the intestines (1).

Several disorders affecting the gastrointestinal tract, such as food intolerances, irritable bowel syndrome (IBS) or celiac disease, can be managed by following highly restrictive diets (7–11). However, over time, these diets can have a negative impact on the composition of the intestinal microbiota, although this is a more complex relationship (11–15). In a recent study by Lenhart et al. IBS patients following low-FODMAP (Fermentable Oligosaccharides, Disaccharides, Monosaccharides, and Polyols), gluten-free or dairy-free diets showed significant differences in bacterial beta diversity and a reduction in the abundance of *Bifidobacterium*, *Lactobacillus,* and *Prevotella* genera (13). Similarly, De Palma et al. reported a reduction in the genera *Bifidobacterium* and *Lactobacillus* in healthy individuals following a gluten-free diet (14). These unwanted effects could be explained by the exclusion of FODMAPs and lactose, described as dietary carbohydrates with prebiotic actions, and gluten-containing foods such as wheat or barley, which are a source of prebiotic fructans (15–17).

Histamine intolerance is a food-related disorder caused by impaired histamine degradation at the intestinal level due to a deficiency in the enzyme diamine oxidase (DAO) (10). Affected individuals suffer a wide range of gastrointestinal and extraintestinal symptoms, such as bloating, diarrhea, abdominal pain, postprandial fullness, constipation, flatulences, headache, tachycardia, hypotonia, pruritus, eczema, urticaria, rhinitis, and nasal congestion. These manifestations usually appear after the consumption of foods containing histamine and/or other biogenic amines (18). In the last decade, new evidence regarding the aetiopathogenesis of histamine intolerance has been published (19–23). Several single nucleotide polymorphisms encoding a DAO enzyme with reduced histamine degradation capacity have been described as potential genetic causes of histamine intolerance (19, 23). Moreover, impaired DAO activity can also be temporary and reversible, being secondary to certain gastrointestinal disorders or as a side effect of some widely used pharmacological drugs (20). More recently, two studies have suggested that dysbiosis of the intestinal microbiota may play a role in this condition (24, 25). In 2018, Schink et al. demonstrated that patients with symptoms of histamine intolerance have an imbalance of the gut microbiota and an impaired intestinal barrier, which could lead to a deficiency in DAO catabolic activity (24). According to these authors, intestinal dysbiosis may contribute to mucosal inflammation and, in turn, favor the development of a leaky gut with the subsequent potential reduction of DAO enzymatic activity. Intestinal inflammation affecting mucosal integrity have been identified as a cause of low DAO activity by other previous works (26–28). In addition, it may be hypothesized that intestinal dysbiosis could be related to histamine intolerance by a possible higher presence of histaminogenic bacteria that could favor the accumulation of histamine at intestinal level, as well as, by a lower presence of bacteria with histaminolytic activity. In fact, the study performed by Mou et al. identified a total of 117 species from the human gut microbiome with the ability to form histamine (29). Moreover, Sánchez-Pérez et al. recently reported a higher proportion of histamine-secreting bacteria (e.g., *Staphylococcus*, *Proteus*, *Clostridium perfringens,* and *Enterococcus faecalis*) in patients with histamine intolerance in comparison with a healthy control group. These authors also reported alterations in gut bacterial diversity in histamine intolerant individuals (25). It would be important to consider that an intestinal dysbiosis could not be, by itself, the only cause of histamine intolerance but would probably aggravate the symptoms derived from other primary causes of a DAO deficiency (genetic or pathological). In fact, a dysbiosis could help explain the varying severity of symptoms frequently reported in individuals with histamine intolerance (30).

The usual dietary management of histamine intolerance is the follow-up of a low-histamine diet. These diets are based on the exclusion of histamine-containing foods (10, 31). As this amine in foods is mainly formed by the bacterial decarboxylation of the precursor aminoacid histidine, the foods susceptible to contain high histamine levels are those fermented or microbiologically altered (by the action of the fermentative bacteria or spoilage bacteria, respectively) (32, 33). Thus, dry-fermented sausages, cured cheese, and other fermented products, together with preserved and semi-preserved fish derivatives, can easily accumulate high histamine levels. Moreover, certain other foods, such as citrus fruits, strawberry, banana and nuts, that do not contain histamine but may contain other biogenic amines (e.g., putrescine and/or cadaverine) are also frequently excluded within low-histamine diets (31). The presence of these other amines can exert an inhibitory effect on the degradation of histamine by DAO enzyme due to the competition for this degradation system (18). Finally, the dietary treatment of histamine intolerance also considers the supplementation with exogenous DAO enzyme to enhance the intestinal degradation of histamine (10, 31).

There is still a lack of information about what happens to the gut microbiota when histamine intolerant patients are treated with a restrictive low-histamine diet and DAO supplementation. Therefore, a first preliminary study was carried out with the

aim to evaluate potential changes on the composition of the intestinal microbiota in a group of five women undergoing the dietary treatment of histamine intolerance.

Materials and methods

Study design

A previous study was conducted in histamine intolerant patients and healthy individuals to characterize and compare the intestinal microbiota composition (25). The current pilot study was carried out with a subgroup of five women from the histamine intolerant group who presented at least three symptoms associated with histamine intolerance. The inclusion criteria were as follows: age between 18 and 65 years; diagnosis of histamine intolerance based on two or more symptoms and negative results for food allergen-specific IgE. The exclusion criteria were pregnancy, lactation, having started a low-histamine diet, and having taken antibiotics and/or probiotics the month before the study. Considering that the female sex seems prevalently affected by histamine intolerance (34), five women were included in the present pilot study. These women were patients from a nutrition and dietetic center specialized in the dietary management of histamine intolerance (DAO Deficiency Clinical Institute, Barcelona, Spain), where the follow-up of the dietary treatment, as well as the evolution of the symptomatology, were monitored. Table 1 displays the baseline characteristics of each patient (age, serum DAO activity, and symptoms). All women showed deficiency in serum DAO activity. The reported clinical manifestations were mainly gastrointestinal (i.e., bloating, heart burn, flatulence, diarrhea, and abdominal pain), followed by headache and dermatological complaints, such as pruritus and eczema. One patient also reported insomnia, muscular pain, and articular pain.

The current study aimed to evaluate the microbiota composition of these patients along 9-month of this dietary treatment (low-histamine diet and DAO supplementation). Figure 1 summarizes the study protocol, in which a total of four stool samples of each patient were collected: at baseline (before starting the dietary treatment) and at 2, 6, and 9 months of the study. Moreover, Figure 1 also details the phases of the dietary treatment prescribed by the nutrition and dietetic center specialized in the dietary management of histamine inolerance. The first phase was the shortest but also the most restrictive, involving the exclusion of all foods with histamine, and/or other biogenic amines. In the second phase, some excluded foods, mainly those without or with low histamine levels but containing other biogenic amines, were gradually reintroduced. Finally, in the last phase, the rest of excluded foods were progressively reintegrated as much as possible according to interindividual tolerance to histamine content in order to achieve a properly long-term balanced diet. During all phases, DAO supplements formulated with porcine kidney protein extract were administered 20 min prior to each main meal (Figure 1).

TABLE 1 Baseline characteristics of each patient with histamine intolerance.

Patient	Age (years)	DAO activity (U/mL)	Clinical symptoms
1	65	9.55	Headache, abdominal bloating, and heart burn.
2	31	6.50	Diarrhea, flatulences, and abdominal bloating.
3	44	9.80	Headache, diarrhea, abdominal bloating, articular pain, muscular pain, and insomnia
4	27	7.70	Diarrhea, abdominal pain, flatulences, and abdominal bloating.
5	56	9.15	Headache, eczema, and pruritus.

Plasma DAO activity was also analyzed using a Radio Extraction Assay (REA) according to the manufacturer's instructions (Sciotec Diagnostic Technologies, Tulln, Austria). Values below 10 U/mL (Histamine Degrading Units) are considered as DAO deficient.

All participants were given detailed information about the aim and procedure of the study and gave their written informed consent prior to study inclusion. The study was approved by the Bioethics Committee of the University of Barcelona (IRB00003099).

Determinations in stool samples

The composition of the gut microbiota was determined by isolation of the bacterial DNA from stool samples (QIAamp Power Fecal Pro DNA kit, QIAGEN, Germantown, MD, USA) and subsequent sequencing of the V3-V4 region of 16S rRNA (Illumina MiSeq platform) at the Genomic and Bioinformatic Service of the Autonomous University of Barcelona. Bioinformatics analysis of the microbiota composition was performed with the EzBiocloud Database (ChunLab, Inc., Seoul, Korea). For the 16S rRNA amplicons, baseline sequence data were previously deposited in the NCBI database by the Bioproject PRJNA811749 (25). The sequence data related to months 2, 6, and 9 of the dietary treatment were deposited in the NCBI database by the Bioproject PRJNA842201.

Statistical analysis

Differences in the microbiota composition during the 9-month study were analyzed by the Kruskal–Wallis test for

non-parametric data. Alpha diversity was measured using the Shannon index and Simpson's index, and beta diversity was calculated by Bray–Curtis dissimilarity analysis and visualized using principal coordinates analysis (PCoA). p-values of $p < 0.05$ were considered statistically significant.

Results and discussion

The follow-up of the improvement of the symptoms derived from the dietary treatment of each patient was carried out by the nutritionist of the dietetic center specialized in the dietary management of histamine intolerance. According to this information, all patients experienced a general improvement in the baseline symptoms. Gastrointestinal manifestations (i.e., bloating, diarrhea, flatulences, abdominal pain, and heart burn) were those that mainly disappeared during the dietary treatment most of them already from the second month. Headache improved in two out of the three patients that reported this symptom. Despite these improvements, any patient achieved a total remission of clinical manifestations.

Regarding the composition of the intestinal microbiota, a gut dysbiosis was evidenced in all the recruited women when compared with the results obtained for a group of

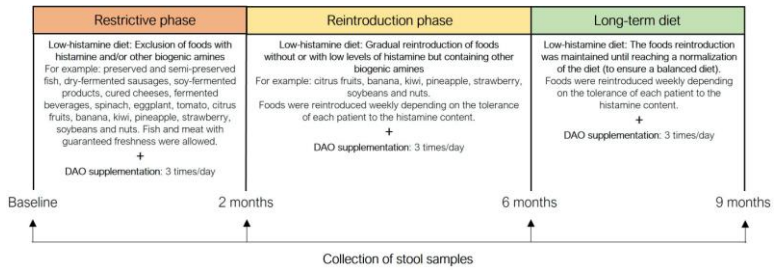


FIGURE 1
The different phases of the dietary treatment and stool sample collection during the 9-month study.

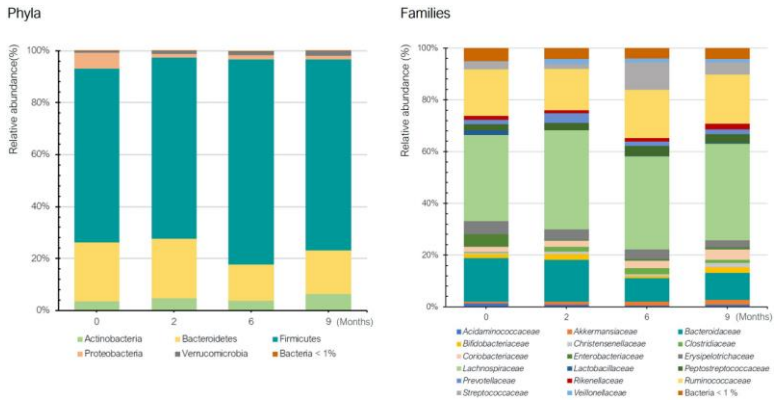


FIGURE 2
Relative abundance (%) of bacteria at phylum and family levels in histamine intolerant patients during the 9-month dietary management.

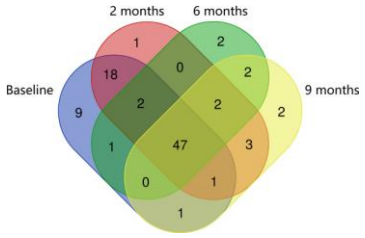


FIGURE 3
Venn diagrams of specific and shared Operational Taxonomic Units (OTUs) detected at the family level at baseline, 2, 6, and 9 months of the study.

healthy individuals used as a control group in the previous study performed by Sánchez-Pérez et al. (25). The observed statistically differences in the relative abundance of bacterial families, genera, and species are shown in **Supplementary Table 1**. In comparison with the control group, histamine intolerant patients showed a significantly higher relative abundance of some bacteria related with histamine-forming capacity (*Morganellaceae*, *Pseudomonadaceae*, *Staphylococcus*, *Proteus*, *Proteus mirabillis,* and *Clostridium perfringens*), as well as a reduction in the relative abundance of the genus *Faecalibacterium*, associated to a healthy gut (**Supplementary Table 1**).

Figure 2 shows the mean relative abundance of bacteria at phylum and family levels in the histamine intolerant patients at baseline and during the dietary treatment (low-histamine diet and DAO supplementation). A similar phylum pattern was observed for all patients at all sampling points, Firmicutes and Bacteroides having the highest relative abundance (**Figure 2**). Overall, no statistically significant differences were found in phyla during the dietary management ($p > 0.05$). The high relative abundance of the phylum Proteobacteria observed at baseline is accounted for by the abnormal abundance of these bacteria in one specific patient (24%) in comparison with the other patients (1–2.5%). It is worth highlighting that this patient (patient 3) also had the highest number of symptoms (**Table 1**). An overgrowth of Proteobacteria has been observed in patients with different intestinal disorders, such as Crohn's disease, ulcerative colitis, IBS and colorectal cancer, and has been postulated as a hallmark of dysbiosis (35–39). A marked reduction (90%) in the relative abundance of Proteobacteria was observed in this patient after 2 months of dietary treatment, when the values were similar to those of the other histamine intolerant patients. Changes in the abundance of Proteobacteria have been previously related to dietary habits (40). Levine et al. also reported a reduced relative abundance of this phylum in pediatric patients with Crohn's disease after they followed a restrictive diet (i.e., excluding foods containing wheat, dairy, animal fats, and additives) and received enteral nutrition (41).

Regarding bacterial families, the distribution pattern in the histamine intolerant patients showed few variations during the dietary treatment (**Figure 2**). Nevertheless, statistically significant changes were observed in the relative abundance of 21 bacterial families, all of them with relative abundances below 1%. These bacterial families are listed in **Supplementary Table 2**. Moreover, a reduction on the total number of families was found, decreasing from a

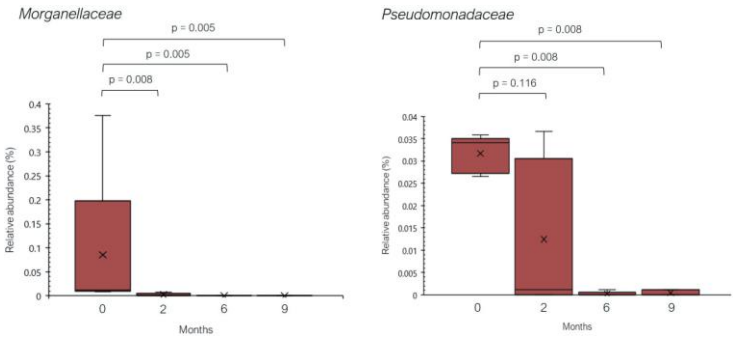


FIGURE 4
Relative abundance (%) of *Morganellaceae* and *Pseudomonadaceae* observed in histamine intolerant patients during the dietary treatment. Mean values are indicated by an ×. $p < 0.05$ indicates statistically significant differences.

10.3389/fnut.2022.1018463

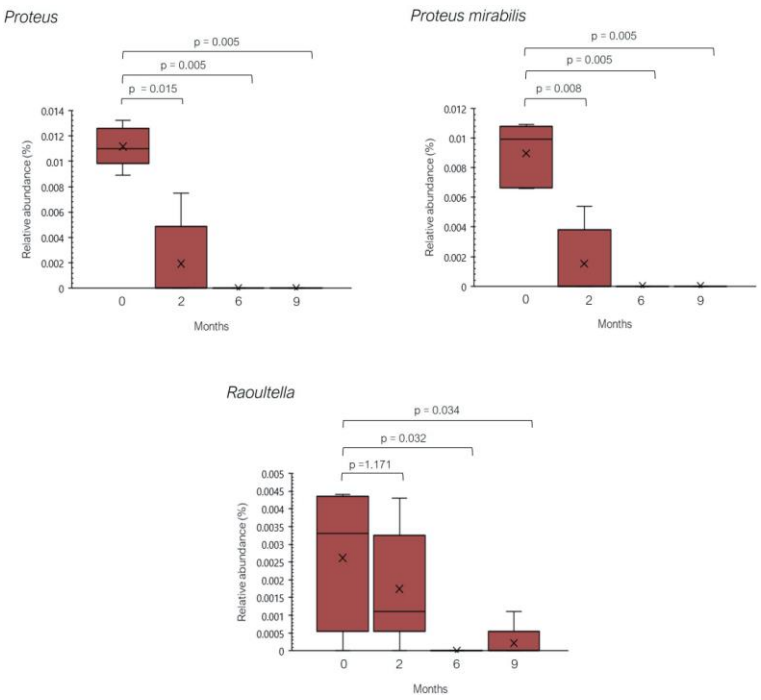


FIGURE 5
Relative abundance (%) of *Proteus*, *Proteus mirabilis*, and *Raoultella* observed in histamine intolerant patients during the dietary management. Mean values are represented with an × . $p < 0.05$ indicates statically significant differences.

total of 79 bacterial families at baseline to 58 at 9 months, some of them related to non-desirable effects (**Figure 3** and **Supplementary Table 2**). For example, the family *Morganellaceae* was considerably reduced in all patients at the second month of the study and absent at 6 months (**Figure 4**). *Morganellaceae* includes bacterial species with a high histamine-formation capacity, such as *Morganella morganii* (29, 42–44). *Pseudomonadaceae* also showed a marked reduction in the relative abundance, especially after 6 months of dietary management (**Figure 4**). High levels of pseudomonas bacteria have been associated with cases of inflammatory bowel disease, also being a bacterial family with several strains related with histamine production (45–50).

In the previous study performed by Sánchez-Pérez et al. a greater relative abundance of several histaminogenic bacteria, mainly belonging to the family *Enterobacteriaceae* and to the genera *Staphylococcus* and *Proteus*, was reported in histamine intolerant individuals in contrast with the healthy control group (25). Thus, an excessive accumulation of bacterial-derived histamine at the intestinal level could account for the onset of symptoms associated with histamine intolerance. In the present study, statistically significant changes were observed in the relative abundance of 44 genera and 64 species during the 9-month dietary treatment (**Supplementary Tables 3**, **4**). Among them, it is worth highlighting the significant reduction observed in the relative abundance of bacteria with recognized histamine-secreting ability (29, 42–44, 51, 52). For example, the relative abundance of the genus *Proteus* and the species *Proteus mirabilis* was dramatically reduced at the second month of the study, being absent in all patients after the sixth month (**Figure 5**). For

10.3389/fnut.2022.1018463

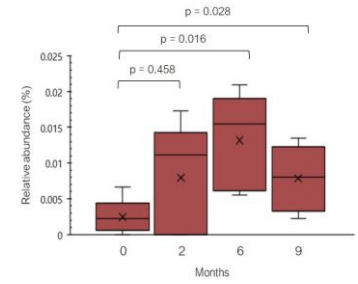


FIGURE 6
Relative abundance (%) of *Roseburia* spp. found in histamine intolerant patients during the dietary treatment. Mean values are represented with an × . $p < 0.05$ indicates statically significant differences.

the genus *Raoultella*, this reduction was statistically significant at 6 and 9 months. In fact, Mou et al. identified some species of the genus *Raoultella* with a putative histamine-secreting capacity within the human gut microbiome (29).

On the other hand, a significant increase in the relative abundance of *Roseburia* spp. was observed throughout the dietary treatment (**Figure 6**). In addition to the reduction of histamine-secreting bacteria, it can be speculated that the decline in number of symptoms reported by the five histamine intolerant women could be partially explained by the increase in *Roseburia* spp. Beneficial properties have been attributed to *Roseburia* species due to its capacity to produce SCFA, which are involved in the maintenance of intestinal homeostasis and the inhibition of a proinflammatory status (53–55). A high abundance of SCFA-producers, among them *Roseburia*, has been found in individuals on diets in which animal-derived products are replaced with plant-based foods (56–58). Low-histamine diets tend to be richer in plant-derived products, as they aim to reduce the consumption of meat and fish derivatives and cured and raw milk cheeses.

Finally, regarding bacterial diversity, no changes were observed for any patient during the dietary treatment in terms of alpha diversity (evaluated by the Shannon and Simpson indices) ($p > 0.05$). In the case of beta diversity (assessed by Bray-Curtis dissimilarity), no significant changes were observed in interindividual differences in the distribution patterns of genera and species along the dietary treatment. In the previous work performed by Sánchez-Pérez et al. statistical differences were observed in the beta diversity between intolerant individuals and the healthy group, this latter showing more homogenous microbial patterns among them (25). In the current study, after 9 months on a low-histamine diet associated with DAO supplementation, 3 out of 5 histamine intolerant patients showed a pattern of microbial species that clustered closely with that previously reported for healthy individuals (25).

These preliminary results prompt further studies to assess the positive effect of the dietary treatment on the gut microbiota composition in histamine intolerant patients, with a larger sample set and involving healthy individuals undergoing the same dietary treatment. The lack of male representation, of data on daily food consumption, as well as regarding the changes in symptom intensity, should also be addressed in future works.

Despite the limitations of the study, preliminary results suggest that the usual dietary treatment of histamine intolerance may influence the composition of intestinal microbiota in histamine intolerant women. According to our results, a reduction in the relative abundance of histamine-secreting bacteria such as *Pseudomonadaceae*, *Proteus*, *Proteus mirabilis*, and *Raoultella* was observed during the dietary treatment. These results would support the hypothesis that a reduction in histamine-secreting bacteria could diminish the accumulation and absorption of histamine at intestinal level and, subsequently, avoid the onset of symptoms. Likewise, it was observed an increase in *Roseburia* spp., bacteria related to an improvement of the inflammation of intestinal mucosa. According to our knowledge, this is the first time that a follow-up study has been performed to assess the influence of the dietary treatment of histamine intolerance on intestinal microbiota composition. The majority of the available studies that evaluate the efficacy of a low-histamine diet on the symptoms of this intolerance considered intervention periods of up to 3 months while in this study, despite the low number of patients, 9-months of treatment were considered. Further studies with a higher number of patients are needed to better understand the relationship between the dietary treatment of histamine intolerance and changes in intestinal microbiota composition. In addition, considering the existence of certain technological bacterial strains with DAO activity it would be interesting to explore the use of DAO-positive probiotic bacteria for the preventive treatment of histamine intolerance (59).

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, Bioproject PRJNA842201.

Ethics statement

The studies involving human participants were reviewed and approved by the Bioethics Committee of the University of

Barcelona (IRB00003099). The patients/participants provided their written informed consent to participate in this study.

Author contributions

MV-N, ML-M, and MV-C: conceptualization. SS-P, OC-B, and AD: investigation. SS-P, OC-B, MB, and ML-M: data curation and writing—original draft preparation. SS-P, OC-B, AD, MV-N, MB, ML-M, and MV-C: writing—review and editing. ML-M and MV-C: supervision. All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by MICIN/AEI/10.13039/501100011033 (grant no. PID2020-117670RB-C21).

Acknowledgments

SS-P was a recipient of a doctoral fellowship from the University of Barcelona (APIF2018).

References

1. Rinninella E, Cintoni M, Raoul P, Lopetuso LR, Scaldaferri F, Pulcini G, et al. Food components and dietary habits: keys for a healthy gut microbiota composition. *Nutrients.* (2019) 11:2393. doi: 10.3390/nu11102393

2. Power SE, O'Toole PW, Stanton C, Ross RP, Fitzgerald GF. Intestinal microbiota, diet and health. *Br J Nutr.* (2014) 111:387–402. doi: 10.1017/S0007114513002560

3. Salonen A, de Vos WM. Impact of diet on human intestinal microbiota and health. *Ann Rev Food Sci Technol.* (2014) 5:239–62. doi: 10.1146/annurev-food-030212-182554

4. Singh A, Zapata RC, Pezeshki A, Reidelberger RD, Chelikani PK. Inulin fiber dose-dependently modulates energy balance, glucose tolerance, gut microbiota, hormones and diet preference in high-fat-fed male rats. *J Nutr Biochem.* (2018) 59:142–52. doi: 10.1016/j.jnutbio.2018.05.017

5. Wolters M, Ahrens J, Romaní-Pérez M, Watkins C, Sanz Y, Benítez-Páez A, et al. Dietary fat, the gut microbiota, and metabolic health – a systematic review conducted within the my new gut project. *Clin Nutr.* (2019) 38:2504–20. doi: 10.1016/j.clnu.2018.12.024

6. Rinninella E, Mele MC, Merendino N, Cintoni M, Anselmi G, Caporossi A, et al. The role of diet, micronutrients and the gut microbiota in age-related macular degeneration: new perspectives from the gut–retina axis. *Nutrients.* (2018) 10:1677. doi: 10.3390/nu10111599

7. Fassio F, Facioni MS, Guagnini F. Lactose maldigestion, malabsorption, and intolerance: a comprehensive review with a focus on current management and future perspectives. *Nutrients.* (2018) 10:1599. doi: 10.3390/nu10111599

8. Bardisi BM, Halawani AKH, Halawani HKH, Alharbi AH, Turkostany NS, Alrehaili TS, et al. Efficiency of diet change in irritable bowel syndrome. *J Family Med Prim Care.* (2018) 7:946–51. doi: 10.4103/jfmpc.jfmpc_173_18

9. Sansotta N, Amirikian K, Guandalini S, Jericho H. Celiac disease symptom resolution: effectiveness of the gluten-free diet. *J Pediatr Gastroenterol Nutr.* (2018) 66:48–52. doi: 10.1097/MPG.0000000000001634

10. Comas-Basté O, Sánchez-Pérez S, Veciana-Nogués MT, Latorre-Moratalla M, Vidal-Carou MC. Histamine intolerance: the current state of the art. *Biomolecules.* (2020) 10:1–26. doi: 10.3390/biom10081181

11. Staudacher HM, Whelan K, Irving PM, Lomer MCE. Comparison of symptom response following advice for a diet low in fermentable carbohydrates (FODMAPs) versus standard dietary advice in patients with irritable bowel syndrome. *J Hum Nutr Diet.* (2011) 24:487–95. doi: 10.1111/j.1365-277X.2011.01162.x

12. Starz E, Wzorek K, Folwarski M, Kaźmierczak-Siedlecka K, Stachowska L, Przewłócka K, et al. The modification of the gut microbiota via selected specific diets in patients with crohn's disease. *Nutrients.* (2021) 13:2125. doi: 10.3390/nu13072125

13. Lenhart A, Dong T, Joshi S, Jaffe N, Choo C, Liu C, et al. Effect of exclusion diets on symptom severity and the gut microbiota in patients with irritable bowel syndrome. *Clin Gastroenterol Hepatol.* (2022) 20:465–83. doi: 10.1016/j.cgh.2021.05.027

14. De Palma G, Nadal I, Collado MC, Sanz Y. Effects of a gluten-free diet on gut microbiota and immune function in healthy adult human subjects. *Br J Nutr.* (2009) 102:1154–60. doi: 10.1017/S0007114509371767

15. Gibson PR, Halmos EP, Muir JG. Review article: fodmaps, prebiotics and gut health-the fodmap hypothesis revisited. *Aliment Pharmacol Ther.* (2020) 52:233–46. doi: 10.1111/apt.15818

16. Salli K, Anglenius H, Hirvonen J, Hibberd AA, Ahonen I, Saarinen MT, et al. The effect of 2'-fucosyllactose on simulated infant gut microbiome and metabolites; a pilot study in comparison to gos and lactose. *Sci Rep.* (2019) 9:13232. doi: 10.1038/s41598-019-49497-z

17. Jackson FW. Effects of a gluten-free diet on gut microbiota and immune function in healthy adult human subjects - comment by jackson. *Br J Nutr.* (2010) 104:773. doi: 10.1017/S0007114510001960

18. Sánchez-Pérez S, Comas-Basté O, Costa-Catala J, Iduriaga-Platero I, Veciana-Nogués MT, Vidal-Carou MC, et al. The rate of histamine degradation by diamine oxidase is compromised by other biogenic amines. *Front Nutr.* (2022) 9:897028.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.1018463/full#supplementary-material

19. Maintz L, Yu CF, Rodríguez E, Baurecht H, Bieber T, Illig T, et al. Association of single nucleotide polymorphisms in the diamine oxidase gene with diamine oxidase serum activities. *Allergy*. (2011) 66:893–902. doi: 10.1111/j.1398-9995.2011.02548.x

20. Leitner R, Zoernpfenning E, Missbichler A. Evaluation of the inhibitory effect of various drugs / active ingredients on the activity of human diamine oxidase in vitro. *Clin Transl Allergy*. (2014) 4:23. doi: 10.1186/2045-7022-4-s3-p23

21. Enko D, Kriegshäuser G, Halwachs-Baumann G, Mangge H, Schnedl WJ. Serum diamine oxidase activity is associated with lactose malabsorption phenotypic variation. *Clin Biochem*. (2017) 50:50–3. doi: 10.1016/j.clinbiochem.2016.08.019

22. Schnedl WJ, Enko D. Considering histamine in functional gastrointestinal disorders. *Crit Rev Food Sci Nutr*. (2020) 61:2960–7. doi: 10.1080/10408398.2020.1791049

23. García-Martín E, García-Menaya J, Sánchez B, Martínez C, Rosendo R, Agúndez JAG. Polymorphisms of histamine-metabolizing enzymes and clinical manifestations of asthma and allergic rhinitis. *Clin Exp Allergy*. (2007) 37:1175–82. doi: 10.1111/j.1365-2222.2007.02769.x

24. Schink M, Konturek PC, Tietz E, Dieterich W, Pinzer TC, Wirtz S, et al. Microbial patterns in patients with histamine intolerance. *J Physiol Pharmacol*. (2018) 69:579–93. doi: 10.26402/jpp.2018.4.09

25. Sánchez-Pérez S, Comas-Basté O, Duelo A, Veciana-Nogués MT, Berlanga M, Latorre-Moratalla ML, et al. Intestinal dysbiosis in patients with histamine intolerance. *Nutrients*. (2022) 14:1774. doi: 10.3390/nu14091774

26. Fukudome I, Kobayashi M, Dabanaka K, Maeda H, Okamoto K, Okabayashi T, et al. Diamine oxidase as a marker of intestinal mucosal injury and the effect of soluble dietary fiber on gastrointestinal tract toxicity after intravenous 5-fluorouracil treatment in rats. *Med Mol Morphol*. (2014) 47:100–7. doi: 10.1007/s00795-013-0055-7

27. Mondovi B, Fogel WA, Federico R, Calinescu C, Mateescu MA, Rosa AC, et al. Effects of amine oxidases in allergic and histamine-mediated conditions. *Recent Pat. Inflamm Allergy Drug Discov*. (2013) 7:20–34.

28. Enko D, Meinitzer A, Mangge H, Kriegshaüser G, Halwachs-Baumann G, Reininghaus EZ, et al. Concomitant prevalence of low serum diamine oxidase activity and carbohydrate malabsorption. *Can J Gastroentero Hepatol*. (2016) 2016:1–4. doi: 10.1155/2016/4893501

29. Mou Z, Yang Y, Hall AB, Jiang X. The taxonomic distribution of histamine-secreting bacteria in the human gut microbiome. *BMC Genomics*. (2021) 22:695. doi: 10.1186/s12864-021-08004-3

30. Schnedl WJ, Lackner S, Enko D, Schenk M, Holasek SJ, Mangge H. Evaluation of symptoms and symptom combinations in histamine intolerance. *Intest Res*. (2019) 17:427–33. doi: 10.5217/ir.2018.00152

31. Sánchez-Pérez S, Comas-Basté O, Veciana-Nogués MT, Latorre-Moratalla ML, Vidal-Carou MC. Low-histamine diets: is the exclusion of foods justified by their histamine content? *Nutrients*. (2021) 13:1395. doi: 10.3390/nu13051395

32. European Food Safety Authority [EFSA]. Panel on biological hazard (BIOHAZ). Scientific opinion on risk based control of biogenic amine formation in fermented foods. *EFSA J*. (2011) 9:1–93. doi: 10.2903/j.efsa.2011.2393

33. Comas-Basté O, Latorre-Moratalla ML, Sánchez-Pérez S, Veciana-Nogués MT, Vidal-Carou MC. Histamine and other biogenic amines in food. In: Proestos C editor. *From scombroid poisoning to histamine intolerance*. Biogenic Amines, London: IntechOpen (2019).

34. Hrubisko M, Dani D, Huorka M, Wawruch M. Histamine intolerance – the more we know the less we know. *Nutrients*. (2021) 13:2228. doi: 10.3390/nu13072228

35. Gophna U, Sommerfeld K, Gophna S, Doolittle WF, Veldhuyzen van Zanten SJ. Differences between tissue-associated intestinal microfloras of patients with crohn's disease and ulcerative colitis. *J Clin Microbiol*. (2006) 44:4136–41. doi: 10.1128/JCM.01004-06

36. Wang T, Cai G, Qiu Y, Fei N, Zhang M, Pang X, et al. Structural segregation of gut microbiota between colorectal cancer patients and healthy volunteers. *ISME J*. (2012) 6:320–9. doi: 10.1038/ismej.2011.109

37. Krogius-Kurikka L, Lyra A, Malinen E, Aarnikunnas J, Tuimala J, Paulin L, et al. Microbial community analysis reveals high level phylogenetic alterations in the overall gastrointestinal microbiota of diarrhoea-predominant irritable bowel syndrome sufferers. *BMC Gastroenterol*. (2009) 9:1–1. doi: 10.1186/1471-230X-9-95

38. Morgan XC, Tickle TL, Sokol H, Gevers D, Devaney KL, Ward DV, et al. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. *Genome Biol*. (2012) 13:79. doi: 10.1186/gb-2012-13-9-r79

39. Litvak Y, Byndloss MX, Tsolis RM, Bäumler AJ. Dysbiotic *Proteobacteria* expansion: a microbial signature of epithelial dysfunction. *Curr Opin Microbiol*. (2017) 39:1–6. doi: 10.1016/j.mib.2017.07.003

40. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci USA*. (2010) 107:14691–6. doi: 10.1073/pnas.1005963107

41. Levine A, Wine E, Assa A, Sigall Boneh R, Shaoul R, Kori M, et al. Crohn's disease exclusion diet plus partial enteral nutrition induces sustained remission in a randomized controlled trial. *Gastroenterology*. (2019) 157:440–50. doi: 10.1053/j.gastro.2019.04.021

42. Bjornsdottir-Butler K, Bolton GE, Mcclellan-Green PD, Jaykus LA, Green DP. Detection of gram-negative histamine-producing bacteria in fish: a comparative study. *J Food Protec*. (2009) 72:1987–91. doi: 10.4315/0362-028x-72.9.1987

43. Bjornsdottir-Butler K, Jones JL, Benner R, Burkhardt W. Development of a real-time PCR assay with an internal amplification control for detection of gram-negative histamine-producing bacteria in fish. *Food Microbiol*. (2011) 28:356–63. doi: 10.1016/j.fm.2010.06.013

44. Ladero V, Calles-Enríquez M, Fernández M, Alvarez MA. Toxicological effects of dietary biogenic amines. *Curr Nutr Food Sci*. (2010) 6:145–56. doi: 10.2174/157340110791233256

45. Mukhopadhya I, Hansen R, El-Omar EM, Hold GL. IBD-what role do *Proteobacteria* play? *Nat Rev Gastroenterol Hepatol*. (2012) 9:219–30. doi: 10.1038/nrgastro.2012.14

46. Wagner J, Short K, Catto-Smith AG, Cameron DJS, Bishop RF, Kirkwood CD. Identification and characterization of *Pseudomonas* 16s ribosomal dna from ileal biopsies of children with Crohn's disease. *PLoS One*. (2008) 3:3578. doi: 10.1371/journal.pone.0003578

47. Parent K, Mitchell P. *Pseudomonas*-like group Va bacteria in Crohn's disease. *Gastroenterology*. (1978) 75:765.

48. Parent K, Mitchell P. Cell wall-defective variants of *Pseudomonas*-like (group Va) bacteria in Crohn's disease. *Gastroenterology*. (1978) 75:368–72. doi: 10.1016/0016-5085(78)90834-x

49. Wei B, Huang T, Dalwadi H, Sutton CL, Bruckner D, Braun J. *Pseudomonas fluorescens* encodes the crohn's disease-associated i2 sequence and t-cell superantigen. *Infect Immun*. (2002) 70:6567–75. doi: 10.1128/IAI.70.12.6567-6575.2002

50. Lavizzari T, Veciana-Nogués MT, Weingart O, Bover-Cid S, Mariné-Font A, Vidal-Carou MC. Occurrence of biogenic amines and polyamines in spinach and changes during storage under refrigeration. *J Agric Food Chem*. (2007) 55:9514–9. doi: 10.1021/jf0713071

51. Kanki M, Yoda T, Tsukamoto T, Shibata T. *Klebsiella pneumoniae* produces no histamine: *Raoultella planticola* and *Raoultella ornithinolytica* strains are histamine producers. *Appl Environ Microbiol*. (2002) 68:3462–6. doi: 10.1128/AEM.68.7.3462-3466.2002

52. Sękowska A. Raoultella spp.—clinical significance, infections and susceptibility to antibiotics. *Folia Microbiol*. (2017) 62:221–7. doi: 10.1007/s12223-016-0490-7

53. Tamanai-Shacoori Z, Smida I, Bousarghin L, Loreal O, Meuric V, Fong SB, et al. *Roseburia* spp.: a marker of health? *Future Microbiol*. (2017) 12:157–70. doi: 10.2217/fmb-2016-0130

54. Venegas DP, de La Fuente MK, Landskron G, González MJ, Quera R, Dijkstra G. Short chain fatty acids (SCFAs) mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. *Front Immunol*. (2019) 10:277. doi: 10.3389/fimmu.2019.00277

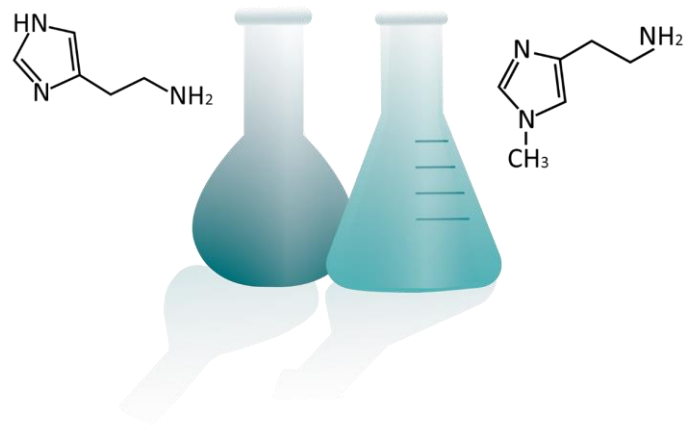
55. Fernández J, Redondo-Blanco S, Gutiérrez-del-Río I, Miguélez EM, Villar CJ, Lombó F. Colon microbiota fermentation of dietary prebiotics towards short-chain fatty acids and their roles as anti-inflammatory and antitumour agents: a review. *J Funct Foods*. (2016) 25:511–22. doi: 10.1016/j.jff.2016.06.032

56. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature*. (2014) 505:559–63. doi: 10.1038/nature12820

57. Tomova A, Bukovsky I, Rembert E, Yonas W, Alwarith J, Barnard ND. The effects of vegetarian and vegan diets on gut microbiota. *Front Nutr*. (2019) 6:47. doi: 10.3389/fnut.2019.00047

58. Dahl WJ, Rivero-Mendoza D, Lambert JM. Diet, nutrients and the microbiome. *Prog Mol Biol Transl Sci*. (2020) 171:237–63. doi: 10.1016/bs.pmbts.2020.04.006

59. Alvarez MA, Moreno-Arribas MV. The problem of biogenic amines in fermented foods and the use of potential biogenic amine-degrading microorganisms as a solution. *Trends Food Sci Technol*. (2014) 39:146–55. doi: 10.1016/j.tifs.2014.07.007



ÀMBIT 3

Intolerància a la histamina i perfil metabòlic en orina

Objectiu 6: Estudiar si els individus amb intolerància a la histamina mostren un perfil d'excreció de la histamina en orina significativament diferent al dels individus sans. Addicionalment, també es planteja avaluar, en un petit subgrup de pacients, el comportament dels nivells urinaris d'histamina i el seu metabòlit, 1-metilhistamina, durant nou mesos del tractament dietètic de la intolerància a la histamina.

3.6. Estudi del perfil d'eliminació d'histamina i 1-metilhistamina en orina en individus amb intolerància a la histamina i avaluació del seu comportament al llarg del tractament dietètic d'aquesta intolerància

PUBLICACIÓ 7

1-methylhistamine as a potential biomarker of food histamine intolerance. A pilot study.

Sònia Sánchez-Pérez, Ricard Celorio-Sardà, M. Teresa Veciana-Nogués, M. Luz Latorre-Moratalla, Oriol Comas-Basté i M. Carmen Vidal-Carou.

Frontiers in Nutrition, 2022, 9, 973682.

Índex d'impacte (JCR 2021): 6,590

Posició en l'àrea "Nutrition & Dietetics": Q1 (16/90)

PLANTEJAMENT I OBJECTIU

Actualment, el diagnòstic de la intolerància a la histamina es realitza mitjançant una combinació de criteris que inclou l'absència d'al·lèrgia alimentària i la presència de símptomes típics d'aquesta intolerància que afectin a dos o més sistemes o òrgans. Aquest diagnòstic se sol complementar amb la determinació de l'activitat DAO en sang o amb la identificació de polimorfismes genètics que afecten el gen que codifica l'enzim DAO, tot i que a dia d'avui l'evidència sobre la seva eficàcia és escassa.

En els últims anys, s'estan duent a terme estudis per identificar un marcador no invasiu que permeti establir un criteri diagnòstic sòlid i clínicament irrefutable de la intolerància a la histamina per dèficit de DAO. En aquest sentit, s'ha proposat l'ús de biomarcadors del metabolisme de la histamina en orina com una possible estratègia diagnòstica. La hipòtesi de treball és que els individus amb intolerància

a la histamina presenten un perfil d'excreció d'histamina i els seus metabòlits en orina diferent al dels individus sans. En un treball previ realitzat per Comas-Basté i col. (2017), es va desenvolupar un mètode cromatogràfic que permet determinar de manera ràpida i inequívoca els nivells urinaris d'histamina i 1-methilhistamina [99]. No obstant, resta pendent estudiar la possible utilitat diagnòstica del perfil metabòlic de la histamina en orina en fenotips d'individus intolerants.

L'objectiu d'aquest estudi va ser avaluar l'excreció urinària d'histamina i 1-methilhistamina en individus amb símptomes compatibles d'intolerància a la histamina i comparar-la amb la d'un grup control d'individus sans. Addicionalment, en un subgrup de pacients també es va avaluar el comportament d'ambdós analits durant nou mesos del tractament dietètic de la intolerància a la histamina.

METODOLOGIA

L'estudi es va dur a terme amb un total de 87 voluntaris, 32 dels quals eren individus amb intolerància a la histamina reclutats en un centre de nutrició i dietètica especialitzat en el tractament dietètic del dèficit de DAO (Institut Clínic del Dèficit de DAO, Barcelona). Els criteris d'inclusió per aquests pacients van ser: tenir entre 18 i 65 anys, un diagnòstic d'intolerància a la histamina basat en la presència de símptomes associats a aquesta intolerància en dos o més sistemes o òrgans i resultats negatius d'al·lèrgia alimentària per IgE. Es van excloure de l'estudi pacients embarassades o en període de lactància i aquells individus que havien començat una dieta baixa en histamina durant el mes anterior a l'inici de l'estudi. Setze dels pacients presentaven un dèficit en l'activitat DAO sanguínia. El grup control estava integrat per 55 adults sans sense símptomes d'intolerància a la histamina, reclutats en el Campus de l'Alimentació de la Universitat de Barcelona.

Per tal de poder valorar la fiabilitat de la recollida d'orina a primera hora del matí (puntual), en un subgrup de 14 voluntaris sans es va comparar l'excreció urinària d'histamina i 1-methilhistamina de primera hora amb la de mostres d'orina

recol·lectades durant 24 hores. A més, també es va avaluar l'excreció urinària dels dos analits en un subgrup de cinc pacients durant nou mesos de tractament dietètic amb una dieta baixa en histamina i suplementació amb enzim DAO exogen (punts de recollida de mostra: 2, 6 i 9 mesos).

Els nivells d'histamina i 1-methilhistamina es van analitzar segons el mètode analític prèviament descrit per Comas-Basté i col. (2017) que es basa en la hidròlisi àcida de les mostres d'orina seguida d'una etapa de purificació i concentració a través d'extracció en fase sòlida (SPE) [99]. La separació cromatogràfica es va dur a terme mitjançant UHPLC-FL. Els nivells d'histamina i 1-methilhistamina es van normalitzar en funció de la concentració de creatinina, determinada mitjançant el mètode colorimètric picrat alcalí [124].

L'estudi va ser aprovat pel Comitè de Bioètica de la Universitat de Barcelona (IRB00003099).

RESULTATS

Els pacients amb intolerància a la histamina van mostrar una mitja de 4,5 símptomes per individu i un 78% d'ells va reportar combinacions de més de 3 símptomes. Les manifestacions clíniques més freqüents van ser de caire gastrointestinal (94% dels pacients), principalment inflor abdominal, flatulències i dolor abdominal, seguit de manifestacions dermatològiques (74%), com per exemple, urticària i pell atòpica. Més del 80% van reportar la presència simultània de símptomes gastrointestinals i dermatològics. En cap cas es va poder correlacionar un major nombre de símptomes amb menor activitat DAO.

Pel que fa a la fiabilitat de la recollida de mostres d'orina puntuals en comparació amb les de 24 hores, no es van observar diferències estadísticament significatives en els valors mitjans d'histamina i 1-metilhistamina ($p>0,05$). De fet, existia una alta correlació en els continguts d'histamina i 1-methilhistamina entre ambdós tipus de mostres ($r=0,952$, $r=0,957$, respectivament).

Pel que fa al perfil d'excreció d'histamina en orina, no es van detectar diferències significatives entre ambdós grups d'estudi, amb valors mitjans de 18,65 ± 11,1 µg/g de creatinina pel grup d'intolerants a la histamina i 26,25 ± 22,2 µg/g de creatinina pel grup control ($p>0,05$). En el cas de la 1-methilhistamina, tot i la gran variabilitat interindividual observada, sí que es va poder demostrar una excreció significativament inferior en el grup de pacients amb intolerància a la histamina (70,29 ± 29,4 µg/g creatinina) en comparació amb el grup control (112,99 ± 55,5 µg/g creatinina) ($p<0,01$). A l'estratificar el grup de pacients amb intolerància a la histamina en funció de l'activitat DAO en sang, no es van trobar diferències significatives en els nivells d'histamina ($p>0,05$) ni 1-metilhistamina ($p>0,05$) entre els que presentaven o no aquest dèficit enzimàtic.

Finalment, pel que fa a l'estudi pilot sobre la influència del tractament dietètic de la intolerància a la histamina en un subgrup de pacients, als dos mesos de seguiment es va veure una reducció en els valors mitjans d'histamina i 1-methilhistamina, tot i que sense diferències significatives ($p>0,05$). En aquest punt, dos pacients van mostrar una reducció molt marcada d'ambdós analits (fins al 86% en el cas de la histamina i al 56% en el cas de la 1-metilhistamina). A partir del segon mes, l'excreció urinària dels analits es va mantenir força constant.

L'elevada variabilitat en els valors d'histamina i 1-methilhistamina en orina dificulta poder establir un llindar a partir del qual poder diferenciar individus sans i intolerants a la histamina. Seria necessari, doncs, realitzar estudis amb més individus i ampliar a altres metabòlits per tenir una imatge més complerta.

TAKE-HOME MESSAGE

Els individus amb símptomes d'intolerància a la histamina mostren un perfil d'excreció urinari diferenciat en comparació amb el grup control a causa d'una menor excreció urinària d'1-methilhistamina. Per tant, la determinació dels metabòlits de la histamina en orina, especialment la 1-methilhistamina, podria esdevenir una possible nova eina diagnòstica no invasiva que complementi els actuals criteris basats en l'avaluació dels simptomes.

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 12 October 2022
DOI 10.3389/fnut.2022.973682

Check for updates

OPEN ACCESS

EDITED BY
Francesco Alessandri,
Sapienza University of Rome, Italy

REVIEWED BY
Ramona Suharoschi,
University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania
Imke Reese,
Independent Researcher, Munich, Germany

*CORRESPONDENCE
M. Carmen Vidal-Carou
mcvidal@ub.edu

†These authors have contributed equally to this work and share first authorship

‡These authors have contributed equally to this work and share last authorship

SPECIALTY SECTION
This article was submitted to Nutrition and Metabolism, a section of the journal Frontiers in Nutrition

RECEIVED 20 June 2022
ACCEPTED 21 September 2022
PUBLISHED 12 October 2022

CITATION
Sánchez-Pérez S, Celorio-Sardà R, Veciana-Nogués MT, Latorre-Moratalla ML, Comas-Basté O and Vidal-Carou MC (2022) 1-methylhistamine as a potential biomarker of food histamine intolerance, A pilot study. *Front. Nutr.* 9:973682. doi: 10.3389/fnut.2022.973682

COPYRIGHT
© 2022 Sánchez-Pérez, Celorio-Sardà, Veciana-Nogués, Latorre-Moratalla, Comas-Basté and Vidal-Carou. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

1-methylhistamine as a potential biomarker of food histamine intolerance. A pilot study

Sònia Sánchez-Pérez[1,2,3†], Ricard Celorio-Sardà[1,2,3†], M. Teresa Veciana-Nogués[1,2,3], M. Luz Latorre-Moratalla[1,2,3], Oriol Comas-Basté[1,2,3‡] and M. Carmen Vidal-Carou[1,2,3*‡]

[1]Departament de Nutrició, Ciències de l'Alimentació i Gastronomia, Facultat de Farmàcia i Ciències de l'Alimentació, Campus de l'Alimentació de Torribera, Universitat de Barcelona, Santa Coloma de Gramenet, Spain, [2]Institut de Recerca en Nutrició i Seguretat Alimentària (INSA-UB), Universitat de Barcelona, Santa Coloma de Gramenet, Spain, [3]Xarxa d'Innovació Alimentària, Barcelona, Spain

Efforts are currently being directed to identify a non-invasive marker that can serve as a solid and clinically irrefutable diagnostic criterion for histamine intolerance associated with diamine oxidase (DAO) deficiency. Accordingly, the identification of biomarkers of histamine (HA) metabolism in urine is proposed as a possible new diagnostic strategy. It is hypothesized that individuals with histamine intolerance could have a different urinary profile of HA and its metabolites in comparison with the healthy population. Thus, the aim of this study was to assess the urinary excretion of HA and 1-methylhistamine (MHA) in individuals diagnosed with histamine intolerance and in a control group. Levels of HA and MHA were compared between 24 h and first morning spot urine in a subgroup of 14 control individuals. Then, HA and MHA concentrations in spot urine of 32 histamine intolerant and 55 control individuals were determined by ultra-high performance liquid chromatography and fluorometric detection (UHPLC-FL) and normalized by creatinine. No differences were found between HA and MHA levels in 24 h and first morning samples. Overall, histamine intolerant patients presented a distinct urinary excretion profile compared to the control group due to lower levels of MHA. No differences in urinary MHA were observed related to serum DAO activity. Spot urine samples were thus validated as a reliable tool to determine the urinary excretion of HA and MHA. These results constitute a starting point for the study of HA metabolomics as a suitable and non-invasive approach to histamine intolerance diagnosis.

KEYWORDS

histamine, 1-methylhistamine, histamine intolerance, diamine oxidase (DAO), DAO deficiency, food intolerance

10.3389/fnut.2022.973682

Introduction

Histamine (2-[4-imidazolyl] ethylamine), a bioactive amine with important physiological activities in the organism, can be found in many frequently consumed foods (1, 2). Dietary histamine (HA) is mainly formed by bacterial decarboxylation of the amino acid histidine. It is found especially in microbiologically spoiled or fermented products, generated by the enzymatic activity of the contaminating or technological bacteria (1, 3).

In healthy people, and under normal conditions, HA is metabolized by two main pathways. One involves oxidative deamination by the enzyme diamine oxidase (DAO) to form imidazole acetaldehyde, which is subsequently transformed into imidazoleacetic acid (IAA) by the action of aldehyde dehydrogenase (ALDH) and combined with a ribose for its excretion. Alternatively, the action of histamine-N-methyltransferase (HNMT) produces 1-methylhistamine (MHA), which is converted by ALDH to N-methylimidazoleacetic acid (MIAA) (2, 4). HNMT is expressed in almost all tissues, while DAO is active mainly in the intestines, kidneys, and placenta. At the intestinal level, DAO plays a key role in the degradation of histamine from food and regulates its passage into the systemic circulation (2, 4).

Histamine intolerance is a disorder arising from reduced histamine degradation capacity in the intestine due to impaired DAO activity, leading to its accumulation in plasma and the appearance of adverse effects. DAO deficiency may be inherited or due to inflammatory and degenerative intestinal disorders, the intake of DAO-blocking drugs or intestinal dysbiosis (5, 6). Histamine intolerant individuals usually show symptoms in two or more non-specific organs or systems, such as gastrointestinal tract (e.g., bloating, diarrhea, abdominal pain, postpandrial fullness, constipation, flatulences, and nausea) nervous system (e.g., headache and dizziness), cardiovascular system (e.g., tachycardia, hypotonia, and collapse), skin (e.g., pruritus, eczema, urticaria, edemas, and flushing) and respiratory system (e.g., rhinorrhea, rhinitis, nasal congestion, and dyspnea) (7). Preventing the onset of symptoms by dietary management involves following a low-histamine diet and supplementation with exogenous DAO (8, 9).

Histamine intolerance is currently diagnosed using a combination of criteria, including the absence of other potential causes of higher HA levels in the organism (i.e., food allergy and mastocytosis) and the presence of two or more typical symptoms, which improve or remit after following a low-histamine diet (5, 10–12). However, there is not a clear consensus in the diagnostic criteria for histamine intolerance. Frequently the diagnosis is complemented by the determination of serum DAO activity, although the evidence for the validity of this measurement is neither abundant nor conclusive (12–17).

In recent years, efforts have been made to identify a non-invasive marker that could serve as a solid and clinically irrefutable diagnostic criterion for DAO-related histamine intolerance. In this context some studies suggest the used of the determination of single-nucleotide polymorphisms related to a lower DAO activity, as well as the identification of biomarkers of histamine metabolism in urine as possible new diagnostic strategies (18). The hypothesis is that individuals with histamine intolerance may have a different excretion profile of HA and its metabolites in urine in comparison with healthy individuals. In a previous study, Comas-Basté et al. developed a Ultra-High Performance Liquid Chromatography (UHPLC) method that allows rapid and accurate analysis of HA and MHA in human urine (18). However, data for the potential diagnostic utility of the HA metabolomic profile in urine to identify histamine intolerant phenotypes are still lacking. Thus, the aim of this study was to analyze the urinary excretion of HA and MHA in individuals with symptoms compatible with histamine intolerance in comparison with a control group. Additionally, the behavior of HA and MHA urinary levels during 9 months of dietary treatment of histamine intolerance was studied in a smaller subgroup of patients.

Materials and methods

Study design and participants

A total of 87 adult volunteers participated in this study. Individuals with histamine intolerance (n = 32, mean age = 43.5 ± 8.8 years, 100% women) were recruited from a nutrition and dietetic center specialized in the dietary management of DAO deficiency (DAO Deficiency Clinical Institute, Barcelona, Spain). All the recruited patients keen to participate were women. In general, histamine intolerance is a clinical frame with a higher prevalence in women (19). The inclusion criteria for the histamine intolerant patients were as follows: aged between 18 and 65 years; diagnosis of histamine intolerance according to Mušič, et al. based on two or more symptoms; and negative results for food allergen-specific IgE (12). The exclusion criteria were pregnancy, lactation, having started a low-histamine diet and having taken antibiotics and/or probiotics the month before the study. Sixteen out of 32 patients with symptoms compatible with histamine intolerance also had plasma DAO deficiency, measured using a radio extraction assay according to the manufacturer's instructions (Sciotec Diagnostic Technologies, Tulln, Austria).

The individuals in the control group (n = 55, mean age = 32.3 ± 5.7 years, 64% women) were volunteers recruited at the Food and Nutrition Campus of the University of Barcelona (Santa Coloma de Gramenet, Barcelona) with no symptoms associated with histamine intolerance (total absence or the

presence of one symptom at most) and without other food intolerances or allergies.

As a preliminary phase, the excretion of HA and MHA was analyzed in urine samples collected over 24 h and in spot samples (first morning urine) in a subgroup of 14 healthy volunteers. For the analysis of urinary HA and MHA, spot samples of every participant were collected without the addition of preservatives and kept under refrigeration until handled in the laboratory. Samples were stored at −80°C until their analysis. Additionally, the urinary excretion of HA and MHA in a subgroup of five histamine intolerance patients with DAO deficiency (< 10 U/ml) was monitored during 9 months of dietary treatment (sampling points: 2, 6, and 9 months). The first 2 months of the dietary management of histamine intolerance were the most restrictive, with the exclusion of all histamine-rich foods and oral supplementation with exogenous DAO. Thereafter, excluded foods were gradually introduced according to the tolerance of the patient, and DAO supplementation was still administered. Recommendations given to the patients on how to follow a low-histamine diet are shown in **Supplementary Table 1**. These recommendations include foods that are likely to contain histamine and those that, although they do not contain histamine, may contain other biogenic amines (8). The presence of these other amines can exert an inhibitory effect on the histamine degradation by DAO enzyme due to the competition for this degradation system (20).

All volunteers received a kit consisting of an information sheet detailing the purpose and conditions of the study, an informed consent document, a questionnaire for data collection and a urine sample collection protocol. The study was approved by the Ethics Committee of the University of Barcelona (Institutional Review Board IRB00003099).

Determination of urinary histamine and 1-methylhistamine by ultra-high performance liquid chromatography and fluorometric detection

Histamine and MHA levels in urine were analyzed as described by Comas-Basté et al. (18). Briefly, the analytical method consists of sample preparation, purification, and concentration by the acidic hydrolysis of urine samples followed by solid phase extraction using mixed cation exchange cartridges. Overall, the analytical procedure allowed for a 50-fold concentration of the analytes in relation to the initial urine content. Chromatographic separation of HA and MHA was performed using UHPLC and a BEH C18 chromatographic column (1.7 μm, 2.1 mm × 50 mm) (Acquity™, Waters Corp., Milford, MA, USA). Fluorometric detection of both analytes was achieved through an online post-column derivatization system using o-Phthalaldehyde (OPA) as the fluorescence reagent. Quantification of HA and MHA levels in urine was accomplished by the external standard procedure, using histamine dihydrochloride and 1-methylhistamine dihydrochloride as the analytical standards (Sigma, St. Louis, MO, USA).

Determination of urinary creatinine

Excreted HA and MHA levels were normalized based on urinary creatinine concentration. This is the recommended approach when estimating the levels of biological substances eliminated in specific urine samples due to the relative stability of creatinine in the absence of renal pathologies (21). Thus, to normalize urinary excretion values, creatinine in urine was determined using the colorimetric alkaline picrate method (Jaffé reaction) adapted to microplates (Thermo Scientific™ 96-Well Microtiter Microplates), as described in Medina-Remón et al. (22). Briefly, 3 μL of urine was mixed with 60 μL of aqueous picric acid solution (1%) and 5 μL of sodium hydroxide (10%). The mixture was stirred and allowed to stand for 15 min in darkness at room temperature. Subsequently, 232 μL of Milli-Q water was added and the absorbance was read at 500 nm on a UV/VIS spectrophotometer. The concentration of HA and MHA in urine was expressed in μg of analyte per g of creatinine.

The excretion of creatinine in urine was within the normal values for all study participants and no significant differences were found between the mean excretion values of the two study groups ($p > 0.05$) (23).

Statistical analysis

The statistical analysis of data was performed with Statistical Software Package for Windows SPSS, version 25.0 (SPSS, Chicago, IL, USA). Student's *t*-test for paired and unpaired samples and the Mann–Whitney *U* Test for non-parametric data were used to assess the significance of differences between urine samples. Pearson's Correlation Coefficient was also used to estimate the goodness of the correlation between 24 h urine samples and specific urine samples.

Results

Reliability of spot urine sample analysis

Spot urine (first morning urine) is the most commonly used type of urine sample in clinical studies, as collecting 24 h urine samples is not patient-friendly. For this reason, in the preliminary phase of the current study, the reliability of spot urine samples was evaluated by comparing the urinary excretion of HA and MHA in spot and 24 h urine samples of 14 subjects.

The mean HA concentration in spot and 24 h samples was 30.9 ± 17.3 μg/g creatinine and 30.0 ± 14.7 μg/g creatinine, respectively and the mean MHA excretion was 143.5 ± 43.0 μg/g creatinine, and 145.1 ± 41.1 μg/g creatinine, respectively. No significant differences were detected in the urinary excretion of HA ($p > 0.05$) and MHA ($p > 0.05$) between the two sample types of each individual. In fact, a high correlation was found between the urinary content of HA and MHA between the spot and 24 h urine samples ($r = 0.952$, $r = 0.957$, respectively) (**Figure 1**).

Urinary histamine and 1-methylhistamine profile in control and histamine intolerant individuals

Patients with histamine intolerance showed an average of 4.5 ± 1.1 symptoms and 78% of them reported combinations of more than three symptoms. The most frequent complaints were gastrointestinal (94% of the patients), mainly bloating, flatulence and abdominal pain, followed by some dermatological manifestations (74%), such as urticaria and atopic skin. More than 80% of patients reported suffering from a simultaneous combination of gastrointestinal and dermatological symptoms. Neurological and respiratory system affections were also reported, although to a lesser extent. Lower serum DAO activity was not correlated with a higher number of symptoms.

Figure 2 shows the distribution of urinary HA and MHA levels in the control and histamine intolerant groups. The values of the two analytes varied widely in both study groups, especially in the case of MHA, which had an interquartile range (IQR = P75–P25) of 85.15 μg/g creatinine and 35.83 μg/g creatinine for the control and histamine intolerant groups, respectively. Histamine levels were not significantly different between the two groups, with mean values of 18.65 ± 11.08 μg/g creatinine for the histamine intolerant group and 26.25 ± 22.17 μg/g creatinine for the control ($p > 0.05$). In contrast, MHA values were significantly lower in patients with symptoms of histamine intolerance (70.29 ± 29.43 μg/g creatinine) compared with the control (112.99 ± 55.48 μg/g creatinine) ($p < 0.01$).

Given that the composition of the two study groups was not homogeneous in terms of sex (the histamine intolerant group consisted entirely of women), potential differences in analyte excretion levels between the sexes were analyzed within the control group. Accordingly, no significant differences in HA and MHA concentration were observed between healthy men and women ($p > 0.05$).

When stratifying individuals with symptoms of histamine intolerance based on the presence or absence of deficient serum DAO activity, no statistically significant differences were found for the levels of urinary HA ($p > 0.05$) or MHA ($p > 0.05$) (**Figure 3**). Moreover, compared with the control group, individuals with deficient serum DAO activity showed similar levels of urinary HA ($p > 0.05$) but significantly lower MHA levels ($p < 0.05$). The same trend was observed when considering the whole histamine intolerant group.

Influence of the dietary treatment of histamine intolerance on the urinary excretion of histamine and 1-methylhistamine

A small-sized study on a subgroup of five patients with symptoms of histamine intolerance was performed to assess the influence of the dietary treatment of histamine intolerance on the urinary excretion of HA and MHA (**Figure 4**). At the initial sampling point (time 0), before the start of the dietary treatment, considerable variability was observed in HA urinary levels (IQR = 10.54 μg/g creatinine).

After 2 months of dietary treatment (i.e., a highly restrictive exclusion of HA-containing foods together with oral supplementation with DAO), a reduction of the mean HA and MHA excretion values was observed, although without statistical significance ($p > 0.05$). At this point, two patients showed marked reductions in the urinary excretion of HA and MHA (up to 86 and 56%, respectively). Thereafter, the urinary excretion of both analytes remained quite constant for all five patients ($p > 0.05$).

Discussion

Early-morning spot urine was found to be suitable for the analysis of urinary HA and MHA, as the content of both analytes was highly correlated with the levels in 24 h samples. This supports the findings of other authors (24, 25), who report that the analyte levels in a spot urine sample correlate with a 24 h collection as long as creatinine values are used to normalize the excretion values. Moreover, Saito et al. found a wide variability in urinary HA values throughout the day and indicated the usefulness of normalizing them as a ratio to creatinine to compensate for these fluctuations (25). As well as their convenience for patients, early-morning urine samples may provide more reliable results due to a higher analyte concentration (including creatinine) (26). Nevertheless, infectious bacterial strains in the urinary tract, especially in women, may potentially influence HA levels in early-morning samples.

In this study, urinary HA excretion values in histamine intolerant and control individuals were very similar (mean values of 18.65 ± 11.08 μg/g creatinine and 26.25 ± 22.17 μg/g creatinine, respectively, $p > 0.05$). Additionally, within the histamine intolerant group, no significant differences were

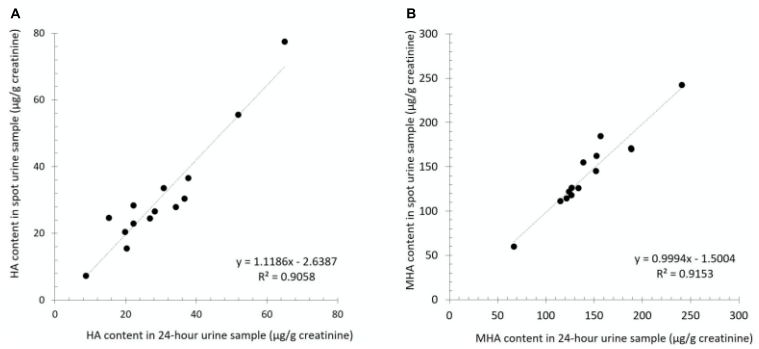


FIGURE 1
Linear correlation for the urinary excretion of histamine (HA, **A**) and 1-methylhistamine (MHA, **B**) between spot urine samples and 24 h urine samples.

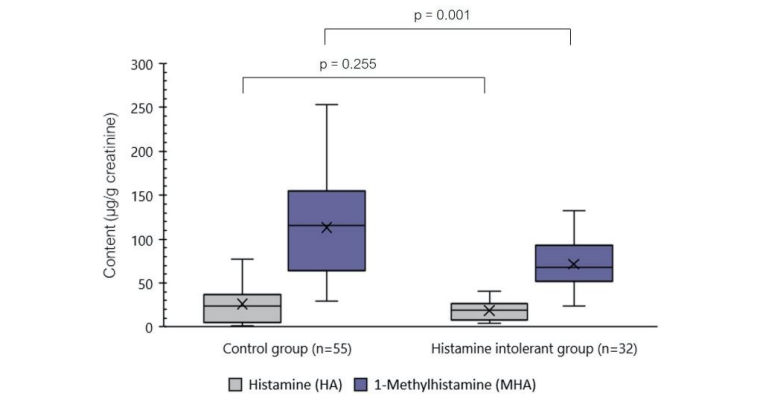


FIGURE 2
Distribution of the levels (µg/g creatinine) of histamine (HA) and 1-methylhistamine (MHA) in spot urine samples of control and histamine intolerant individuals. $p < 0.05$ indicates statically significant differences in the urinary content of the analytes between study groups.

found in excreted HA between individuals with and without deficient serum DAO activity ($p > 0.05$). In all cases, the HA values observed in the different individuals of both study groups fell within the ranges previously described by Keyzer et al. (9.8–73.6 µg/g creatinine) and Oosting et al. (8.8–55.9 µg/g creatinine) in healthy individuals (26, 27). Likewise, Lamale et al. reported normal HA excretion levels in healthy adult women, ranging between 22.4 and 58.2 µg/g creatinine, but found a significant increase in women with interstitial cystitis (28).

In contrast, we observed significant differences in the urinary concentration of MHA, the levels being markedly lower in histamine intolerant individuals (71.58 ± 29.43 µg/g creatinine) compared to the control population (112.99 ± 55.48 µg/g creatinine) ($p < 0.05$).

10.3389/fnut.2022.973682

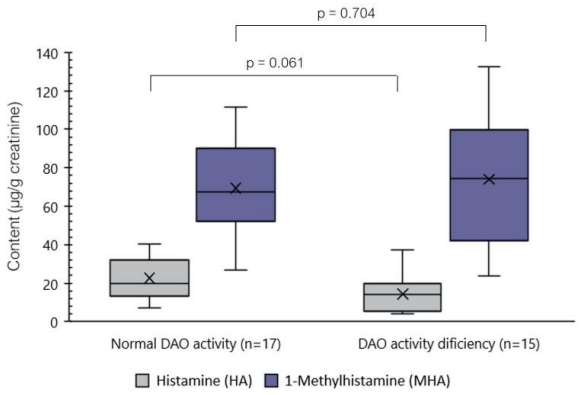


FIGURE 3
Distribution of the levels (µg/g creatinine) of histamine (HA) and 1-methylhistamine (MHA) in spot urine samples of individuals with symptoms of histamine intolerance with or without serum DAO enzyme deficiency. $p < 0.05$ indicates statically significant differences in the urinary content of the analytes between study groups.

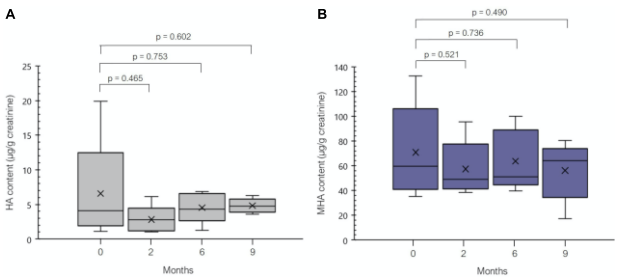


FIGURE 4
Distribution of the levels of (µg/g creatinine) of histamine (HA, **A**) and 1-methylhistamine (MHA, **B**) during 9 months of dietary treatment (low-histamine diet and DAO enzyme supplementation) in five patients.

Within the histamine intolerant group, urinary MHA values were very similar between those with or without deficient serum DAO activity ($p > 0.05$); moreover, there was no apparent relationship between levels of urinary MHA and the frequency or type of symptomatology. Regardless of the differences observed between groups, the urinary MHA excretion values of most individuals in both groups match those reported by several authors in previous studies (55–230 µg/g of creatinine) (24, 27, 29). This great variability in MHA excretion could be partially explained by interindividual differences in the functionality of HA degradation systems in the organism (29).

The observed reduction in MHA levels in the histamine intolerant group indicates that the urinary HA and MHA profile could be a more reliable biomarker of histamine intolerance than serum DAO activity. In this study, only 50% of patients diagnosed with histamine intolerance based on the presence of symptoms were identified by DAO activity measurement.

As MHA is an HA-derived metabolite arising from the action of the enzyme HNMT, lower MHA levels in histamine

intolerant individuals with DAO deficiency were not expected. A possible explanation is that impaired DAO activity could lead to a compensatory activation of HA metabolism by HNMT (30, 31), and ultimately an increase in the pathway end-product (i.e., MIAA). Thus, as well as potentially confirming the current results, the measurement of IAA and MIAA levels in urine would provide a more complete profile of HA excretion and its possible alteration in histamine intolerant individuals. New insight into the HA metabolome in this population would also be obtained.

Besides the use of the HA and MHA as a potential biomarker for histamine intolerance, a smaller preliminary study was performed with a subgroup of five histamine intolerance patients, in order to assess the behavior of HA and MHA urinary levels during 9 months follow-up of a low-histamine diet and oral DAO supplementation. To date, only a few studies have assessed the effect of dietary HA on the urinary levels of HA and its metabolites and they have been focused on healthy individuals (32–35). For example, in the study performed by Granerus, histamine was given orally to three healthy subjects, resulting in higher levels of urinary HA and the metabolites MHA and especially MIAA. Moreover, the urinary excretion of MIAA was found to be a dose-dependent on the administration of exogenous HA (32). Similarly, Keyzer et al. reported an increase in the excretion of MIAA after the oral administration of 200 μM of HA in three healthy individuals, with no significant changes in MHA. A high correlation was also found between protein intake and the urinary excretion of HA metabolites (33). According to our knowledge the current work is the first study with histamine intolerant patients. The results of the current study revealed a decrease in the mean urinary levels of HA and MHA after the first 2 months, although without statistical significance. The reduction was especially marked in two patients. These results can be explained by the highly restrictive nature of the diet at the beginning of the treatment, when a wide range of foods with typically high HA contents were excluded (e.g., fermented foods and beverages, preserved and semi-preserved fish products, spinach, eggplant, tomato, citrus, and nuts) (8). No clear trend was observed for HA and MHA levels during the reintroduction phase of the dietary treatment. The determination of a wider range of metabolites, including MIAA, could allow a more accurate assessment of the influence of the dietary treatment of histamine intolerance on the urinary profile of HA in patients with histamine intolerance.

Limitations

The main limitations of the present study were the rather small size of each sample group and the lack of male representation in the histamine intolerance group (although no differences were found between men and women in the control group). Moreover, it would have been of interest to measure the intensity of the clinical symptoms of the patients to elucidate any correlation with the urinary HA profile. Lastly, it is important to highlight that the preliminary study on the influence of a dietary treatment of histamine intolerance on the urinary excretion of HA and MHA was only assessed in five individuals, thus limiting the extrapolation of the results.

Conclusion

To the best of our knowledge, this is the first time that the determination of urinary HA and its metabolites, particularly MHA, is proposed as a diagnostic tool for histamine intolerance to complement the current criteria based on the assessment of symptoms. The validity of using spot urine samples to measure the urinary excretion of HA and MHA has been confirmed. According to the obtained results, the hypothesis that the excretion profile in individuals with symptoms of histamine intolerance differed from that of the healthy individuals was demonstrated due to lower urinary levels of MHA in histamine intolerant patients. However, no differences were observed in urinary MHA related to the level of serum DAO activity. These results constitute a starting point for the study of HA metabolomics as a suitable and non-invasive approach to histamine intolerance diagnosis. An area for further research is the influence of a low-histamine diet and DAO supplementation on the urinary excretion of HA in histamine intolerant patients.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the University of Barcelona (Institutional Review Board IRB00003099). The patients/participants provided their written informed consent to participate in this study.

Author contributions

MV-N, ML-M, and MV-C: conceptualization. SS-P, RC-S, and OC-B: investigation and writing – original draft preparation. SS-P, RC-S, MV-N, ML-M, OC-B, and MV-C: writing – review and editing. OC-B and MV-C: supervision.

All authors have read and agreed to the published version of the manuscript.

Funding

This research was supported by Grant PID2020-117670RB-C21, funded by MICIN/AEI/10.13039/501100011033.

Acknowledgments

SS-P was a recipient of a doctoral fellowship from the University of Barcelona (APIF2018).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.973682/full#supplementary-material

References

1. European Food Safety Authority [EFSA]. Panel on Biological Hazard (BIOHAZ). Scientific opinion on risk based control of biogenic amine formation in fermented foods. *EFSA J.* (2011) 9:1–93. doi: 10.2903/j.efsa.2011.2393

2. Comas-Basté O, Latorre-Moratalla ML, Sánchez-Pérez S, Veciana-Nogués MT, Vidal-Carou MC. Histamine and other biogenic amines in food. From scombroid poisoning to histamine intolerance. In: Proestos C, editor. *Biogenic Amines*. London: IntechOpen (2019).

3. Bover-Cid S, Latorre-Moratalla ML, Veciana-Nogués MT, Vidal-Carou MC. Processing contaminants: biogenic amines. In: Motarjemi Y, Moy GG, Todd ECD, editors. *Encyclopedia of Food Safety*. Waltham: Elsevier (2014). p. 381–91. doi: 10.1016/B978-0-12-378612-8.00216-X

4. Schwelberger HG. Histamine intolerance: overestimated or underestimated? *Inflamm Res.* (2009) 58:51–2. doi: 10.1007/s00011-009-2004-4

5. Comas-Basté O, Sánchez-Pérez S, Veciana-Nogués MT, Latorre-Moratalla M, Vidal-Carou MC. Histamine intolerance: the current state of the art. *Biomolecules.* (2020) 10:1–26. doi: 10.3390/biom10081181

6. Sánchez-Pérez S, Comas-Basté O, Duelo A, Veciana-Nogués MT, Berlanga M, Latorre-Moratalla ML, et al. Intestinal dysbiosis in patients with histamine intolerance. *Nutrients.* (2022) 14:1774. doi: 10.3390/nu14091774

7. Schnedl WJ, Enko D. Considering histamine in functional gastrointestinal disorders. *Crit Rev Food Sci Nutr.* (2020) 61:2960–7. doi: 10.1080/10408398.2020.1791049

8. Sánchez-Pérez S, Comas-Basté O, Veciana-Nogués MT, Latorre-Moratalla ML, Vidal-Carou MC. Low-histamine diets: is the exclusion of foods justified by their histamine content? *Nutrients.* (2021) 13:1395. doi: 10.3390/nu13051395

9. Schnedl WJ, Schenk M, Lackner S, Enko D, Mangge H, Forster F. Diamine oxidase supplementation improves symptoms in patients with histamine intolerance. *Food Sci Biotechnol.* (2019) 28:1779–84. doi: 10.1007/s10068-019-00627-3

10. Tuck CJ, Biesiekierski JR, Schmid-Grendelmeier P, Pohl D. Food intolerances. *Nutrients.* (2019) 11:1–16. doi: 10.3390/nu11071684

11. Reese I, Ballmer-Weber B, Beyer K, Dölle-Bierke S, Kleine-Tebbe J, Klimek L, et al. Guideline on management of suspected adverse reactions to ingested histamine – Guideline of the German Society for Allergology and Clinical Immunology (DGAKI), the Society for Pediatric Allergology and Environmental Medicine (GPA), the Medical Association of German Allergologists (AeDA) as well as the Swiss Society for Allergology and Immunology (SGAI) and the Austrian Society for Allergology and Immunology (ÖGAI). *Allergol Select.* (2021) 5:305–14. doi: 10.5414/alx02269e

12. Mušič E, Korošec P, Šilar M, Adamic K, Košnik M, Rijavec M. Serum diamine oxidase activity as a diagnostic test for histamine intolerance. *Wien Klin Wochenschr.* (2013) 125:239–43. doi: 10.1007/s00508-013-0354-y

13. Pinzer TC, Tietz E, Waldmann E, Schink M, Neurath MF, Zopf Y. Circadian profiling reveals higher histamine plasma levels and lower diamine oxidase serum activities in 24% of patients with suspected histamine intolerance compared to food allergy and controls. *Allergy Eur J Allergy Clin. Immunol.* (2018) 73:949–57. doi: 10.1111/all.13361

14. Manzotti G, Breda D, Di Gioacchino M, Burastero SE. Serum diamine oxidase activity in patients with histamine intolerance. *Int J Immunopathol Pharmacol.* (2016) 29:105–11. doi: 10.1177/0394632015617170

15. Töndury B, Wüthrich B, Schmid-Grendelmeier P, Seifert B, Ballmer-Weber B. Histamine intolerance: is the determination of diamine oxidase activity in the serum useful in routine clinical practice? *Allergologie.* (2008) 31:350–6. doi: 10.5414/ALP31350

16. Kofler H, Aberer W, Deibl M, Hawranek T, Klein G, Reider NF. Diamine oxidase (DAO) serum activity: not a useful marker for diagnosis of histamine intolerance. *Allergologie.* (2009) 32:105–9. doi: 10.5402/2011/353045

17. Schnoor HS, Mosbech H, Skov PS, Poulsen LK, Jensen BM. Diamine oxidase determination in serum. *Allergo J.* (2013) 22:108–11. doi: 10.1007/s15007-013-0063-x

18. Comas-Basté O, Latorre-Moratalla ML, Bernacchia R, Veciana-Nogués MT, Vidal-Carou MC. New approach for the diagnosis of histamine intolerance based on the determination of histamine and methylhistamine in urine. *J Pharm Biomed Anal.* (2017) 145:379–85. doi: 10.1016/j.jpba.2017.06.029

19. Hrubisko M, Dani D, Huorka M, Wawruch M. Histamine intolerance – The more we know the less we know. *Nutrients.* (2021) 13:2228. doi: 10.3390/nu13072228

20. Sánchez-Pérez S, Comas-Basté O, Costa-Catala J, Iduriaga-Platero I, Veciana-Nogués MT, Vidal-Carou MC, et al. The rate of histamine degradation by diamine oxidase is compromised by other biogenic amines. *Front Nutr.* (2022) 9:897028. doi: 10.3389/fnut.2022.897028

21. Ruiz N, Segarra AB, Lara L, Ramírez-Sánchez M, Prieto I. Diet and oxidative status. The dietary pattern and urinary 8-isoprostane in healthy Spanish women. *Antioxidants.* (2019) 8:271. doi: 10.3390/antiox8080271

22. Medina-Remón A, Barrionuevo-González A, Zamora-Ros R, Andres-Lacueva C, Estruch R, Martínez-González MÁ, et al. Rapid Folin-Ciocalteu method using microtiter 96-well plate cartridges for solid phase extraction to assess urinary total phenolic compounds, as a biomarker of total polyphenols intake. *Anal Chim Acta.* (2009) 634:54–60. doi: 10.1016/j.aca.2008.12.012

23. Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, Pirkle JL. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. *Environ Health Perspect.* (2005) 113:192–200. doi: 10.1289/ehp.7337

24. Oosting E, Keyzer J, Wolthers BG. Correlation between urinary levels of histamine metabolites in 24-hour urine and morning urine samples of man: influence of histamine-rich food. *Agents Actions.* (1989) 27:205–7. doi: 10.1007/BF02222240

25. Saito K, Horie M, Nakazawab H. Determination of urinary excretion of histamine and 1-methylhistamine by liquid chromatography. *J Chromatogr B.* (1994) 654:270–5. doi: 10.1016/0378-4347(94)00056-5

26. Oosting E, Keyzer J, Wolthers BG, Scholtis RJH. Age dependent normal values of histamine and histamine metabolites in human urine. *Agents Actions.* (1988) 23:307–10. doi: 10.1007/BF02142572

27. Keyzer JJ, de Monchy JGR, van Doormaal JJ, van Voorst Vader PC. Improved diagnosis of mastocytosis by measurement of urinary histamine metabolites. *N Engl J Med.* (1983) 309:1603–5. doi: 10.1056/NEJM198312293092603

28. Lamale LM, Lutgendorf SK, Zimmerman MB, Kreder KJ. Interleukin-6, histamine, and methylhistamine as diagnostic markers for interstitial cystitis. *Urology.* (2006) 68:702–6. doi: 10.1016/j.urology.2006.04.033

29. Van Toorenenbergen AW, Oranje AP. Comparison of serum tryptase and urine N-methylhistamine in patients with suspected mastocytosis. *Clin Chim Acta.* (2005) 359:72–7. doi: 10.1016/j.cccn.2005.03.041

30. He GH, Cai WK, Zhang JB, Ma CY, Yan F, Lu J, et al. Associations of polymorphisms in HRH2, HRH3, DAO, and HNMT genes with risk of chronic heart failure. *Biomed Res Int.* (2016) 2016:1208476. doi: 10.1155/2016/1208476

31. Butterfield JH, Pongdee T, Ravi A. Urinary markers of mast cell disease and their role in diagnosis and management. In: Cem A, editor. *Mastocytosis.* Warren, MI: Springer International Publishing (2020). doi: 10.1007/978-3-030-27820-5

32. Granerus G. Effects of oral histamine, histidine and diet on urinary excretion of histamine, methylhistamine and 1-methyl-4-imidazoleacetic acid in man. *Scand J Clin Lab Invest.* (1968) 22:49–58.

33. Keyzer JJ, Breukelman H, Wolthers BG, van den Heuvel M, Kromme N, Berg WC. Urinary excretion of histamine and some of its metabolites in man: influence of the diet. *Agents Actions.* (1984) 15:189–94. doi: 10.1007/BF01972348

34. Evans E, Nicholls J. A method for the gas chromatographic determination of urinary 1,4-methyl-imidazoleacetic acid. *J Chromatogr.* (1973) 82:394–7. doi: 10.1016/s0021-9673(01)91850-3

35. Mitchell RG, Code CF. Effect of diet on urinary excretion of histamine. *J Appl Physiol.* (1954) 6:387–92. doi: 10.1152/jappl.1954.6.7.387

4. DISCUSSIÓ

4. DISCUSSIÓ

Àmbit 1. Intolerància a la histamina i dieta

De les aproximacions actualment utilitzades pel tractament de la intolerància a la histamina, de la que existeixen més treballs publicats sobre la seva eficàcia és de les dietes baixes en histamina [32,45]. En una primera fase de la dieta s'exclou un elevat nombre d'aliments, tots ells relacionats pels pacients com desencadenants de la simptomatologia, fet que la fa altament restrictiva [85]. Durant aquesta fase, també es recomana la presa diària del complement DAO, abans de cada àpat principal. No obstant això, el fet que existeixin diferents propostes de dieta (no totes exclouen els mateixos aliments), junt amb que no s'ha establert un llindar d'histamina que caracteritzi els aliments baixos en aquesta amina, fa que la gestió dietètica d'aquesta intolerància sigui complexa.

En aquesta tesi es va realitzar una revisió crítica d'un total de 10 dietes baixes en histamina en base als continguts d'histamina i altres amines biògenes dels aliments exclosos, utilitzant per aquest propòsit les dades de continguts d'histamina i altres amines biògenes del propi grup de recerca. En primer lloc, d'aquesta revisió es confirma la falta de consens en la composició d'aquestes dietes, ja que es va trobar una gran heterogeneïtat en el tipus i nombre d'aliments a excloure (Figura 7). Dels 34 aliments o tipus d'aliments identificats pels pacients com a desencadenants de la intolerància, 23 s'eliminaven només en la meitat o menys de les dietes revisades.

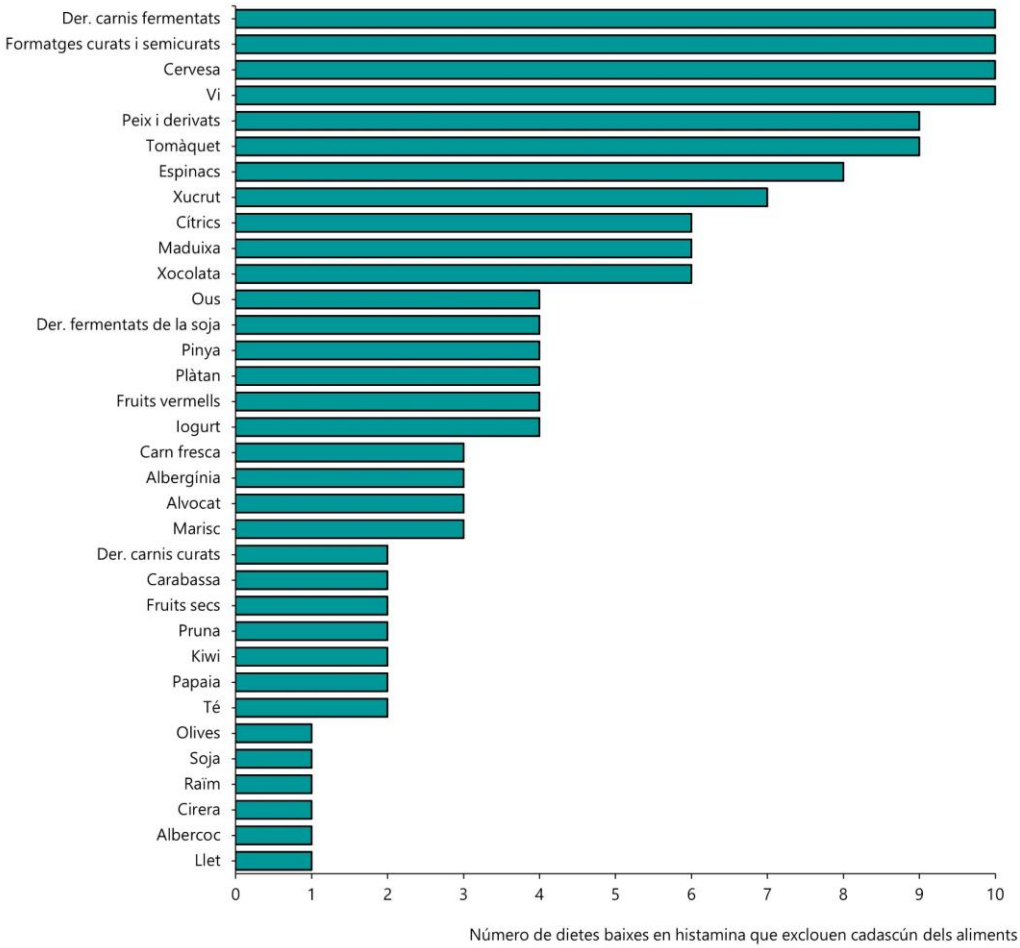


Figura 7. Llistat dels aliments que s'exclouen de les dietes baixes en histamina i el número de dietes que proposen la seva exclusió [50–52,102–107,121].

Els únics aliments exclosos en el 100% de les dietes van ser els derivats carnis crus-curats fermentats, els formatges curats, el vi i la cervesa, tots ells amb una elevada susceptibilitat de contenir histamina (Figura 7). En general, segons les dades del grup de recerca sobre continguts d'amines en productes espanyols, els nivells d'histamina que es troben en tots aquests tipus d'aliments són relativament baixos o moderats, en el cas concret dels derivats carnis fermentats i formatges, els nivells poden ser extremadament elevats [7]. Cal destacar que en el vi i la cervesa, encara que els continguts d'histamina no acostumen a ser alts, la presència d'alcohol pot

augmentar els efectes adversos d'aquesta amina, ja que alcohol i histamina competeixen per un enzim responsable de la seva metabolització [6]. Curiosament, productes fermentats com el xucrut i els derivats de la soja, que sovint presenten continguts d'histamina més elevats que els altres productes fermentats esmentats anteriorment, només s'eliminaven en set i quatre de les dietes revisades, respectivament [7,125]. L'origen bacterià de la histamina en els productes fermentats explicaria l'elevada variabilitat observada. L'activitat descarboxilasa de bacteris alterants procedents d'una manca d'higiene de les matèries primeres i/o dels processos d'elaboració, i/o dels propis bacteris fermentatius, seria la responsable d'aquesta formació [16,17].

A part d'histamina, en els aliments fermentats també solen haver-hi altres amines, principalment tiramina. De fet, segons Bover-Cid i col. (2014) i Toro-Funes i col. (2015) és freqüent trobar alts continguts d'aquesta amina entre els diferents tipus de productes fermentats, arribant-se a acumular, en algunes mostres de formatges i fermentats de la soja, nivells de fins als 1500 mg/kg i 1700 mg/kg, respectivament [7,125]. La formació de tiramina en aquests productes està fortament lligada a l'activitat de bacteris de l'àcid làctic, sovint presents pel seu rol fermentatiu però, en el cas de valors tant elevats també solen ser responsables aquells microorganismes contaminants presents a les matèries primeres o procedents dels processos d'elaboració d'aquests productes [22,126]. Finalment, en els aliments fermentats també destaca la presència de putrescina i cadaverina, diamines que, com en el cas de la histamina, procedeixen de l'activitat de bacteris fermentatius i/o alterants [6]. Per exemple, en el 90% dels derivats carnis fermentats crus-curats i en el 85% dels formatges analitzats per Bover-Cid i col. (2014), contenien putrescina i/o cadaverina, i sovint a concentracions al menys 4 vegades superiors a les de la histamina [7]. Aquest perfil amínic trobat en mostres de productes fermentats del mercat espanyol ha estat també descrit per altres mostres de diferents procedències [6,127,128].

El 90% de les dietes baixes en histamina exclouen el peix i les seves conserves i semiconserves (Figura 7). En principi, el que s'espera en aquests tipus d'aliments seria no trobar histamina ni cap altra amina. De fet, en la majoria de les mostres de peix fresc i dels seus derivats comercialitzats a Espanya no es va trobar histamina o els nivells detectats van ser molt baixos (P95 inferior als 20 mg/kg) [7]. Tanmateix, segons dades de l'EFSA sobre diferents productes derivats de la pesca europeus, només el 27% de 6329 mostres van presentar histamina i, en continguts generalment baixos [6]. No obstant, una manca de frescor en el peix o unes condicions higièniques inadequades en l'elaboració de productes derivats, ocasionaria un important increment en els continguts d'histamina, com per exemple, els trobats en algunes mostres de salmó fresc (111 mg/kg) i en mostres de sardines en conserva (657 mg/kg d'histamina) [7]. Per aquest motiu, en les dietes baixes en histamina se sol aconsellar l'exclusió de peix (especialment si no es pot assegurar la seva frescor) i dels seus derivats. A més, en algunes dietes només es recomana l'eliminació de peix blau com la sardina, l'anxova, la tonyina i el verat, els quals serien més susceptibles d'acumular elevades quantitats d'histamina perquè presenten elevats continguts d'histidina, el seu aminoàcid precursor [18]. La presència d'altres amines, especialment putrescina i cadaverina derivades de l'acció de bacteris alterants, sol ser també habitual davant d'una manca de frescor [13,129].

Dels 22 aliments no fermentats d'origen vegetal que es van identificar en les dietes baixes en histamina, els espinacs, l'albergínia i el tomàquet van ser els únics que presentaren, generalment, continguts rellevants d'histamina. De fet, la histamina es poc freqüent en aliments d'origen vegetal i, sovint es troba en nivells baixos o moderats [7]. No obstant, per algunes mostres d'albergínia i espinacs s'han descrit valors màxims de fins a 100 mg/kg [7]. Així doncs, tot i que l'albergínia pot contenir nivells tant o més elevats d'histamina que els espinacs o els tomàquets, aquesta hortalissa només s'excloïa en el 30% de les dietes, front del 80-90% en el cas dels dos anteriors (Figura 7). Segons Lavizzari i col. (2007), la presència de nivells baixos d'histamina en espinacs tindria un origen natural, cas que també podria succeir en l'albergínia i en el tomàquet [27]. Aquest fet explicaria que aquesta amina es trobi

pràcticament sempre en aquests productes vegetals [27]. D'altra banda, de la mateixa manera que succeeix en els aliments d'origen animal, la presència d'alts continguts d'histamina i altres amines pot ser conseqüència d'una activitat microbiològica [7,27,28].

L'elevada variabilitat en els continguts d'histamina, tant en aliments d'origen animal com vegetal, es deu a la influència de múltiples factors, tant intrínsecs com extrínsecs [8]. D'entre aquests, la temperatura d'emmagatzematge s'ha mostrat com un dels factors més importants en la formació d'histamina [8,13,14,38]. Naila i col. (2012) i Jiang i col. (2013) han descrit temperatures d'entre 25-30ºC com a òptimes per a la majoria de microorganismes histaminogènics, encara que també s'ha observat una formació important d'histamina en aliments refrigerats (4-10ºC), especialment en peix [130,131]. De fet, en el cas del peix, l'emmagatzematge amb gel a temperatures al voltant de 0ºC va ser l'única condició ambiental capaç de retardar la formació d'histamina [131]. La formació d'histamina i altres amines biògenes al llarg de processos de fermentació es veu també disminuïda quan aquesta es realitza a temperatures inferiors als 15ºC [16,21]. Similarment, altres autors han reportat increments d'histamina i putrescina durant l'emmagatzematge de vegetals de fulla verda a temperatures de refrigeració (5-6ºC) [27,132].

El pH també manté una estreta relació amb l'acumulació d'histamina en els aliments, la qual es veu generalment afavorida a pH àcids [8]. Altres factors com ara la formulació (p.ex. addició de sal, espècies o nitris) i els diferents processos tecnològics de conservació (p.ex. pasteurització, altes pressions hidrostàtiques o irradiacions) i/o sistemes d'envasat aplicats (p.ex. buit o atmosferes modificades), també han estat àmpliament estudiats, ja que també mostren influència en la capacitat microbiana per formar histamina [8,16,17,133]. A més, un altre factor que també pot influir en el contingut d'histamina és la cocció domèstica. Per exemple, segons Latorre-Moratalla i col. (2015) el contingut d'histamina en espinacs es va arribar a reduir fins a un 83% respecte al producte en cru, passant aquesta amina a l'aigua de cocció [134].

A part de tots aquests aliments que acabem d'esmentar susceptibles de contenir histamina, un elevat nombre d'aliments que també s'exclouen no presenta *a priori* quantitats importants d'histamina (68% dels aliments identificats entre les diferents dietes) [7]. És important destacar que part d'aquests aliments tot i no contenir histamina sí que acostumen a presentar nivells molt rellevants d'altres amines, especialment, putrescina. Aquest seria el cas, per exemple, dels cítrics, plàtan, carbassa, fruits secs i brots de soja. Paradoxalment, aliments com el carabassó, els pèsols, el pebrot verd i el blat de moro, que es caracteritzen pels seus alts nivells en putrescina, no es van trobar exclosos en cap de les dietes baixes en histamina. La putrescina és una amina àmpliament distribuïda entre els aliments d'origen vegetal que sol tenir, amb algunes excepcions, un origen fisiològic, essent precursora de les poliamines espermidina i espermina. A la putrescina se li atribueixen diferents funcions a la planta, actuant, per exemple, com a factor de creixement durant la germinació o en la protecció front situacions de sequera o de temperatures extremes [26,28,125,135,136]. En definitiva, la varietat, l'origen, el mètode de cultiu i factors lligats al transport o l'emmagatzematge podrien tenir certa influència en l'acumulació d'aquesta amina en els aliments d'origen vegetal [137,138].

Finalment, a les dietes revisades es recomana l'eliminació d'aliments que ni contenen histamina ni cap altra amina en quantitats destacables, representant més del 35% del total d'aliments identificats en aquesta revisió (Figura 7). No obstant això, la gran majoria només es consideren en un nombre molt baix de dietes. Diferents articles científics llisten certs aliments amb una suposada capacitat per alliberar histamina endògena i per aquesta condició s'han considerat també capaços de desencadenar els símptomes de la intolerància, incloent-t'hi fruites cítriques, papaia, tomàquet, maduixes, pinya, nous, xocolata i marisc, entre altres [29,38,139]. No obstant això, el mecanisme pel qual es donaria aquest efecte encara no ha estat dilucidat. De fet, l'única revisió disponible realitzada per Vlieg-Boerstra i col. (2005) respecte a la capacitat d'alliberar histamina de certs aliments, afirma clarament que no existeix cap evidència que recolzi aquest mecanisme. Aquesta

hipòtesi només es basa en uns pocs, i no concloents, estudis *in vitro*, a part de no existir cap estudi clínic en humans que li doni suport [139].

En definitiva, a partir d'aquest estudi s'ha pogut comprovar que entre els aliments que normalment s'exclouen de les dietes baixes en histamina es troben aquells en els que és freqüent una elevada presència d'histamina, però també, d'altres que si bé no tenen o tenen poca histamina, poden contenir nivells elevats d'altres diamines, especialment de putrescina. Així doncs, podria ser factible pensar que aquestes altres amines potenciessin l'absorció de la histamina exògena al competir per la DAO per la seva degradació a nivell intestinal i, ser, en certa manera també, corresponsables de l'aparició dels efectes adversos de la histamina.

Els estudis experimentals existents sobre la interferència d'altres amines en la degradació de la histamina per part de la DAO són molt escassos i desactualitzats, essent la majoria dels anys 50 i 80 [81–84]. Per aquest motiu, en el marc d'aquesta tesi, i per tal de donar resposta a la hipòtesi plantejada, es va avaluar, en primer lloc, la diferent capacitat que presenta l'enzim DAO per degradar *in vitro* la histamina i altres substrats amino (putrescina, cadaverina, tiramina, espermidina i espermina) per, a continuació, estudiar la influència d'aquests últims en la velocitat de degradació de la histamina.

La histamina va ser l'amina que es va degradar a una major velocitat per part de l'enzim DAO (28 mU), mentre que aquesta afinitat va ser significativament menor per la putrescina i la cadaverina. La tiramina, l'espermidina i l'espermina van ser també substrats de l'enzim DAO, encara que amb taxes de degradació extremadament baixes (per sota dels 2 mU). Ignesti i col. (2003) també van descriure una major afinitat de l'enzim DAO d'origen porcí per la histamina que per la resta d'amines estudiades, essent aquest enzim 9, 12 i 300 vegades menys afí per la putrescina, la cadaverina i l'espermidina, respectivament [140].

L'afinitat de la DAO pels diferents substrats amino sembla estar influenciada per la naturalesa química d'aquests, especialment per la longitud de la molècula i per la

presència de components alifàtics o aromàtics [33]. Segons Blaschko i Hawkins (1950) durant l'oxidació enzimàtica d'una diamina, el substrat és ancorat a l'enzim mitjançant els grups amino formant un anell intermedi. S'ha suggerit que com més llarga és la separació entre els dos grups amino, més feble és aquesta unió de la molècula amb la DAO [141]. A més, la preferència de l'enzim DAO per un substrat o un altre sembla ser que també depèn de l'origen d'aquest enzim. Diferents estudis han reportat una major afinitat per la histamina que per altres amines en el cas d'enzims DAO de rosegadors, porcí o humà, mentre que quan la DAO té un origen vegetal, aquesta afinitat és major per la putrescina i la cadaverina [33,35,140,142]. En aquest sentit, Pietrangeli i col. (2007) van observar que l'enzim DAO procedent de pèsol vermell (*Lathyrus cicera L.*) degradava la histamina a una menor velocitat que la putrescina i la cadaverina [33].

Un cop demostrat que, a part de la histamina, la putrescina, la cadaverina, la tiramina, l'espermidina i l'espermina són també substrat de l'enzim DAO d'origen porcí, es va valorar quantitativament la influència d'aquestes amines sobre la degradació de la histamina (Figura 8). Aquest estudi ens va permetre confirmar que la presència de putrescina i/o cadaverina reduïa significativament la velocitat de degradació de la histamina, i aquesta reducció era dependent de la proporció en la que es trobaven aquestes amines. Com es pot observar en la Figura 8, quan major era la quantitat de putrescina i/o cadaverina present en el medi, major era l'efecte inhibitori sobre la velocitat de degradació de la histamina, observant-se així el màxim efecte quan aquestes diamines es trobaven en quantitats 20 vegades superiors a la de la histamina. Aquest fet pot ser atribuït a que els dos substrats presenten afinitats similars per l'enzim DAO [140]. A diferència d'aquestes diamines, tiramina, espermidina i espermina només van ser capaces d'inhibir la degradació de la histamina quan es trobaven en altes quantitats, que correspon al color taronja dels histogrames de la Figura 8. Tots aquests resultats demostrarien que la intensitat en la que altres amines interfereixen en la degradació de la histamina per part de la DAO, es troba directament relacionada amb l'activitat d'aquest enzim per cada un dels substrat amino, és a dir, quan més activitat més inhibició.

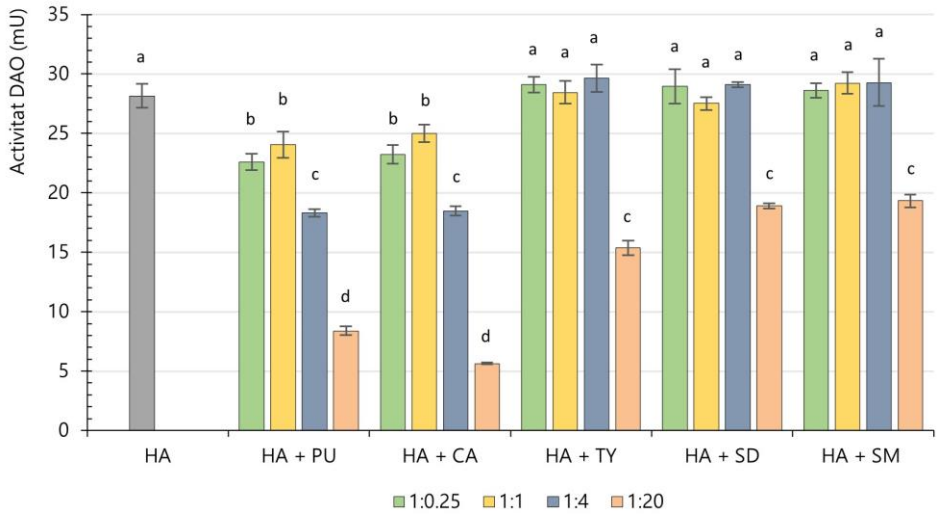


Figura 8. Comparació de l'activitat (mU) de l'enzim DAO sobre la histamina sola (gris) i sobre la histamina quan es troba amb diferents proporciones de putrescina, cadaverina, tiramina, espermidina o espermina: 1:0,25 (verd), 1:1 (groc), 1:4 (blau) i 1:20 (taronja). Les lletres denoten diferències estadísticament significatives ($p<0,05$). HA: Histamina, PU: Putrescina, CA: Cadaverina, TY: Tiramina, SD: Espermidina i SM: Espermina.

Com s'ha dit anteriorment, l'efecte inhibitori de certs composts amino sobre l'acció de la DAO per reduir la histamina ha estat molt poc estudiat, existint, segons el nostre coneixement, només quatre estudis que a més es van realitzar moltes dècades enrere [81–84]. Són uns exemples els estudis portats a terme per Arunlakshana i col. l'any 1954 i Mongar l'any 1957 on es va observar com la cadaverina era capaç de potenciar els efectes de la histamina sobre la contracció de l'ili, la tràquea i l'úter en animals de laboratori [81,82]. Lyons i col. (1983) van descriure un increment del 45% en l'absorció de la histamina en segments intestinals de rata després d'una perfusió de cadaverina i, al mateix temps, una reducció del 20% en els nivells d'àcid imidazolacetic, metabòlit de la histamina generat per l'activitat de la DAO [83]. Uns anys més tard, Hui i Taylor, van reportar un increment de la histamina no metabolitzada en orina de rates a les que se'ls hi havia administrat putrescina i/o cadaverina [84]. En aquest cas, van ser necessàries concentracions de putrescina i/o cadaverina 4-5 vegades més elevades que les

d'histamina per causar aquest efecte inhibitori. Per contra, l'administració de tiramina no va provocar cap canvi en els nivells d'histamina en orina [84].

Tot i que els resultats obtinguts en aquesta tesi van en la mateixa línia que els reportats prèviament per altres autors, creiem que aquesta és la primera vegada que es confirma l'efecte inhibitori per part d'altres amines (principalment putrescina i cadaverina) en la degradació de la histamina per la DAO utilitzant la determinació directa d'aquesta activitat enzimàtica *in vitro.* La intensitat d'aquest efecte inhibitori dependrà tant del substrat amino com de la seva concentració.

Cal recordar que, en el context d'una dieta, és factible trobar aliments, com per exemple, els productes fermentats, els cítrics o els fruits secs, que independentment de si contenen o no histamina, poden presentar nivells elevats d'altres amines, essent en darrer terme, les responsables de potenciar els efectes tòxics de la histamina. Aquest fet pot justificar el perquè alguns pacients relacionen certs aliments que no contenen histamina amb l'aparició dels símptomes de la intolerància a la histamina.

Àmbit 2. Intolerància a la histamina i microbiota intestinal

Habitualment es descriuen tres possibles orígens per la intolerància a la histamina per dèficit de DAO. D'una banda, el genètic, pel qual s'han descrit diferents polimorfismes en el gen que codifica l'enzim DAO relacionats amb una menor activitat [32,55]. Aquest dèficit enzimàtic també pot ser causat per l'acció de diferents fàrmacs que poden bloquejar l'enzim DAO de forma temporal i reversible [79]. I per últim, aquesta intolerància pot aparèixer també de forma secundaria a una afectació gastrointestinal [54]. Per exemple, Enko i col. (2017) van trobar que el dany en la mucosa intestinal provocat per diferents patologies, com gastroenteritis i síndrome d'intestí irritable (SII), entre d'altres, comportava una disminució en les activitats enzimàtiques de la DAO i la lactasa [73].

A més d'aquestes tres possibles causes, un estudi recent ha suggerit una possible relació entre l'alteració en la composició de la microbiota intestinal i l'aparició de la intolerància a la histamina [36]. Aquests autors han proposat que una disbiosi intestinal en pacients amb intolerància a la histamina pot contribuir a una inflamació de la mucosa intestinal i, conseqüentment a una reducció de l'activitat de l'enzim DAO [36]. A més, tenint en compte que la microbiota intestinal pot incloure bacteris capaços de formar histamina [37], seria factible pensar que una sobreexpressió d'aquests bacteris en pacients intolerants a la histamina expliqués l'aparició de la simptomatologia. En aquest context, es va plantejar estudiar la microbiota intestinal d'individus amb intolerància a la histamina i si una disbiosi intestinal podria estar relacionada amb l'aparició d'aquesta intolerància. L'estudi va comparar el perfil i la composició de la microbiota intestinal de 12 individus amb intolerància a la histamina amb el de 14 individus sans sense símptomes d'aquesta intolerància.

A nivell de fílums, els dos grups d'estudi van presentar un perfil molt similar, amb predomini de Firmicutes i Bacteroidetes (Figura 9). No obstant això, Proteobacteria va ser lleugerament superior en el grup d'intolerants a la histamina encara que sense diferències estadísticament significatives. Schink i col. (2018) també van reportar nivells més elevats d'aquest fílum en pacients amb intolerància a la histamina [36]. Segons aquests autors, un sobrecreixement de Proteobacteria podria resultar en una lleugera inflamació intestinal que a la vegada comportaria una disfunció de l'epiteli. També s'han trobat elevades proporcions d'aquest fílum en pacients amb altres desordres o patologies intestinals, com per exemple, en malalties inflamatòries intestinals (MII), en càncer colorectal i SII [143–146]. Una possible explicació del perquè el fílum Proteobacteria es troba augmentat en processos d'inflamació intestinal seria que aquesta inflamació pot incrementar la quantitat d'oxigen disponible en el lumen, fet que canviaria la proporció de bacteris anaerobis obligats per bacteris anaerobis facultatius, com és el cas de Proteobacteria. D'aquesta manera, un increment en la seva abundància s'ha postulat com a marcador de disbiosi intestinal [147,148].

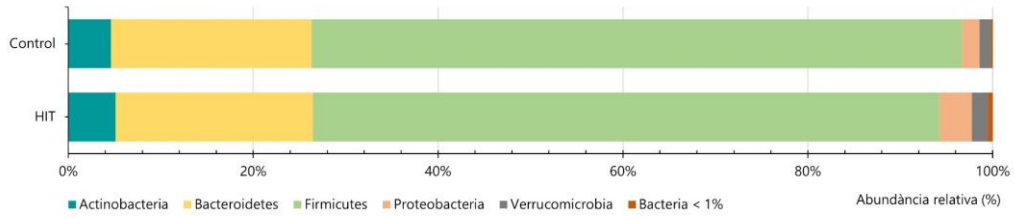


Figura 9. Abundància relativa (%) dels diferents fílums trobats en el grup control i en el grup d'intolerants a la histamina (HIT).

Com es mostra en la Figura 10, les famílies bacterianes més abundants en ambdós grups van ser *Lachnospiraceae* (Firmicutes), *Ruminococcaceae* (Firmicutes) i *Bacteroidaceae* (Bacteroidetes), totes elles representant més del 50% del total de famílies. A aquest nivell, dins de les diferències significatives trobades, destacar que el grup d'individus intolerants a la histamina va mostrar una menor abundància relativa de *Prevotellaceae* en comparació amb el grup control. Segons la literatura, una menor proporció de bacteris pertanyents a aquesta família es pot relacionar amb una reducció en la síntesi de mucina que pot provocar un increment de la permeabilitat intestinal [149]. En aquest sentit, Schink i col. (2018) van reportar uns valors de zonulina, marcador de permeabilitat intestinal, en individus amb intoleràncIa a la histamina per sobre dels valors normals en població sana, suggerint certa alteració en la permeabilitat intestinal d'aquests pacients [36]. Un increment de la permeabilitat facilitaria, entre d'altres, la penetració de metabòlits microbians, com la histamina, i en conseqüència, l'aparició dels seus efectes adversos [36].

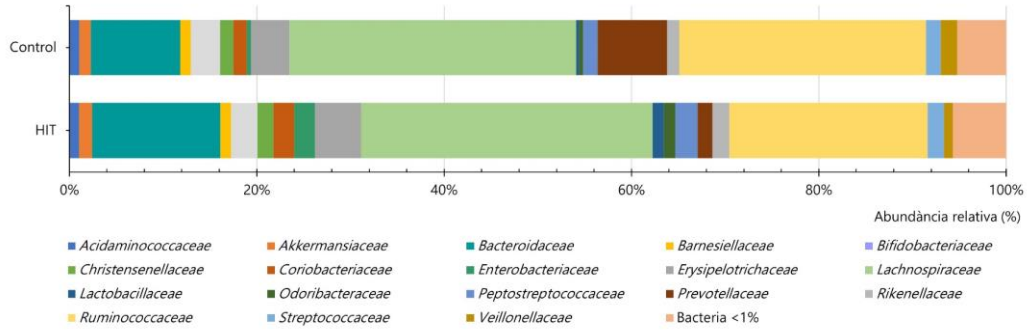


Figura 10. Abundància relativa (%) de les diferents famílies trobades en el grup control i en el grup d'intolerants a la histamina (HIT).

Les majors diferències trobades entre els dos grups d'estudi van ser en gèneres i espècies. Per exemple, en el primer *boxplot* representat en la Figura 11 es mostra l'abundància relativa de *Ruminococcus*, la qual va ser menor en el grup de pacients amb intolerància a la histamina en comparació amb el grup control. A aquest gènere bacterià se li ha atribuït un paper important en el manteniment d'una bona salut intestinal, ja que alguns dels seus membres tenen la capacitat de degradar polisacàrids complexes en diferents sucres simples, fent-los més disponibles per les cèl·lules epitelials de l'intestí [150,151]. Un altre gènere com *Faecalibacterium,* i especialment la seva espècie *Faecalibacterium prausnitzii*, trobats en proporcions significativament inferiors en el grup d'intolerants (Figura 11), són un dels principals formadors de butirat en l'intestí humà [152–154]. El butirat és un AGCC que representa la principal font d'energia dels colonòcits i també exerceix efectes protectores en front a càncer colorectal i a MII [152–154]. Per aquests motius, *F. prausnitzii* s'ha proposat com un bon marcador de salut intestinal.

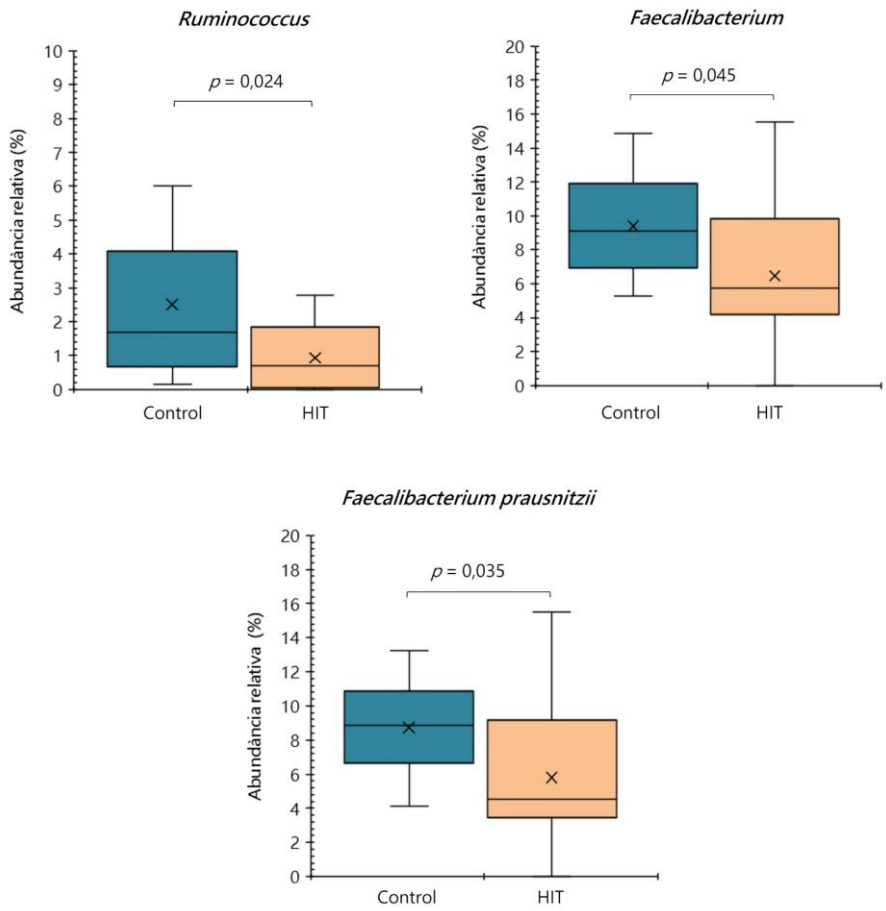


Figura 11. Abundància relativa (%) de diferents bacteris amb propietats beneficioses per l'organisme. HIT: Grup d'intolerants a la histamina.

Altrament, en la Figura 12 es representen tots aquells gèneres i espècies formadors d'histamina que van ser significativament superiors en el grup d'intolerants a la histamina. Aquest es el cas de *Staphylococcus*, *Proteus* i gèneres no identificats de la família *Enterobacteriaceae*. Moltes espècies d'aquests gèneres s'han descrit com a importants formadores d'histamina [155–157]. És important destacar però, que la capacitat histaminogènica és soca depenent [18].

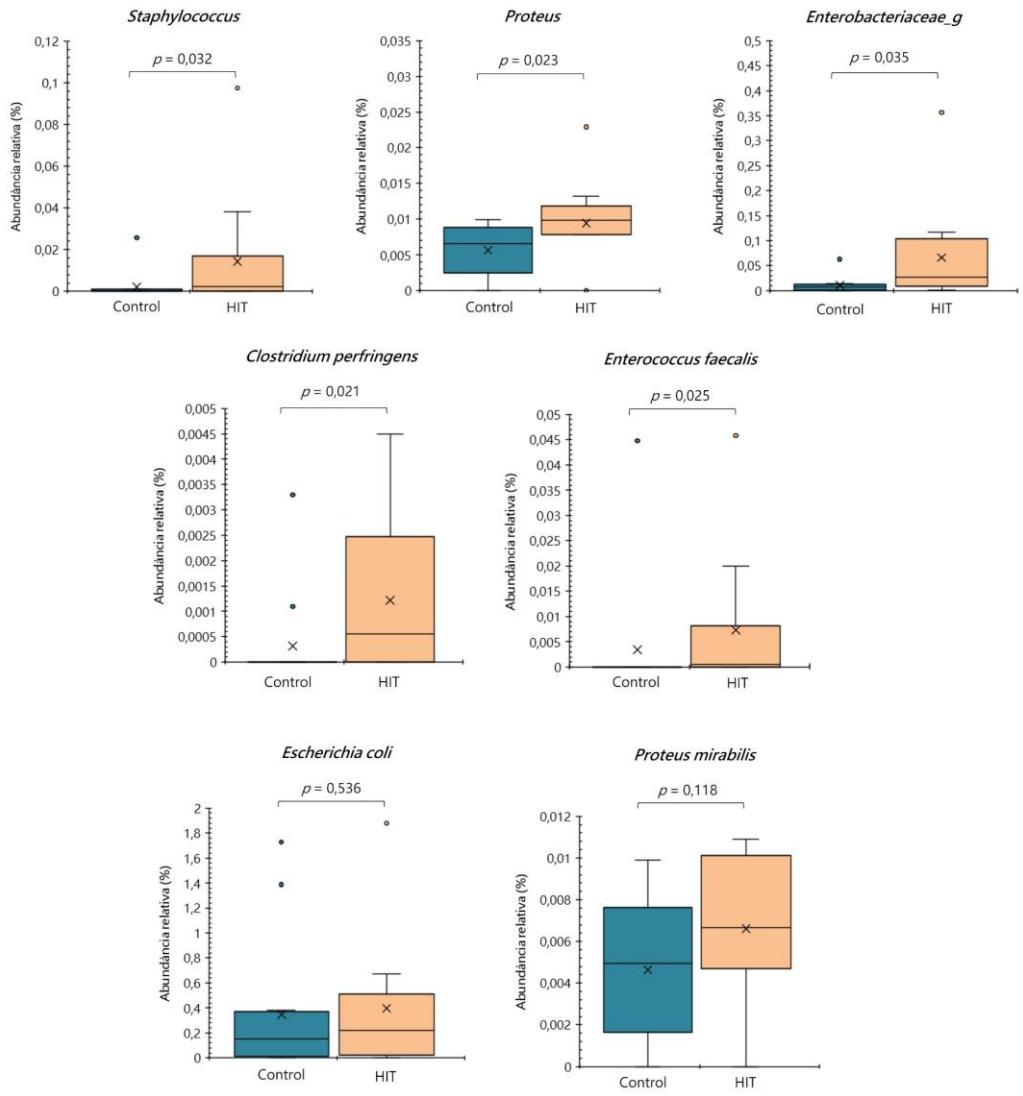


Figura 12. Abundància relativa (%) de diferents bacteris relacionats amb la formació d'histamina. HIT: Grup d'intolerants a la histamina.

Actualment, existeix força informació en la literatura científica sobre la capacitat de diferents soques bacterianes per formar histamina en aliments, però és poca, i molt recent, la informació sobre aquesta capacitat per part de la microbiota intestinal [148,158,159]. L'any 2021, Mou i col. van identificar 117 espècies de la microbiota intestinal humana potencialment capaces de formar histamina, la majoria pertanyents als gèneres *Morganella*, *Lactobacillus*, *Staphylococcus*,

Photobacterium, i *Clostridium*, àmpliament descrits com histaminogènics [148]. Segons aquests autors, *Clostridium perfringens* és una de les espècies més freqüentment associada a aquesta capacitat de formar histamina, independentment de la soca [148]. En el present estudi, la presència de *C. perfringens,* bacteri que també s'ha relacionat amb desordres gastrointestinals severs, va ser més freqüent en el grup de pacients amb intolerància a la histamina que en els controls sans, on només es va detectar en dos individus (Figura 12). En aquesta mateixa figura també es pot observar una tendència a l'alça en l'abundància de les espècies *Enterococcus faecalis*, *Escherichia coli* i *Proteus mirabilis* en el grup d'intolerants. Pugin i col. (2017) van descriure una forta activitat aminogènica en aquestes espècies aïllades de l'intestí humà, mostrant l'habilitat de formar no només histamina sinó també altres amines com, putrescina, cadaverina i tiramina [37].

La major abundància de bacteris amb capacitat de formar histamina a nivell intestinal que s'ha trobat en els pacients amb intolerància a la histamina podria resultar en una acumulació excessiva d'histamina a nivell intestinal i, per tant, en una potencial major absorció d'aquesta amina i un increment de la seva presència en plasma. A més, tenint en compte que aquests pacients pateixen, en principi, una deficiència de l'enzim DAO, la toxicitat d'aquesta amina es veuria potenciada en aquests individus. Per un altre costat, s'ha suggerit també, que un excés d'histamina podria afectar negativament la mucosa intestinal. D'acord amb alguns estudis en animals d'experimentació, nivells elevats d'histamina procedents de bacteris intestinals es relacionen amb una resposta pro-inflamatòria [158,160,161]. Segons Mishima i col. (2020), una disbiosi intestinal amb una sobreexpressió de bacteris formadors d'histamina i uns elevats nivells d'histamina intestinal s'associarien amb el desenvolupament i l'agreujament del SII [162]. En l'estudi *in silico* realitzat per Mou i col. (2021) amb 2451 mostres de femta de pacients amb MII (colitis ulcerosa i malaltia de Crohn), també es va trobar una sobreexpressió de bacteris amb una potencial capacitat histaminogènica [148]. Contràriament als resultats obtinguts per tots aquests estudis, d'altres, realitzats tant *in vitro* com en animals de

laboratori, suggereixen que la histamina a nivell intestinal estaria exercint efectes immunomoduladors degut a la supressió de la producció d'interleuquines proinflamatòries [163,164].

Malgrat la sobreexpressió de bacteris histaminogènics observada en el grup d'intolerants a la histamina, no es va trobar que aquests presentessin una major concentració d'histamina en femta en comparació amb els controls sans. De fet, la majoria dels individus, tant del grup control com dels pacients intolerants (71% i 92%, respectivament) van mostrar nivells d'histamina en femta dins dels valors considerats normals (<959 ng/g femta). Aquests resultats estan en concordança amb els descrits per Schink i col. (2018), els quals també van trobar valors molt similars d'histamina en les mostres de femta d'individus sans i d'intolerants a la histamina, i pràcticament tots dins del rang de normalitat [36].

A part de l'abundància relativa dels diferents grups bacterians, un altre concepte que es té en compte en l'estudi de la microbiota és la diversitat o riquesa en gèneres i espècies. En aquest estudi, aquesta diversitat bacteriana es va determinar mitjançant l'alfa-diversitat (índex de Shannon i índex de Simpson) i la beta-diversitat (índex de dissimilitud de Bray-Curtis). Malgrat que no es van observar diferències significatives per l'alfa-diversitat (referida a la riquesa d'espècies i l'abundància de cada una d'elles que presenta cada individu) entre els dos grups d'estudi, sí que el grup d'intolerants va mostrar una menor riquesa d'espècies pertanyents als gèneres *Bifidobacterium* i *Lactobacillus*, amb un 31% i un 41% menys d'espècies que el grup control, respectivament. Pel que fa a la beta-diversitat, entesa com les diferències interindividuals en el perfil de distribució de gèneres i espècies, l'índex de Bray-Curtis va apuntar diferències significatives entre els dos grups estudiats, tant per gèneres ($p=0,024$) com per espècies ($p=0,029$). A més, el grup d'individus intolerants va mostrar una major heterogeneïtat en aquest perfil de distribució que l'observat pel grup control.

Si comparem els nostres resultats sobre diversitat bacteriana amb els de l'únic estudi existent sobre microbiota intestinal en pacients intolerants a la histamina,

veiem que aquests són contradictoris. A diferència del nostre treball, Schink i col. (2018) sí que van trobar una menor alfa-diversitat en el grup d'individus diagnosticats d'intolerància a la histamina en comparació als individus sans, mentre que no van observar diferències significatives en la beta-diversitat [36]. Aquesta discrepància també es fa extensible a d'altres estudis centrats en diferents intoleràncies alimentàries o desordres gastrointestinals, en alguns dels quals es va observar una menor alfa-diversitat en el grup de malalts, mentre que en d'altres no es van trobar diferències significatives en aquest paràmetre respecte les poblacions sanes [165–169].

En definitiva, els resultats obtinguts en el marc d'aquesta tesi van evidenciar que els individus intolerants a la histamina estudiats presentaven una disbiosi o alteració de la microbiota intestinal caracteritzada per un costat, per una disminució d'alguns bacteris beneficiosos per la salut intestinal i per l'altre, per un sobrecreixement de bacteris formadors d'histamina. Aquesta sobreexpressió pot comportar un increment dels nivells d'histamina a l'intestí, fet que fa més probable una major absorció cap a plasma i l'aparició dels efectes provocats per aquesta amina.

Actualment, s'està estudiant com diferents factors ambientals, alimentaris o fisiològics, entre d'altres, influeixen en el perfil i composició de la microbiota intestinal [170,171]. De fet, s'està demostrant que els hàbits alimentaris serien un dels principals factors externs que més estarien influint en la seva composició [170–172]. Són diversos els estudis que han avaluat l'impacte de diferents dietes restrictives emprades pel tractament d'intoleràncies alimentàries o desordres gastrointestinals sobre els patrons microbians intestinals [173–177]. En un estudi recent dut a terme per Lenhart i col. (2022) es va observar com un grup de pacients amb SII i adherits a diferents dietes restrictives baixes en FODMAPs, en gluten o en lactosa presentaven una menor abundància dels gèneres *Bifidobacterium* i *Lactobacillus*, bacteris que exerceixen efectes positius per la salut intestinal [175].

En el cas de la intolerància a la histamina, no existeix fins a la data, cap treball centrat en estudiar els possibles canvis en la microbiota intestinal d'individus amb

intolerància a la histamina, els quals podrien tenir una disbiosi intestinal tal i com s'ha demostrat en l'estudi anterior, durant el tractament dietètic d'aquesta intolerància. Per aquest motiu, en el marc d'aquesta tesi també es va plantejar avaluar els potencials canvis en la composició de la microbiota intestinal en un subgrup de cinc pacients amb disbiosi intestinal, procedents de l'anterior estudi, durant el tractament dietètic de la intolerància a la histamina (és a dir, dieta baixa en histamina i suplementació amb DAO). Aquest subgrup de pacients estava format per dones procedents i tractades en un centre de nutrició i dietètica especialitzat en la gestió dietètica del dèficit de DAO. Aquest centre va monitoritzar el seguiment del tractament i l'evolució dels símptomes al llarg dels nou mesos de tractament dietètic.

Els principals símptomes reportats per aquestes pacients abans de l'inici del tractament dietètic van ser principalment a nivell del tracte gastrointestinal, tot i que algunes també, manifestaven mal de cap i símptomes dermatològics. Aquesta simptomatologia va millorar al final de l'estudi en totes les pacients (després dels nous mesos de seguiment del seu tractament dietètic), encara que en cap cas es va revertir totalment.

Quant a la composició de la microbiota intestinal al llarg del tractament dietètic, totes les pacients van presentar un perfil de fílums similar no observant-se canvis significatius al llarg de l'estudi. No obstant, a l'inici, una pacient mostrava una proporció anormalment elevada del fílum Proteobacteria en comparació amb les altres pacients. Cal destacar que aquesta mateixa pacient també presentava un major nombre de símptomes. Passats dos mesos de tractament dietètic, l'abundància de Proteobacteria d'aquesta pacient es va veure fortament reduïda arribant a valors similars als de les altres intolerants. Com s'ha mencionat anteriorment, alguns autors han proposat que un elevat creixement de Proteobacteria es podria utilitzar com a marcador de disbiosi intestinal [143–147]. A la vegada, també existeixen estudis que han relacionat canvis en l'abundància de Proteobacteria amb els hàbits alimentaris [178,179]. Per exemple, Levine i col.

(2019) va reportar una reducció en l'abundància relativa d'aquest fílum en pacients pediàtrics amb malaltia de Crohn després de seguir una dieta restrictiva amb exclusió d'aliments que contenien blat, làctics, greixos animals i additius i, amb l'administració de nutrició enteral [179].

A diferència dels fílums, sí que hi van haver diferències estadísticament significatives en l'abundància relativa de diferents famílies bacterianes al llarg dels tractament dietètic ($p<0,05$). En el diagrama de Venn, representat en la Figura 13, es pot observar la pèrdua d'algunes famílies, passant d'un total de 79 a l'inici, a 58 als nou mesos d'estudi, algunes d'elles relacionades amb efectes no desitjables per la salut intestinal.

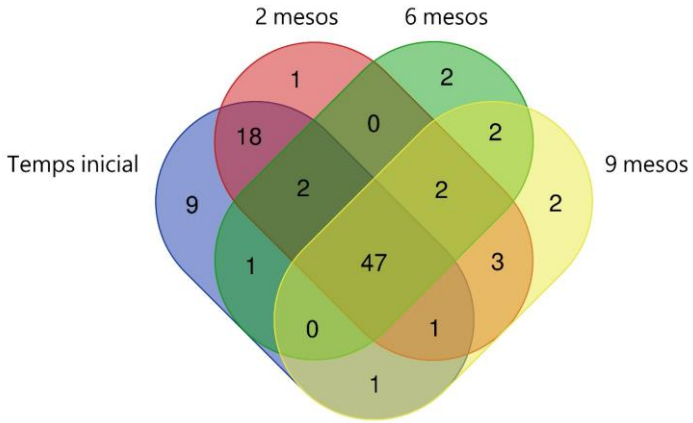


Figura 13. Diagrama de Venn del total de famílies trobades al llarg del tractament dietètic de la intoleràncIa a la histamina.

Un exemple destacable, és la reducció en la proporció de la família *Morganellaceae* en totes les pacients al segon mes de l'estudi, arribant a ser totalment absent als sis mesos de tractament (Figura 14). *Morganellaceae* inclou espècies bacterianes amb una reconeguda capacitat de formar histamina, com ara *Morganella morganii* [180]. *Pseudomonadaceae* va ser una de les altres famílies amb una marcada reducció en la seva abundància, especialment després dels sis mesos d'estudi (Figura 14). Alts nivells de pseudomonas s'han associat amb casos de MII, sent també una família bacteriana amb diverses soques relacionades amb la producció d'histamina

[27,181–185]. Tanmateix, en la Figura 14 també es mostren importants reduccions en gèneres i espècies bacterianes amb reconeguda capacitat secretora d'histamina [12,148,180,186–188]. Per exemple, l'abundància del gènere *Proteus* i de la seva espècie *Proteus mirabilis* es va veure reduïda dràsticament al segon mes de tractament, no arribant-se a detectar en cap de les pacients a partir del sisè mes. En el cas de *Raoultella*, un altre gènere descrit com histaminogènic, aquesta disminució va ser estadísticament significativa als sis i nou mesos.

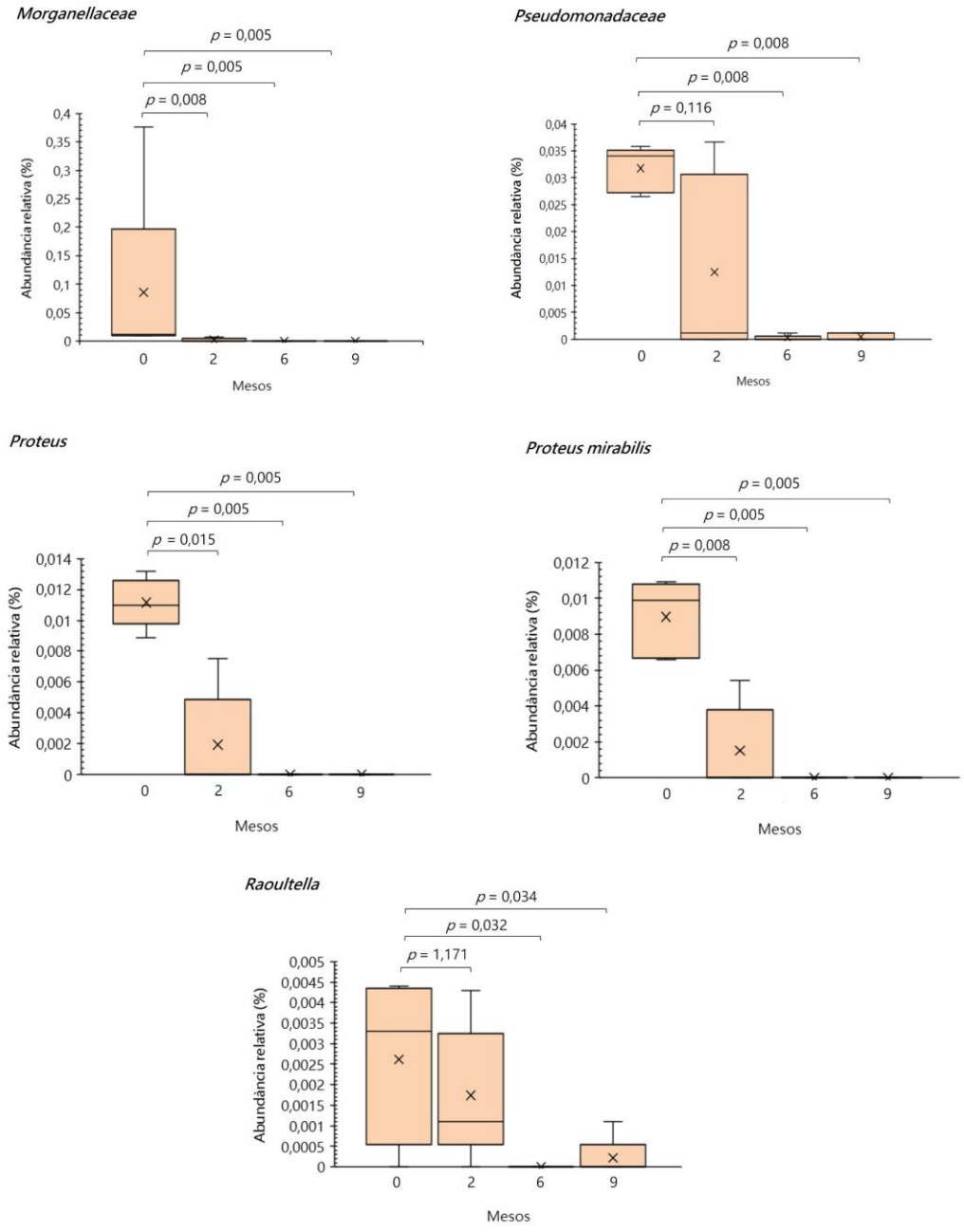


Figura 14. Evolució de l'abundància relativa (%) de diferents bacteris relacionats amb la formació d'histamina observada en individus intolerants al llarg del tractament dietètic de la intolerància a la histamina.

D'altra banda, aquestes pacients van mostrar un augment significatiu en l'abundància relativa de *Roseburia spp* al llarg del tractament dietètic. A aquesta espècie bacteriana se li han atribuït propietats beneficioses a causa de la seva capacitat per produir AGCC, els quals es troben involucrats en el manteniment de l'homeòstasi intestinal i en la inhibició dels estats d'inflamació [189–191]. Alguns autors han assenyalat que individus que segueixen dietes baixes en productes d'origen animal i enriquides en aliments d'origen vegetal presenten una major abundància de bacteris productors d'AGCC, entre ells *Roseburia* [192–194]. Com ja s'ha vist en apartats anteriors d'aquesta tesi, en el cas de les dietes baixes en histamina existeix un predomini dels aliments d'origen vegetal ja que se sol reduir el consum de derivats de la carn i el peix i, determinats làctics. Es podria hipotetitzar, per tant, que aquest augment observat en la proporció de *Roseburia* en aquestes pacients podria ser, en part, efecte del seguiment d'una dieta baixa en histamina.

Quant a la diversitat bacteriana, tot i no observar-se canvis estadísticament significatius en cap de les pacients al llarg del tractament dietètic, al final de l'estudi, tres de les cinc pacients van mostrar un perfil d'espècies microbianes molt similar al descrit pel grup d'individus sans de l'anterior estudi (apartat 3.4.).

Els principals resultats d'aquest estudi preliminar semblen indicar una millora en el desequilibri en quant a certs bacteris histaminogènics al llarg del tractament dietètic seguit per aquestes pacients. Aquest fet podria contribuir a explicar, en part, la disminució en el nombre de símptomes reportats per aquestes pacients intolerants a la histamina.

Malgrat aquests resultats prometedors, hem de tenir en compte que tot just s'està començant a estudiar el rol de la microbiota intestinal en la intolerància a la histamina, i per tant, encara s'ha de seguir aprofundint en aquest aspecte mitjançant assajos clínics més ambiciosos que estudiïn tant aquesta relació etiològica com el potencial efecte que pot tenir la gestió dietètica d'aquesta intolerància.

Àmbit 3. Intolerància a la histamina i perfil metabolòmic en orina

Fins al moment, la presència de manifestacions clíniques associades a la intolerància a la histamina que afectin dos o més òrgans o sistemes i, el fet de no presentar una al·lèrgia alimentària són els principals mètodes de diagnòstic de la intolerància a la histamina [2,32]. Addicionalment, també s'estan realitzant proves complementàries com la determinació de l'activitat DAO en sang i/o la identificació de SNPs del gen que codifica la DAO. Encara que durant aquests anys han augmentat els estudis que hi sumen evidència, aquestes proves diagnòstiques necessiten reforçar la seva validesa clínica ja que, sovint, aquests estudis presenten resultats controvertits [85,87]. Per tant, avui en dia encara seria necessari trobar una eina que permetés establir un criteri de diagnòstic sòlid i clínicament irrefutable de la intolerància a la histamina per dèficit de DAO.

Recentment s'ha proposat l'ús de biomarcadors del metabolisme de la histamina en orina com un nou mètode diagnòstic no invasiu d'aquesta intolerància [99]. La hipòtesi de treball és que els individus amb intolerància a la histamina presenten un perfil d'excreció d'histamina i els seus metabòlits en orina diferent al dels individus sans. Prèviament, i amb aquest objectiu, el grup de recerca va desenvolupar un mètode cromatogràfic que permet identificar de manera ràpida i inequívoca els nivells urinaris d'histamina i 1-methilhistamina [99]. Ara, en el marc d'aquesta tesi, es planteja estudiar la possible utilitat diagnòstica del perfil metabòlic de la histamina en orina en individus amb intolerància a la histamina. Concretament, es va avaluar l'excreció urinària d'histamina i 1-methilhistamina de 32 individus diagnosticats d'intolerància a la histamina a través de manifestacions clíniques i que relacionaven l'aparició o exacerbació de la simptomatologia després del consum d'aliments susceptibles de contenir histamina. A més, d'entre aquests pacients, 16 presentaven un dèficit de DAO sanguini. Per aquest estudi, es va considerar també un grup control que incloïa 55 individus sans. Addicionalment, en un subgrup de cinc pacients es va monitoritzar l'excreció urinària d'ambdós analits al llarg del tractament dietètic de la intolerància a la histamina (dieta baixa en histamina i

suplementació amb enzim DAO exogen) per tal de valorar la seva influència en el perfil urinari de la histamina.

Prèviament a l'estudi dels metabòlits de la histamina en orina dels pacients intolerants, es va avaluar en 14 individus sans, la fiabilitat de l'ús de mostres d'orina de primera hora del matí en comparació a la recollida durant 24 hores. L'elevada correlació observada entre ambdós tipus de mostres va demostrar que l'ús d'orina de primera hora del matí era també adequada pel propòsit d'aquest estudi. Els treballs publicats per Oosting i col. (1989) i Saito i col. (1994) van trobar també que els nivells d'histamina i 1-methilhistamina en mostres d'orina puntuals correlacionaven amb els de mostres recollides durant 24 hores [195,196]. Saito i col. (1994) van trobar una àmplia variabilitat en els valors urinaris d'histamina al llarg del dia i van indicar la utilitat de normalitzar-los en proporció a la creatinina per a compensar aquestes fluctuacions [196]. A més de la comoditat per als pacients, les mostres d'orina de primera hora del matí poden proporcionar resultats més fiables a causa d'una major concentració de l'analit (inclosa la creatinina) [197]. Segons aquests i altres autors, caldria també considerar en la interpretació dels resultats, la possible influència de la formació d'histamina per part de soques bacterianes infeccioses presents en el tracte urinari, especialment en dones, en els nivells d'histamina en mostres d'orina de primera hora del matí [197–199].

Com es pot observar en els *boxplots* de la Figura 15, els valors d'excreció urinària d'histamina en el grup d'intolerants a la histamina i el grup control van ser molt similars ($p>0,05$). A més, dins del grup d'intolerants a la histamina, tampoc es van trobar diferències significatives en l'excreció d'histamina entre individus amb i sense un dèficit de l'enzim DAO sanguini ($p>0,05$) (Figura 16). Independentment del grup d'estudi, els valors d'histamina es trobaven dins dels rangs prèviament descrits per altres autors en individus sans (8,8 - 73,6 µg/g creatinina) [197,199]. Així mateix, Lamale i col. (2006) van reportar nivells normals d'excreció d'histamina en dones adultes sanes, que oscil·laven entre 22,4 i 58,2 µg/g de creatinina, a diferència

de les dones amb cistitis intersticial que presentaven un augment significatiu de l'excreció urinària d'aquest compost [200].

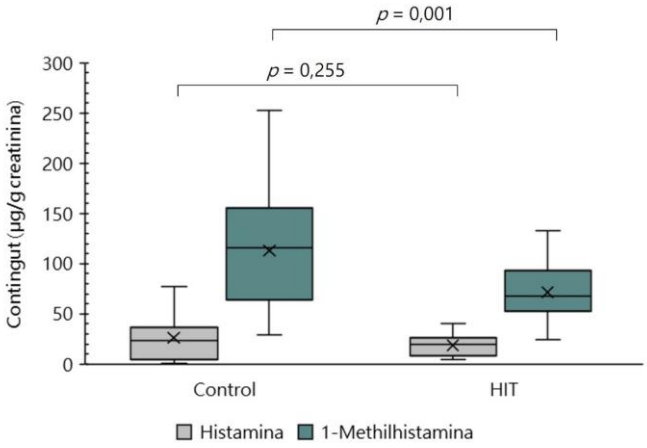


Figura 15. Distribució dels nivells d'histamina i 1-methilhistamina (µg/g creatinina) en mostres de primera hora del matí d'individus sans i d'intolerants a la histamina.

Per contra, el grup d'intolerants a la histamina sí que va mostrar nivells notablement inferiors d'1-methilhistamina en comparació amb el grup control (Figura 15). No obstant això, al comparar els individus intolerants a la histamina amb i sense dèficit de DAO sanguini, no es van observar diferències en els valors d'aquest analit en orina ($p>0,05$) (Figura 16). A més, tampoc hi va haver una relació aparent entre els nivells d'1-metilhistamina urinària i la freqüència o el tipus de simptomatologia presentada per aquests pacients.

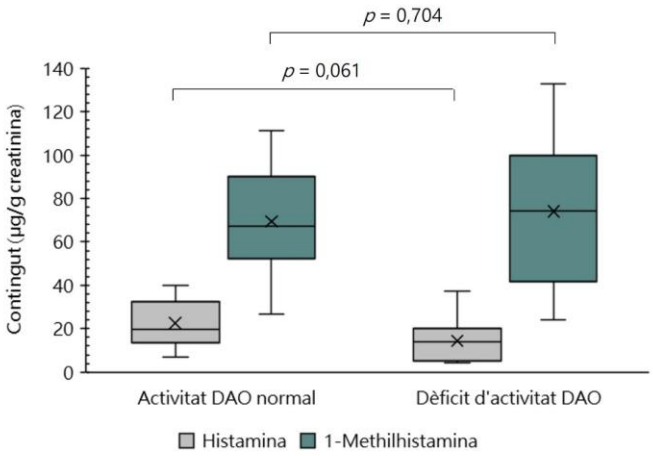


Figura 16. Distribució dels nivells d'histamina i 1-methilhistamina (µg/g creatinina) en mostres de primera hora del matí d'individus intolerants amb i sense dèficit de DAO sanguini.

Malgrat s'hagin observat diferències en l'excreció urinària d'1-methilhistamina entre els dos grups, els valors d'aquest compost en la majoria dels individus (intolerants i controls) coincideixen amb els reportats en persones sanes per altres autors (55 - 230 µg/g de creatinina) [195,199,201]. Segons Van Toorenenbergen i Oranje (2005), aquesta gran variabilitat en l'excreció d'1-methilhistamina podria explicar-se en part per les diferències interindividuals en la funcionalitat dels sistemes de degradació de la histamina en l'organisme [201].

En definitiva, la reducció observada en els nivells d'1-methilhistamina en el grup intolerants a la histamina indicaria que el perfil urinari de la histamina i el seu metabòlit podria ser un biomarcador de la intolerància a la histamina més fiable que l'activitat DAO en sang. Recordar que en aquest estudi, només el 50% dels pacients diagnosticats d'intolerància a la histamina en funció de la simptomatologia van mostrar una deficiència de l'activitat de l'enzim DAO en sang.

La 1-metilhistamina és un metabòlit derivat de la histamina que sorgeix de l'acció de l'enzim HNMT, i per tant, no s'esperava trobar nivells més baixos d'aquest metabòlit en individus intolerants a la histamina, que *a priori*, tenen un dèficit de DAO. Una possible explicació seria que una alteració en l'activitat DAO podria

conduir a una activació compensatòria del metabolisme de la histamina per l'enzim HNMT i, en última instància, a un augment en el producte final d'aquesta via (és a dir, de l'àcid N-metilimidazol acètic) [198,202]. Per tant, la determinació dels nivells d'àcid imidazol acètic i de l'àcid N-metilimidazol acètic en orina complementaria el perfil d'excreció d'histamina i proporcionaria una imatge més acurada de les possibles alteracions produïdes en individus amb intoleràncIa a la histamina. Aquests resultats també aportarien nous coneixements sobre el metabolisme de la histamina en aquesta població.

A més de l'ús de la histamina i l'1-metilhistamina com a potencials biomarcadors de la intoleràncIa a la histamina, es van avaluar els nivells d'aquests dos analits en un subgrup de pacients durant el tractament dietètic de la intoleràncIa a la histamina. Fins a dia d'avui, són molt pocs els estudis que han avaluat l'efecte de la histamina dietètica sobre els nivells urinaris d'histamina i els seus metabòlits i, només, en individus sans [203–206]. Per exemple, en l'estudi realitzat per Granerus (1968), on es va administrar histamina per via oral a tres subjectes sans, es va descriure un increment important en els nivells urinaris d'histamina, 1-metilhistamina, i especialment l'àcid N-metilimidazol acètic en orina. A més, l'excreció urinària de l'àcid N-metilimidazol acètic depenia de la dosi d'histamina administrada [203]. Uns anys més tard, Keyzer i col. (1984) també van reportar un augment en l'excreció de l'àcid N-metilimidazol acètic després de l'administració oral de 200 µM d'histamina en tres individus sans, però sense mostrar canvis significatius en la 1-metilhistamina. El mateix estudi va descriure una alta correlació entre la ingesta de proteïnes i l'excreció urinària dels metabòlits de la histamina [204]. Segons el nostre coneixement el present treball és el primer realitzat amb pacients intolerants a la histamina. Els resultats d'aquest estudi preliminar van mostrar que els nivells mitjans d'histamina i 1-metilhistamina en orina van patir una disminució després dels dos primers mesos, malgrat que sense ser estadísticament significatius ($p>0,05$). Aquest descens va ser especialment marcat en dos dels pacients, amb una reducció de fins al 86% en el cas de la histamina i al 56% en el cas de la 1-metilhistamina. Aquests resultats podrien explicar-se per la naturalesa altament

restrictiva de la dieta al començament del tractament, en la que s'exclou una àmplia gamma d'aliments susceptibles de contenir elevats nivells d'histamina (per exemple, aliments i begudes fermentats, productes derivats del peix, espinacs, albergínia i tomàquet) [85]. Durant les fases de reintroducció d'aliments no es va observar cap tendència clara ni per la histamina ni per la 1-metilhistamina.

Malgrat es tracten de resultats preliminars d'un estudi pilot amb només cinc individus, el tractament dietètic actualment utilitzat per la intoleràncIa a la histamina podria estar influint en l'excreció d'histamina i 1-methilhistamina en orina. Caldrà, per tant, determinar també altres metabòlits com l'àcid imidazol acètic i l'àcid N-metilimidazol acètic per conèixer el perfil complert d'excreció de la histamina i valorar millor aquest efecte.

5. CONCLUSIONS

5. CONCLUSIONS

[CAT]

1. A diferència del que habitualment es creu, es poden trobar quantitats rellevants d'histamina en aliments d'origen vegetal no fermentats, destacant entre aquests, l'albergínia, els espinacs i el tomàquet. En contrast amb la histamina, la putrescina (diamino-butà) es troba en gairebé tots els aliments d'origen vegetal revisats, i sovint en quantitats elevades, destacant en aquest cas els cítrics, el pebrot verd i els pèsols, entre d'altres.

2. Existeix una notable falta de consens quant als aliments que s'exclouen en les dietes baixes en histamina proposades en la literatura científica. Els aliments susceptibles de contenir histamina suposen únicament un 32% dels aliments exclosos. L'eliminació d'altres aliments que no tenen histamina es podria explicar perquè presenten un elevat contingut d'altres amines, principalment, putrescina.

3. La presència de putrescina i cadaverina (diamino-pentà) redueix significativament la velocitat de degradació de la histamina per l'enzim DAO, essent aquest efecte dependent de la quantitat d'amines present. Les dues diamines van reduir un 80% l'activitat de l'enzim DAO sobre la histamina quan es trobaven en concentracions 20 vegades superiors. Aquests resultats contribueixen a explicar per què certs aliments sense histamina, però amb continguts rellevants de putrescina o cadaverina, es relacionen amb l'aparició de símptomes de la intolerància a la histamina.

4. Els individus amb intoleràncIa a la histamina van presentar un perfil i composició de la microbiota intestinal diferent als dels individus sans. La disbiosi en el grup d'intolerants es caracteritzà per una major abundància de bacteris formadors d'histamina (*Staphylococcus*, *Proteus*, alguns gèneres no identificats de la família *Enterobacteriaceae*, *Clostridium perfringens* i *Enteroccus faecalis*) i una menor proporció de certs bacteris associats a una bona salut intestinal (*Prevotellaceae*, *Ruminococcus*, *Faecalibacterium* i *Faecalibacterium prausnitzii*).

5. Es va observar un canvi en el perfil i composició de la microbiota intestinal de cinc dones amb intoleràncIa a la histamina al llarg de nou mesos de dieta baixa en histamina i suplementació amb enzim DAO. Durant aquest període, es va veure reduïda l'abundància relativa de bacteris histaminogènics, particularment de les famílies *Morganellaceae* i *Pseudomanadaceae*, dels gèneres *Raoultella* i *Proteus* i de la seva espècie *Proteus mirabillis*. Cal confirmar aquests resultats en futurs estudis que incloguin un major nombre de pacients i un grup d'individus sans que segueixin també una dieta baixa en histamina.

6. Els individus intolerants a la histamina van presentar un perfil metabòlic de la histamina en orina diferent al del grup control, caracteritzat per una reducció significativa dels nivells d'1-metilhistamina. Aquests resultats obririen la possibilitat de que els metabòlits de la histamina es puguin utilitzar com a biomarcadors no invasius de la intoleràncIa a la histamina. Cal, però augmentar l'evidència d'aquesta possible eina diagnòstica amb un major nombre d'individus i ampliant el nombre de metabòlits de la histamina a monitoritzar.

5. CONCLUSIONS

[ENG]

1. Contrary to popular belief, significant amounts of histamine can be found in non-fermented plant-origin foods, notably eggplant, spinach and tomato. In contrast to histamine, putrescine (diamino-butane) is found in nearly all the reviewed plant foods, and often in high amounts, especially in citrus fruits, green peppers, and peas, among others.

2. Among the low-histamine diets described in the scientific literature, there is a notable lack of consensus regarding which foods should be eliminated. Notably, foods likely to contain histamine account for only 32% of the excluded items. The exclusion of histamine-free foods is based on a high content of other amines, mainly putrescine (diamino-butane).

3. The presence of putrescine and cadaverine (diamino-pentane) significantly reduces the rate of histamine degradation by the DAO enzyme, the effect being dependent on the amount of the different amines. Putrescine and cadaverine reduced DAO activity on histamine by 80% when their concentrations were 20-fold higher than that of histamine. These results help to explain why certain histamine-free foods, but with relevant contents of these other diamines, are associated with the appearance of histamine intolerance symptoms.

4. The gut microbiota profile and composition differed between histamine intolerant and healthy individuals. The dysbiosis in the intolerant group was characterized by a greater abundance of histamine-secreting bacteria (*Staphylococcus*, *Proteus*, some unidentified genera belonging to the family *Enterobacteriaceae*, *Clostridium perfringens* and *Enteroccus faecalis*) and a lower proportion of certain bacteria associated with good intestinal health (*Prevotellaceae*, *Ruminococcus*, *Faecalibacterium* and *Faecalibacterium prasunitzii*).

5. A change in the gut microbiota profile and composition was observed in five histamine intolerant women following a low-histamine diet with DAO supplementation for nine months. During this period, the relative abundance of histaminogenic bacteria, particularly of the families *Morganellaceae* and *Pseudomanadaceae*, the genera *Raoultella* and *Proteus* and the species *Proteus mirabillis*, was reduced. These results need to be confirmed in future studies including a larger number of patients and a group of healthy individuals also following a low-histamine diet.

6. The histamine intolerant individuals presented a different histamine metabolic profile in urine compared to the control group, characterized by significantly lower levels of 1-methylhistamine. These results would open perspectives for the use of these metabolites as non-invasive biomarkers of histamine intolerance. However, further evidence of this possible diagnostic tool needs to be obtained in studies with larger populations and monitoring a wider range of histamine metabolites.

6. BIBLIOGRAFIA

6. BIBLIOGRAFIA

1. Johansson, S.G.O.; Bieber, T.; Dahl, R.; Friedmann, P.S.; Lanier, B.Q.; Lockey, R.F.; et al. Revised Nomenclature for Allergy for Global Use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. *J Allergy Clin Immunol* **2004**, *113*, 832–836, doi:10.1016/j.jaci.2003.12.591.

2. Tuck, C.J.; Biesiekierski, J.R.; Schmid-Grendelmeier, P.; Pohl, D. Food Intolerances. *Nutrients* **2019**, *11*, 1–16, doi:10.3390/nu11071684.

3. Onyimba, F.; Crowe, S.E.; Johnson, S.; Leung, J. Food Allergies and Intolerances: A Clinical Approach to the Diagnosis and Management of Adverse Reactions to Food. *Clin Gastroenterol Hepatol* **2021**, *19*, 2230-2240, doi:10.1016/j.cgh.2021.01.025.

4. Arasi, S.; Nurmatov, U.; Turner, P.J.; Ansotegui, I.J.; Daher, S.; Dunn-Galvin, A.; et al. Consensus on DEfinition of Food Allergy SEverity (DEFASE): Protocol for a Systematic Review. *World Allergy Organ J* **2020**, *13*, 100493, doi:10.1016/j.waojou.2020.100493.

5. Zugasti Murillo, A. Intolerancia Alimentaria. *Endocrinol Nutr* **2009**, *56*, 241–250, doi:10.1016/S1575-0922(09)71407-X.

6. EFSA Panel on Biological Hazard (BIOHAZ). Scientific Opinion on Risk Based Control of Biogenic Amine Formation in Fermented Foods. *EFSA J* **2011**, *9*, 2393, doi: 10.2903/j.efsa.2011.2393.

7. Bover-Cid, S.; Latorre-Moratalla, M.L.; Veciana-Nogués, M.T.; Vidal-Carou, M.C. Processing Contaminants: Biogenic Amines. In *Encyclopedia of Food Safety*, Motarjemi, Y., Moy, G., Todd, E., Eds.; Academic Press, 2014; Vol. 2, pp. 381–391, ISBN 9780123786128.

8. Gardini, F.; Özogul, Y.; Suzzi, G.; Tabanelli, G.; Özogul, F. Technological Factors Affecting Biogenic Amine Content in Foods: A Review. *Front Microbiol* **2016**, *7*, 1–18, doi:10.3389/fmicb.2016.01218.

9. Russo, P.; Spano, G.; Arena, M.P.; Capozzi, V.; Fiocco, D.; Grieco, F.; et al. Are Consumers Aware of the Risks Related to Biogenic Amines in Food. *Curr Res Technol Edu Top Appl Microbiol Microb Biotechnol* **2010**, 1087–1095.

10. Zhao, Y.; Zhang, X.; Jin, H.; Chen, L.; Ji, J.; Zhang, Z. Histamine Intolerance—A Kind of Pseudoallergic Reaction. *Biomolecules* **2022**, *12*, 454, doi:10.3390/biom12030454.

11. Linares, D.M.; Martín, M.C.; Ladero, V.; Alvarez, M.A.; Fernández, M. Biogenic Amines in Dairy Products. *Crit Rev Food Sci Nutr* **2011**, *51*, 691–703, doi:10.1080/10408398.2011.582813.

12. Ladero, V.; Calles-Enríquez, M.; Fernández, M.; Alvarez, M.A. Toxicological Effects of Dietary Biogenic Amines. *Curr Nutr Food Sci* **2010**, *6*, 145–156, doi:10.2174/157340110791233256.

13. Visciano, P.; Schirone, M.; Tofalo, R.; Suzzi, G. Histamine Poisoning and Control Measures in Fish and Fishery Products. *Front Microbiol* **2014**, *5*, 1–3, doi:10.3389/fmicb.2014.00500.

14. Biji, K.B.; Ravishankar, C.N.; Venkateswarlu, R.; Mohan, C.O.; Gopal, T.K.S. Biogenic Amines in Seafood: A Review. *J Food Sci Technol* **2016**, *53*, 2210–2218, doi:10.1007/s13197-016-2224-x.

15. Hungerford, J.M. Scombroid Poisoning: A Review. *Toxicon* **2010**, *56*, 231–243, doi:10.1016/j.toxicon.2010.02.006.

16. Naila, A.; Flint, S.; Fletcher, G.; Bremer, P.; Meerdink, G. Control of Biogenic Amines in Food - Existing and Emerging Approaches. *J Food Sci* **2010**, *75*, doi:10.1111/j.1750-3841.2010.01774.x.

17. Vidal-Carou, M.C.; Veciana-Nogués, M.T.; Latorre-Moratalla, M.L.; Bover-Cid, S. Biogenic Amines: Risks and Control. In *Handbook of Fermented Meat and Poultry*; Toldrá, F., Hui, Y., Astiasarán, I., Sebranek, J., Talon, R., Eds.; John Wiley & Sons, Ltd: Oxford, 2014; pp. 413–428, ISBN 9781118522653.

18. Comas-Basté, O.; Latorre-Moratalla, M.L.; Sánchez-Pérez, S.; Veciana-Nogués, M.T.; Vidal-Carou, M.C. Histamine and Other Biogenic Amines in Food. From Scombroid Poisoning to Histamine Intolerance. In *Biogenic Amines*; Proestos C, Ed.; London, UK, 2019, doi: 0.5772/intechopen.84333.

19. Latorre-Moratalla, M.L.; Veciana-Nogués, T.; Bover-Cid, S.; Garriga, M.; Aymerich, T.; Zanardi, E.; et al. Biogenic Amines in Traditional Fermented Sausages Produced in Selected European Countries. *Food Chem* **2008**, *107*, 912–921, doi:10.1016/j.foodchem.2007.08.046.

20. Alvarez, M.A.; Moreno-Arribas, M.V. The Problem of Biogenic Amines in Fermented Foods and the Use of Potential Biogenic Amine-Degrading Microorganisms as a Solution. *Trends Food Sci Technol* **2014**, *39*, 146–155, doi:10.1016/j.tifs.2014.07.007.

21. Latorre-Moratalla, M.L.; Bover-Cid, S.; Veciana-Nogués, M.T.; Vidal-Carou, M.C. Control of Biogenic Amines in Fermented Sausages: Role of Starter Cultures. *Front Microbiol* **2012**, *3*, 169, doi: 10.3389/fmicb.2012.00169.

22. Latorre-Moratalla, M.L.; Comas-Basté, O.; Bover-Cid, S.; Vidal-Carou, M.C. Tyramine and Histamine Risk Assessment Related to Consumption of Dry Fermented Sausages by the Spanish Population. *Food and Chemical Toxicology* **2017**, *99*, 78–85, doi:10.1016/j.fct.2016.11.011.

23. Visciano, P.; Schirone, M. Update on Biogenic Amines in Fermented and Non-Fermented Beverages. *Foods* **2022**, *11*, 353, doi:10.3390/foods11030353.

24. European Comission Regulation 1019/2013/ EU. Amending Annex I to Regulation (EC) No 2073/2005 as Regards Histamine in Fishery Products. *Off J Eur Union* **2013**, *282*, 46–47.

25. Food and Drug Administration (FDA). Fish and Fishery Products Hazards and Controls Guidance; 4th ed.; U.S. Department of Health and Human Services: Gainesville, **2011**.

26. Muñoz-Esparza, N.C.; Costa-Catala, J.; Comas-Basté, O.; Toro-Funes, N.; Latorre-Moratalla, M.L.; Veciana-Nogués, M.T.; et al. Occurrence of Polyamines in Foods and the Influence of Cooking Processes. *Foods* **2021**, *10*, 1752, doi:10.3390/foods10081752.

27. Lavizzari, T.; Veciana-Nogués, M.T.; Weingart, O.; Bover-Cid, S.; Mariné-Font, A.; Vidal-Carou, M.C. Occurrence of Biogenic Amines and Polyamines in Spinach and Changes during Storage under Refrigeration. *J Agric Food Chem* **2007**, *55*, 9514–9519, doi:10.1021/jf071307l.

28. Moret, S.; Smela, D.; Populin, T.; Conte, L.S. A Survey on Free Biogenic Amine Content of Fresh and Preserved Vegetables. *Food Chem* **2005**, *89*, 355–361, doi:10.1016/j.foodchem.2004.02.050.

29. Maintz, L.; Novak, N. Histamine and Histamine Intolerance. *Am J Clin Nutr* **2007**, *85*, 1185–1196, doi:10.1093/ajcn/85.5.1185.

30. Klocker, J.; Mätzler, S.A.; Huetz, G.N.; Drasche, A.; Kolbitsch, C.; Schwelberger, H.G. Expression of Histamine Degrading Enzymes in Porcine Tissues. *Inflammation Research* **2005**, *54*, 54–57, doi:10.1007/s00011-004-0425-7.

31. Bieganski, T.; Kusche, J.; Lorenz, W.; Hesterberg, R.; Stahlknecht, C.D.; Feussner, K.-D. Distribution and Properties of Human Intestinal Diamine Oxidase and its Relevance for the Histamine Catabolism. **1983**, *756*, 196–203, doi:10.1016/0304-4165(83)90092-2.

32. Comas-Basté, O.; Sánchez-Pérez, S.; Veciana-Nogués, M.T.; Latorre-Moratalla, M.; Vidal-Carou, M.C. Histamine Intolerance: The Current State of the Art. *Biomolecules* **2020**, *10*, 1–26, doi:10.3390/biom10081181.

33. Pietrangeli, P.; Federico, R.; Mondovì, B.; Morpurgo, L. Substrate Specificity of Copper-Containing Plant Amine Oxidases. *J Inorg Biochem* **2007**, *101*, 997–1004, doi:10.1016/j.jinorgbio.2007.03.014.

34. Šebela, M.; Luhová, L.; Frébort, I.; Faulhammer, H.G.; Hirota, S.; Zajoncová, L.; et al. Analysis of the Active Sites of Copper/Topa Quinone-Containing Amine Oxidases from Lathyrus Odoratus and L. Sativus Seedlings. *Phytochem Anal* **1998**, *9*, 211–222, doi: 10.1002/(SICI)1099-1565(199809/10)9:5<211::AID-PCA407>3.0.CO;2-X.

35. Elmore, B.O.; Bollinger, J.A.; Dooley, D.M. Human Kidney Diamine Oxidase: Heterologous Expression, Purification, and Characterization. *J Biol Inorg Chem* **2002**, *7*, 565–579, doi:10.1007/s00775-001-0331-1.

36. Schink, M.; Konturek, P.C.; Tietz, E.; Dieterich, W.; Pinzer, T.C.; Wirtz, S.; et al. Microbial Patterns in Patients with Histamine Intolerance. *J Physiol Pharmacol* **2018**, *69*, 579–593, doi:10.26402/jpp.2018.4.09.

37. Pugin, B.; Barcik, W.; Westermann, P.; Heider, A.; Wawrzyniak, M.; Hellings, P.; et al. A Wide Diversity of Bacteria from the Human Gut Produces and Degrades Biogenic Amines. *Microb Ecol Health Dis* **2017**, *28*, 1353881, doi:10.1080/16512235.2017.1353881.

38. Kovacova-Hanuskova, E.; Buday, T.; Gavliakova, S.; Plevkova, J. Histamine, Histamine Intoxication and Intolerance. *Allergol Immunopathol (Madr)* **2015**, *43*, 498–506, doi: 10.1016/j.aller.2015.05.001.

39. European Food Safety Authority (EFSA) & European Centre of Disease Prevention and Control (ECDC). The European Union summary report of trends and sources of zoonosis, zoonotic agents and food-borne outbreaks in 2020. *EFSA J* **2021**, *19*, 6971, doi: 10.2903/j.efsa.2021.6971.

40. Reese, I.; Ballmer-Weber, B.; Beyer, K.; Fuchs, T.; Kleine-Tebbe, J.; Klimek, L.; et al. German Guideline for the Management of Adverse Reactions to Ingested Histamine. *Allergo Journal* **2017**, *26*, 51–61, doi:10.1007/s40629-017-0011-5.

41. Sattler, J.; Lorenz, W. Intestinal Diamine Oxidases and Enteral-Induced Histaminosis: Studies on Three Prognostic Variables in an Epidemiological Model. In *Amine Oxidases and Their Impact on Neurobiology*. Riederer, P., Youdim, M.B.H., Eds.; Journal of Neural Transmission, Springer Vienna: Vienna, 1990; pp. 291–314, doi: doi.org/10.1007/978-3-7091-9113-2_39.

42. Sattler, J.; Häfner, D.; Klotter, H.J.; Lorenz, W.; Wagner, P.K. Food-Induced Histaminosis as an Epidemiological Problem: Plasma Histamine Elevation and Haemodynamic Alterations after Oral Histamine Administration and Blockade of Diamine Oxidase (DAO). *Agents Actions* **1988**, *23*, 361–365, doi:10.1007/BF02142588.

43. Sattler, J.; Lorenz, W.; Kubo, K.; Schmal, A.; Sauer, S.; Lüben, L. Food-Induced Histaminosis under Diamine Oxidase (DAO) Blockade in Pigs: Further Evidence of the Key Role of Elevated Plasma Histamine Levels as Demonstrated by Successful Prophylaxis with Antihistamines. *Agents Actions* **1989**, *27*, 212–214, doi:10.1007/BF02222242.

44. Jarisch, R.; Wantke, F.; Raithel, M.; Hemmer, W. Histamine and Biogenic Amines. In *Histamine intolerance,histamine and seasickness.*; Jarisch, R., Ed.; Springer Berlin, Germany, 2014; Vol. 55, pp. 3–43, ISBN 9783642554469.

45. Hrubisko, M.; Danis, R.; Huorka, M.; Wawruch, M. Histamine Intolerance — The More We Know the Less We Know . *Nutrients* **2021**, *13*, 2228, doi:10.3390/nu13072228.

46. Schnedl, W.J.; Lackner, S.; Enko, D.; Schenk, M.; Holasek, S.J.; Mangge, H. Evaluation of Symptoms and Symptom Combinations in Histamine Intolerance. *Intest Res* **2019**, *17*, 427–433, doi:10.5217/ir.2018.00152.

47. Pinzer, T.C.; Tietz, E.; Waldmann, E.; Schink, M.; Neurath, M.F.; Zopf, Y. Circadian Profiling Reveals Higher Histamine Plasma Levels and Lower Diamine Oxidase Serum Activities in 24% of Patients

with Suspected Histamine Intolerance Compared to Food Allergy and Controls. *Allergy: Eur J Allergy Clin Immunol* **2018**, *73*, 949–957, doi:10.1111/all.13361.

48. Izquierdo-Casas, J.; Comas-Basté, O.; Latorre-Moratalla, M.L.; Lorente-Gascón, M.; Duelo, A.; Vidal-Carou, M.C.; et al. Low Serum Diamine Oxidase (DAO) Activity Levels in Patients with Migraine. *J Physiol Biochem* **2018**, *74*, 93–99, doi:10.1007/s13105-017-0571-3.

49. Manzotti, G.; Breda, D.; di Gioacchino, M.; Burastero, S.E. Serum Diamine Oxidase Activity in Patients with Histamine Intolerance. *Int J Immunopathol Pharmacol* **2016**, *29*, 105–111, doi:10.1177/0394632015617170.

50. Maintz, L.; Benfadal, S.; Allam, J.P.; Hagemann, T.; Fimmers, R.; Novak, N. Evidence for a Reduced Histamine Degradation Capacity in a Subgroup of Patients with Atopic Eczema. *J Allergy Clin Immunol* **2006**, *117*, 1106–1112, doi:10.1016/j.jaci.2005.11.041.

51. Rosell-Camps A; Zibetti S; Pérez-Esteban G; Vila-Vidal M; Ferrés-Ramis L; García-T.; et al. Histamine Intolerance as a Cause of Chronic Digestive complaints in Pediatric Patients. *Rev Esp Enferm Dig* **2013**, *105*, 201–207, doi: 10.4321/s1130-01082013000400004.

52. Mušič, E.; Korošec, P.; Šilar, M.; Adamič, K.; Košnik, M.; Rijavec, M. Serum Diamine Oxidase Activity as a Diagnostic Test for Histamine Intolerance. *Wien Klin Wochenschr* **2013**, *125*, 239–243, doi:10.1007/s00508-013-0354-y.

53. Guida, B.; de Martino, C.; de Martino, S.; Tritto, G.; Patella, V.; Trio, R.; et al. Histamine Plasma Levels and Elimination Diet in Chronic Idiopathic Urticaria. *Eur J Clin Nutr* **2000**, *54*, 155–158, doi: 10.1038/sj.ejcn.1600911.

54. Enko, D.; Meinitzer, A.; Mangge, H.; Kriegshaüser, G.; Halwachs-Baumann, G.; Reininghaus, E.Z.; et al. Concomitant Prevalence of Low Serum Diamine Oxidase Activity and Carbohydrate Malabsorption. *Can J Gastroenterol Hepatol* **2016**, *2016*, 1–4, doi:10.1155/2016/4893501.

55. Maintz, L.; Yu, C.F.; Rodríguez, E.; Baurecht, H.; Bieber, T.; Illig, T.; et al. Association of Single Nucleotide Polymorphisms in the Diamine Oxidase Gene with Diamine Oxidase Serum Activities. Allergy: Eur J Allergy Clin Immunol **2011**, *66*, 893–902, doi:10.1111/j.1398-9995.2011.02548.x.

56. Mayo-Yáñez, M.; Díaz-Díaz, A.; Vázquez-Barro, J.C.; González-Botas, J.H.; Figueroa, A.; Martín-Martín, C.S. Relationship between Allergic Rhinitis and Diamine Oxidase Activity: A Preliminary Report. *Allergol Select* **2021**, *5*, 187–194, doi:10.5414/als400537.

57. Kucher, A.N. Association of Polymorphic Variants of Key Histamine Metabolism Genes and Histamine Receptor Genes with Multifactorial Diseases. *Russ J Genet* 2019, *55*, 794–814, doi: 10.1134/S102279541907010X.

58. García-Martín, E.; García-Menaya, J.; Sánchez, B.; Martínez, C.; Rosendo, R.; Agúndez, J.A.G. Polymorphisms of Histamine-Metabolizing Enzymes and Clinical Manifestations of Asthma and Allergic Rhinitis. *Clin Exp Allergy* **2007**, *37*, 1175–1182, doi:10.1111/j.1365-2222.2007.02769.x.

59. Ayuso, P.; García-Martín, E.; Martínez, C.; Agúndez, J.A.G. Genetic Variability of Human Diamine Oxidase: Occurrence of Three Nonsynonymous Polymorphisms and Study of Their Effect on Serum Enzyme Activity. *Pharmacogenet Genom* **2007**, *17*, 687–693, doi:10.1097/FPC.0b013e328012b8e4.

60. Kaur, S.; Ali, A.; Siahbalaei, Y.; Ahmad, U.; Nargis, F.; Pandey, A.K.; et al. Association of Diamine Oxidase (DAO) Variants with the Risk for Migraine from North Indian Population. *Meta Gene* **2020**, *24*, 100619, doi:10.1016/j.mgene.2019.100619.

61. García-Martín, E.; Ayuso, P.; Martínez, C.; Blanca, M.; Agúndez, J.A.G. Histamine Pharmacogenomics. *Pharmacogenomics* **2009**, *10*, 867–883, doi:10.2217/pgs.09.26.

62. García-Martín, E.; Martínez, C.; Serrador, M.; Alonso-Navarro, H.; Ayuso, P.; Navacerrada, F.; et al. Diamine Oxidase Rs10156191 and Rs2052129 Variants Are Associated With the Risk for Migraine. *Headache: J Headache Pain* **2015**, *55*, 276–286, doi:10.1111/eci.13757.

63. Meza-Velázquez, R.; López-Márquez, F.; Espinosa-Padilla, S.; Rivera-Guillen, M.; Ávila-Hernández, J.; Rosales-González, M. Asociación de Polimorfismos de Diaminoxidasa e Histamina N Metiltransferasa Con La Presencia, Discapacidad y Severidad de Migraña En Un Grupo de Madres Mexicanas de Niños Alérgicos. *Neurologia* **2017**, *32*, 500–507, doi:10.1016/j.nrl.2016.02.025.

64. García-Martín, E.; Navarro-Muñoz, S.; Amo, G.; Rodriguez, C.; Serrador, M.; Alonso-Navarro, H.; et al. Increased Serum Diamine Oxidase Activity in Nonallergic Patients with Migraine. *Eur J Clin Invest* **2022**, *52*, 13757, doi:10.1111/eci.13757.

65. Schnedl, W.J.; Enko, D. Histamine Intolerance Originates in the Gut. *Nutrients* **2021**, *13*, 1262, doi:10.3390/nu13041262.

66. Raithel, M.; Matek, M.; Baenkler, H.W.; Jorde, W.; Hahn, E.G. Mucosal Histamine Content and Histamine Secretion in Crohn's Disease, Ulcerative Colitis and Allergic Enteropathy. *Int Arch Allergy Immunol* **1995**, *108*, 127–133, doi:10.1159/000237129.

67. Kuefner, M.A.; Schwelberger, H.G.; Hahn, E.G.; Raithel, M. Decreased Histamine Catabolism in the Colonic Mucosa of Patients with Colonic Adenoma. *Dig Dis Sci* **2008**, *53*, 436–442, doi:10.1007/s10620-007-9861-x.

68. Schmidt, W.U.; Sattler, J.; Hesterberg, R.; Röher, H.D.; Zoedler, T.; Sitter, H.; et al. Human Intestinal Diamine Oxidase (DAO) Activity in Crohn's Disease: A New Marker for Disease Assessment? *Agents Actions* **1990**, *30*, 267–270, doi:10.1007/BF01969057.

69. Miyoshi, J.; Miyamoto, H.; Goji, T.; Taniguchi, T.; Tomonari, T.; Sogabe, M.; et al. Serum Diamine Oxidase Activity as a Predictor of Gastrointestinal Toxicity and Malnutrition Due to Anticancer Drugs. *J Gastroenterol Hepatol (Aust)* **2015**, *30*, 1582–1590, doi:10.1111/jgh.13004.

70. Fukudome, I.; Kobayashi, M.; Dabanaka, K.; Maeda, H.; Okamoto, K.; Okabayashi, T.; et al. Diamine Oxidase as a Marker of Intestinal Mucosal Injury and the Effect of Soluble Dietary Fiber on Gastrointestinal Tract Toxicity after Intravenous 5-Fluorouracil Treatment in Rats. *Med Mol Morphol* **2014**, *47*, 100–107, doi:10.1007/s00795-013-0055-7.

71. Raithel, M.; Ulrich, P.; Hochberger, J.; Hahn, E.G. Measurament of Gut Diamine Oxidase Activity. *Ann N Y Acad Sci* **1998**, *859*, 262–266, doi:10.1111/j.1749-6632.1998.tb11142.x.

72. Schnedl, W.J.; Enko, D. Considering Histamine in Functional Gastrointestinal Disorders. *Crit Rev Food Sci Nutr* **2020**, *61*, 2960–2967, doi:10.1080/10408398.2020.1791049.

73. Enko, D.; Kriegshäuser, G.; Halwachs-Baumann, G.; Mangge, H.; Schnedl, W.J. Serum Diamine Oxidase Activity Is Associated with Lactose Malabsorption Phenotypic Variation. *Clin Biochem* **2017**, *50*, 50–53, doi:10.1016/j.clinbiochem.2016.08.019.

74. Schnedl, W.J.; Meier-Allard, N.; Lackner, S.; Enko, D.; Mangge, H.; Holasek, S.J. Increasing Expiratory Hydrogen in Lactose Intolerance Is Associated with Additional Food Intolerance/Malabsorption. *Nutrients* **2020**, *12*, 3690, doi:10.3390/nu12123690.

75. Schnedl, W.J.; Meier-Allard, N.; Michaelis, S.; Lackner, S.; Enko, D.; Mangge, H.; et al. Serum Diamine Oxidase Values, Indicating Histamine Intolerance, Influence Lactose Tolerance Breath Test Results. *Nutrients* **2022**, *14*, 2026, doi:10.3390/nu14102026.

76. Schnedl, W.J.; Lackner, S.; Enko, D.; Schenk, M.; Mangge, H.; Holasek, S.J. Non-Celiac Gluten Sensitivity: People without Celiac Disease Avoiding Gluten—Is It Due to Histamine Intolerance? *Inflammation Research* **2018**, *67*, 279–284, doi: 10.1007/s00011-017-1117-4.

77. Griauzdaitė, K.; Maselis, K.; Žvirblienė, A.; Vaitkus, A.; Jančiauskas, D.; Banaitytė-Baleišienė, I.; et al. Associations between Migraine, Celiac Disease, Non-Celiac Gluten Sensitivity and Activity of Diamine Oxidase. *Med Hypotheses* **2020**, *142*, 109738, doi:10.1016/j.mehy.2020.109738.

78. Sattler, J.; Hesterberg, R.; Lorenz, W.; Schmidt, U.; Crombach, M.; Stahlknecht, C.D. Inhibition of Human and Canine Diamine Oxidase by Drugs Used in an Intensive Care Unit: Relevance for Clinical Side Effects? *Agents Actions* **1985**, *16*, 91–94, doi:10.1007/BF01983109.

79. Leitner, R.; Zoernpfenning, E.; Missbichler, A. Evaluation of the Inhibitory Effect of Various Drugs / Active Ingredients on the Activity of Human Diamine Oxidase in Vitro. *Clin Transl Allergy* **2014**, *4*, 23, doi:10.1186/2045-7022-4-s3-p23.

80. McGrath, A.P.; Hilmer, K.M.; Collyer, C.A.; Shepard, E.M.; Elmore, B.O.; Brown, D.E.; et al. Structure and Inhibition of Human Diamine Oxidase. *Biochemistry* **2009**, *48*, 9810–9822, doi:10.1021/bi9014192.

81. Mongar, J.L. Effect of Chain Length of Aliphatic Amines on Histamine Potentiation and Release. *Br J Pharmacol Chemother* **1957**, *12*, 140–148, doi:10.1111/j.1476-5381.1957.tb00112.x.

82. Arunlakshana, O.; Mongar, J.L.; Schild, H.O. Potentiation of Pharmacological Effects of Histamine by Histaminase Inhibitors. *J Physiol* **1954**, *123*, 32–54, doi:10.1113/jphysiol.1954.sp005032.

83. Lyons, D.E.; Beery, J.T.; Lyons, S.A.; Taylor, S.L.; Lyons, D.E.; Lyons, J.T. Cadaverine and Aminoguanidine Potentiate the Uptake of Histamine in Vitro in Perfused Intestinal Segments of Rats. *Toxicol Appl Pharmacol* **1983**, *70*, 445–458, doi: 10.1016/0041-008x(83)90162-x.

84. Hui, J.Y.; Taylor, S.L. Inhibition of in Vivo Histamine Metabolism in Rats by Foodborne and Pharmacologic Inhibitors of Diamine Oxidase, Histamine N-Methyltransferase, and Monoamine Oxidase. *Toxicol Appl Pharmacol* **1985**, *81*, 241–249, doi: 10.1016/0041-008x(85)90160-7.

85. Reese, I.; Ballmer-Weber, B.; Beyer, K.; Dölle-Bierke, S.; Kleine-Tebbe, J.; Klimek, L.; et al. Guideline on Management of Suspected Adverse Reactions to Ingested Histamine - Guideline of the German Society for Allergology and Clinical Immunology (DGAKI), the Society for Pediatric Allergology and Environmental Medicine (GPA), the Medical Association of German Allergologists (AeDA) as Well as the Swiss Society for Allergology and Immunology (SGAI) and the Austrian Society for Allergology and Immunology (ÖGAI). *Allergol Select* **2021**, *5*, 305–314, doi:10.5414/alx02269e.

86. Cucca, V.; Ramirez, G.A.; Pignatti, P.; Asperti, C.; Russo, M.; Della-Torre, E.; et al. Basal Serum Diamine Oxidase Levels as a Biomarker of Histamine Intolerance: A Retrospective Cohort Study. *Nutrients* **2022**, *14*, 1513, doi:10.3390/nu14071513.

87. Schnoor, H.S.; Mosbech, H.; Skov, P.S.; Poulsen, L.K.; Jensen, B.M. Diamine Oxidase Determination in Serum. *Allergo J* **2013**, *22*, 108–111, doi:10.1007/s15007-013-0063-x.

88. Töndury, B.K.; Töndury, B.; Wüthrich, B.; Schmid-Grendelmeier, P.; Seifert, B.; Ballmer-Weber, B. Histaminintoleranz : Wie Sinnvoll Ist Die Bestimmung Der Diaminoxidase-Aktivität Im Serum in Der Alltäglichen Klinischen Praxis? *Allergologie* **2008**, *31*, 350–356, doi: 10.5414/ALP31350.

89. Kofler, H.; Aberer, W.; Deibl, M.; Hawranek, T.; Klein, G.; Reider, N.F. Diamine Oxidase (DAO) Serum Activity: Not a Useful Marker for Diagnosis of Histamine Intolerance. *Allergologie* **2009**, *32*, 105–109, doi: 10.5414/ALP32105.

90. Kuefner, M.A.; Schwelberger, H.G.; Hahn, E.G.; Raithel, M. Decreased Histamine Catabolism in the Colonic Mucosa of Patients with Colonic Adenoma. *Dig Dis Sci* **2008**, *53*, 436–442, doi:10.1007/s10620-007-9861-x.

91. Kuefner, M.A.; Schwelberger, H.G.; Weidenhiller, M.; Hahn, E.G.; Raithel, M. Both Catabolic Pathways of Histamine via Histamine-N-Melhyl-Transferase and Diamine Oxidase Are Diminished in the Colonic Mucosa of Patients with Food Allergy. *Inflammation Research* **2004**, *53*, 31–32, doi:10.1007/s00011-003-0314-5.

92. Kuefner, M.A.; Schwelberger, H.G.; Ulrich, P.; Hahn, E.G.; Raithel, M. Total Histamine Degradation Capacity (THDC) as an Important Biological Marker of Histamine Metabolism in Human Colonic Mucosa. *Inflammation Research* **2002**, *51*, 87–88, doi:10.1007/pl00022461.

93. Raithel, M.; Küfner, M.; Ulrich, P.; Hahn, E.G. The Involvement of the Histamine Degradation Pathway by Diamine Oxidase in Manifest Gastrointestinal Allergies. *Inflammation Research* **1999**, *48*, 75–76, doi:10.1007/s000110050414.

94. Lessof, M.H.; Gant, V.; Hinuma, K.; Murphy, G.M.; Dowling, R.H. Recurrent Urticaria and Reduced Diamine Oxidase Activity. *Clin Exp Allergy* **1990**, *20*, 373–376, doi:10.1111/j.1365-2222.1990.tb02796.x.

95. Kofler, L.; Ulmer, H.; Kofler, H. Histamine 50-Skin-Prick Test: A Tool to Diagnose Histamine Intolerance. *ISRN Allergy* **2011**, *2011*, 1–5, doi:10.5402/2011/353045.

96. Wagner, A.; Buczyłko, K.; Zielińska-Bliźniewska, H.; Wagner, W. Impaired Resolution of Wheals in the Skin Prick Test and Low Diamine Oxidase Blood Level in Allergic Patients. *Postepy Dermatol Alergol* **2019**, *36*, 538–543, doi:10.5114/ada.2019.89504.

97. Komericki, P.; Klein, G.; Reider, N.; Hawranek, T.; Strimitzer, T.; Lang, R.; et al. Histamine Intolerance: Lack of Reproducibility of Single Symptoms by Oral Provocation with Histamine: A Randomised, Double-Blind, Placebo-Controlled Cross-over Study. *Wien Klin Wochenschr* **2011**, *123*, 15–20, doi:10.1007/s00508-010-1506-y.

98. Wöhrl, S.; Hemmer, W.; Focke, M.; Rappersberger, K.; Jarisch, R. Histamine Intolerance-like Symptoms in Healthy Volunteers after Oral Provocation with Liquid Histamine. *Allergy Asthma Proc* **2004**, *25*, 305–311.

99. Comas-Basté, O.; Latorre-Moratalla, M.L.; Bernacchia, R.; Veciana-Nogués, M.T.; Vidal-Carou, M.C. New Approach for the Diagnosis of Histamine Intolerance Based on the Determination of Histamine and Methylhistamine in Urine. *J Pharm Biomed Anal* **2017**, *145*, 379–385, doi:10.1016/j.jpba.2017.06.029.

100. Comas-Basté, O. Intoleràncía a la Histamina: Noves Estratègies de Diagnòstic i Tractament, Tesi doctoral. Universitat de Barcelona (UB), Barcelona.

101. Schnedl, W.J.; Schenk, M.; Lackner, S.; Enko, D.; Mangge, H.; Forster, F. Diamine Oxidase Supplementation Improves Symptoms in Patients with Histamine Intolerance. *Food Sci Biotechnol* **2019**, *28*, 1779–1784, doi:10.1007/s10068-019-00627-3.

102. Wagner, N.; Dirk, D.; Peveling-Oberhag, A.; Reese, I.; Rady-Pizarro, U.; Mitzel, H.; et al. A Popular Myth – Low-Histamine Diet Improves Chronic Spontaneous Urticaria – Fact or Fiction? *J Eur Acad Dermatol Venereol* **2017**, *31*, 650–655, doi:10.1111/jdv.13966.

103. Wantke, F.; Gotz, M.; Jarisch, R. Histamine-Free Diet: Treatment of Choice for Histamine-Induced Food Intolerance and Supporting Treatment for Chronical Headaches. *Clin Exp Allergy* **1993**, *23*, 982–985, doi:10.1111/j.1365-2222.1993.tb00287.x.

104. Son, J.H.; Chung, B.Y.; Kim, H.O.; Park, C.W. A Histamine-Free Diet Is Helpful for Treatment of Adult Patients with Chronic Spontaneous Urticaria. *Ann Dermatol* **2018**, *30*, 164–172, doi:10.5021/ad.2018.30.2.164.

105. King W; McCargar L; Joneja J.M; Barr S. I Benefits of a Histamine-Reducing Diet for Some Patients with Chronic Urticaria and Angiodema. Can J Diet Pract Res **2000**, *61*, 24–26.

106. Böhn, L.; Störsrud, S.; Törnblom, H.; Bengtsson, U.; Simrén, M. Self-Reported Food-Related Gastrointestinal Symptoms in IBS Are Common and Associated with More Severe Symptoms and Reduced Quality of Life. *Am J Gastroenterol* **2013**, *108*, 634–641, doi:10.1038/ajg.2013.105.

107. Lefèvre, S.; Astier, C.; Kanny, G. Intolérance à l'histamine Ou Fausses Allergies Alimentaires de Mécanisme Histaminique. *Rev Fr Allergol* **2017**, *57*, 24–34, doi:10.1016/j.reval.2016.10.004.

108. Steinbrecher, I.; Jarisch, R. Histamin Und Kopfschmerz. *Allergologie* **2005**, *28*, 85–91, doi: 10.5414/ALP28085.

109. Park, C.; Park, C.; Choi, J.; Lee, H. A Study of Elimination Diet for Chronic Idiopathic Urticaria in Korea. *J Am Acad Dermatol* **2008**, *58*, AB38, doi:10.1016/j.jaad.2007.10.180.

110. Worm, M.; Fiedler, E.M.; Dölle, S.; Schink, T.; Hemmer, W.; Jarisch, R.; et al. Exogenous Histamine Aggravates Eczema in a Subgroup of Patients with Atopic Dermatitis. *Acta Derm Venereol* **2009**, *89*, 52–56, doi:10.2340/00015555-0565.

111. Siebenhaar, F.; Melde, A.; Magerl, M.; Zuberbier, T.; Church, M.K.; Maurer, M. Histamine Intolerance in Patients with Chronic Spontaneous Urticaria. *J Eur Acad Dermatol Venereol* **2016**, *30*, 1774–1777, doi:10.1111/jdv.13778.

112. Hoffmann, K.M.; Gruber, E.; Deutschmann, A.; Jahnel, J.; Hauer, A.C. Histamine Intolerance in Children with Chronic Abdominal Pain. *Arch Dis Child* 2013, *98*, 832–833, doi:10.1136/archdischild-2013-305024.

113. Lackner, S.; Malcher, V.; Enko, D.; Mangge, H.; Holasek, S.J.; Schnedl, W.J. Histamine-Reduced Diet and Increase of Serum Diamine Oxidase Correlating to Diet Compliance in Histamine Intolerance. *Eur J Clin Nutr* **2019**, *73*, 102–104, doi:10.1038/s41430-018-0260-5.

114. Vassilopoulou, E.; Konstantinou, G.N.; Dimitriou, A.; Manios, Y.; Koumbi, L.; Papadopoulos, N.G. The Impact of Food Histamine Intake on Asthma Activity: A Pilot Study. *Nutrients* **2020**, *12*, 1–13, doi:10.3390/nu12113402.

115. Comas-Basté, O.; Latorre-Moratalla, M.L.; Rabell-González, J.; Veciana-Nogués, M.T.; Vidal-Carou, M.C. Lyophilised Legume Sprouts as a Functional Ingredient for Diamine Oxidase Enzyme Supplementation in Histamine Intolerance. *LWT-Food Sci Technol* **2020**, *125*, 109-201, doi:10.1016/j.lwt.2020.109201.

116. European Commission Commission Implementing Regulation (EU) 2017/2470 of 20 December 2017 establishing the Union list of novel foods in accordance with Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. Off J Eur Union **2017**, L 351, 72–201.

117. Yacoub, M.R.; Ramirez, G.A.; Berti, A.; Mercurio, G.; Breda, D.; Saporiti, N.; et al. Diamine Oxidase Supplementation in Chronic Spontaneous Urticaria: A Randomized, Double-Blind Placebo-Controlled Study. *Int Arch Allergy Immunol* **2018**, *176*, 268–271, doi:10.1159/000488142.

118. Izquierdo-Casas, J.; Comas-Basté, O.; Latorre-Moratalla, M.L.; Lorente-Gascón, M.; Duelo, A.; Soler-Singla, L.; et al. Diamine Oxidase (DAO) Supplement Reduces Headache in Episodic Migraine Patients with DAO Deficiency: A Randomized Double-Blind Trial. *Clin Nutr* **2019**, *38*, 152–158, doi:10.1016/j.clnu.2018.01.013.

119. Comas-Basté, O.; Latorre-Moratalla, M.L.; Sánchez-Pérez, S.; Veciana-Nogués, M.T.; Vidal-Carou, M.C. In Vitro Determination of Diamine Oxidase Activity in Food Matrices by an Enzymatic Assay Coupled to UHPLC-FL. *Anal Bioanal Chem* **2019**, *411*, 7595-7602, doi:10.1007/s00216-019-02178-2.

120. San Mauro Martin, I.; Brachero, S.; Garicano Vilar, E. Histamine Intolerance and Dietary Management: A Complete Review. *Allergol Immunopathol (Madr)* **2016**, *44*, 475–483, doi:10.1016/j.aller.2016.04.015.

121. Joneja, J.M.; Carmona-Silva, C. Outcome of a Histamine-Restricted Diet Based on Chart Audit. *J Nutr Environ Med* **2001**, *11*, 249–262, doi:10.1080/1359084012010309.

122. Lehane, L.; Olley, J. Histamine Fish Poisoning Revisited. *Int J Food Microbiol* **2000**, *58*, 1–37, doi: 10.1016/s0168-1605(00)00296-8.

123. Veciana-Nogués M T; Vidal-Carou M C Dieta Baja En Histamina. In *Dieta y nutrición clínica* ; Salas-Salvadó J.; Bonada-Sanjaume A.; Trallero-Casaña R.; Trallero-Casaña R.; Burgos-Peláez R., Eds.; Elsevier: Barcelona, Spain, 2008; pp. 443–448.

124. Medina-Remón, A.; Barrionuevo-González, A.; Zamora-Ros, R.; Andres-Lacueva, C.; Estruch, R.; Martínez-González, M.Á.; et al. Rapid Folin-Ciocalteu Method Using Microtiter 96-Well Plate Cartridges for Solid Phase Extraction to Assess Urinary Total Phenolic Compounds, as a

Biomarker of Total Polyphenols Intake. *Anal Chim Acta* **2009**, *634*, 54–60, doi:10.1016/j.aca.2008.12.012.

125. Toro-Funes, N.; Bosch-Fuste, J.; Latorre-Moratalla, M.L.; Veciana-Nogués, M.T.; Vidal-Carou, M.C. Biologically Active Amines in Fermented and Non-Fermented Commercial Soybean Products from the Spanish Market. *Food Chem* **2015**, *173*, 1119–1124, doi:10.1016/j.foodchem.2014.10.118.

126. Barbieri, F.; Montanari, C.; Gardini, F.; Tabanelli, G. Biogenic Amine Production by Lactic Acid Bacteria: A Review. *Foods* **2019**, *8*, 17, doi:10.3390/foods8010017.

127. Schirone, M.; Esposito, L.; D'Onofrio, F.; Visciano, P.; Martuscelli, M.; Mastrocola, D.; et al. Biogenic Amines in Meat and Meat Products: A Review of the Science and Future Perspectives. *Foods* **2022**, *11*, 788, doi:10.3390/foods11060788.

128. Zdolec, N.; Bogdanović, T.; Severin, K.; Dobranić, V.; Kazazić, S.; Grbavac, J.; et al. Biogenic Amine Content in Retailed Cheese Varieties Produced with Commercial Bacterial or Mold Cultures. *Processes* **2021**, *10*, 10, doi:10.3390/pr10010010.

129. FAO/WHO (Food and Agriculture Organitzation of the United Nations/World Health Organitzation) *Public Health Risks of Histamine and Other Biogenic Amines from Fish and Fishery Products. Meeting Report*; Rome, **2013**, ISBN: 9789240691919.

130. Naila, A.; Flint, S.; Fletcher, G.C.; Bremer, P.J.; Meerdink, G. Histamine Stability in Rihaakuru at −80, 4 and 30°C. *Food Chem* **2012**, *135*, 1226–1229, doi:10.1016/j.foodchem.2012.05.066.

131. Jiang, Q.Q.; Dai, Z.Y.; Zhou, T.; Wu, J.J.; Bu, J.Z.; Zheng, T.L. Histamine Production and Bacterial Growth in Mackerel (Pneumotophorus Japonicus) during Storage. *J Food Biochem* **2013**, *37*, 246–253, doi:10.1111/jfbc.12021.

132. Simon-Sarkadi, L.; Holzapfel, W.H.; Halasz, A. Biogenic Amines Contents and Microbial Contamination of Leafy Vegetables during Storage at 5C. *J Food Biochem* **1993**, *17*, 407–418, doi:10.1111/j.1745-4514.1993.tb00483.x.

133. Doeun, D.; Davaatseren, M.; Chung, M.-S. Biogenic Amines in Foods. *Food Sci Biotechnol* **2017**, *26*, 1463–1474, doi:10.1007/s10068-017-0239-3.

134. Latorre-Moratalla, M.L.; Comas-Basté, O.; Veciana-Nogués, M.T.; Vidal-Carou, M.C. La Cocción Reduce El Contenido de Histamina de Las Espinacas. *11a Reunión de la Sociedad Española de Seguridad Alimentaria (SESAL),* Pamplona, 2015.

135. Kalač, P. Health Effects and Occurrence of Dietary Polyamines: A Review for the Period 2005-Mid 2013. *Food Chem* **2014**, *161*, 27–39, doi:10.1016/j.foodchem.2014.03.102.

136. Kalač, P.; Švecová S.; Pelikánová T. Levels of Biogenic Amines in Typical Vegetable Products. *Food Chem* **2002**, *77*, 349–351, doi: 10.1016/S0308-8146(01)00360-0.

137. Preti, R.; Bernacchia, R.; Vinci, G. Chemometric Evaluation of Biogenic Amines in Commercial Fruit Juices. *Eur Food Res Technol* **2016**, *242*, 2031–2039, doi:10.1007/s00217-016-2701-5.

138. Esti, M.; Volpe, G.; Massignan, L.; Compagnone, D.; la Notte, E.; Palleschi, G. Determination of Amines in Fresh and Modified Atmosphere Packaged Fruits Using Electrochemical Biosensors. *J Agric Food Chem* **1998**, *46*, 4233–4237, doi:10.1021/jf980159j.

139. Vlieg-Boerstra, B.J.; van der Heide, S.; Oude Elberink, J.N.G.; Kluin-Nelemans, J.C.; Dubois, A.E.J. Mastocytosis and Adverse Reactions to Biogenic Amines and Histamine-Releasing Foods: What Is the Evidence? *J Med* **2005**, *63*, 244–249.

140. Ignesti, G. Equations of Substrate-Inhibition Kinetics Applied to Pig Kidney Diamine Oxidase (DAO, E.C. 1.4.3.6). *J Enzyme Inhib Med Chem* **2003**, *18*, 463–473, doi:10.1080/14756360310001605543.

141. Blaschko, H.; Hawkins, J. Enzymic Oxidation of Aliphatic Diamines. *J. Pharmacol* **1950**, *5*, 625-632, doi: 10.1111/j.1476-5381.1950.tb00612.x.

142. Lortie, M.J.; Novotny, W.F.; Peterson, O.W.; Vallon, V.; Malvey, K.; Mendonca, M.; et al. Agmatine, a Bioactive Metabolite of Arginine: Production, Degradation, and Functional Effects in the Kidney of the Rat. *J Clin Investig* **1996**, *97*, 413–420, doi:10.1172/JCI118430.

143. Gophna, U.; Sommerfeld, K.; Gophna, S.; Doolittle, W.F.; Veldhuyzen van Zanten, S.J.O. Differences between Tissue-Associated Intestinal Microfloras of Patients with Crohn's Disease and Ulcerative Colitis. *J Clin Microbiol* **2006**, *44*, 4136–4141, doi:10.1128/JCM.01004-06.

144. Wang, T.; Cai, G.; Qiu, Y.; Fei, N.; Zhang, M.; Pang, X.; et al. Structural Segregation of Gut Microbiota between Colorectal Cancer Patients and Healthy Volunteers. *ISME J* **2012**, *6*, 320–329, doi:10.1038/ismej.2011.109.

145. Krogius-Kurikka, L.; Lyra, A.; Malinen, E.; Aarnikunnas, J.; Tuimala, J.; Paulin, L.; et al. Microbial Community Analysis Reveals High Level Phylogenetic Alterations in the Overall Gastrointestinal Microbiota of Diarrhoea-Predominant Irritable Bowel Syndrome Sufferers. *BMC Gastroenterol* **2009**, *9*, 1–11, doi:10.1186/1471-230X-9-95.

146. Morgan, X.C.; Tickle, T.L.; Sokol, H.; Gevers, D.; Devaney, K.L.; Ward, D. V.; et al. Dysfunction of the Intestinal Microbiome in Inflammatory Bowel Disease and Treatment. *Genome Biol* **2012**, *13*, 79, doi:10.1186/gb-2012-13-9-r79.

147. Litvak, Y.; Byndloss, M.X.; Tsolis, R.M.; Bäumler, A.J. Dysbiotic Proteobacteria Expansion: A Microbial Signature of Epithelial Dysfunction. *Curr Opin Microbiol* **2017**, *39*, 1–6, doi:10.1016/j.mib.2017.07.003.

148. Mou, Z.; Yang, Y.; Hall, A.B.; Jiang, X. The Taxonomic Distribution of Histamine-Secreting Bacteria in the Human Gut Microbiome. *BMC Genom* **2021**, *22*, doi:10.1186/s12864-021-08004-3.

149. Scheperjans, F.; Aho, V.; Pereira, P.A.B.; Koskinen, K.; Paulin, L.; Pekkonen, E.; et al. Gut Microbiota Are Related to Parkinson's Disease and Clinical Phenotype. *Movement Disorders* **2015**, *30*, 350–358, doi:10.1002/mds.26069.

150. La Reau, A.J.; Suen, G. The Ruminococci: Key Symbionts of the Gut Ecosystem. *J Microbiol* **2018**, *56*, 199–208, doi:10.1007/s12275-018-8024-4.

151. Cockburn, D.W.; Koropatkin, N.M. Polysaccharide Degradation by the Intestinal Microbiota and Its Influence on Human Health and Disease. *J Mol Biol* 2016, *428*, 3230–3252, doi:10.1016/j.jmb.2016.06.021.

152. Lopez-Siles, M.; Duncan, S.H.; Garcia-Gil, L.J.; Martinez-Medina, M. Faecalibacterium Prausnitzii: From Microbiology to Diagnostics and Prognostics. *ISME J* **2017**, *11*, 841–852, doi:10.1038/ismej.2016.176.

153. Martín, R.; Miquel, S.; Benevides, L.; Bridonneau, C.; Robert, V.; Hudault, S.; et al. Functional Characterization of Novel Faecalibacterium Prausnitzii Strains Isolated from Healthy Volunteers: A Step Forward in the Use of F. Prausnitzii as a next-Generation Probiotic. *Front Microbiol* **2017**, *8*, 1–13, doi:10.3389/fmicb.2017.01226.

154. Laursen, M.F.; Laursen, R.P.; Larnkjær, A.; Mølgaard, C.; Michaelsen, K.F.; Frøkiær, H.; et al. Faecalibacterium Gut Colonization Is Accelerated by Presence of Older Siblings. *mSphere* **2017**, *2*, 2–7, doi:10.1128/msphere.00448-17.

155. Durak-Dados, A.; Michalski, M.; Osek, J. Histamine and Other Biogenic Amines in Food. *J Vet Res* **2020**, *64*, 281–288, doi:10.2478/jvetres-2020-0029.

156. Landete, J.M.; de Las Rivas, B.; Marcobal, A.; Muñoz, R. Updated Molecular Knowledge about Histamine Biosynthesis by Bacteria. *Crit Rev Food Sci Nutr* **2008**, *48*, 697–714, doi:10.1080/10408390701639041.

157. Refai, M.A.; El-Hariri, M.; Ahmed, Z.A.M.; Jakee, J. E. Histamine Producing Bacteria in Fish. *Egypt J Aquat Biol Fish* **2020**, *24*, 1-11, doi:10.21608/EJABF.2020.117856.

158. Krell, T.; Gavira, J.A.; Velando, F.; Fernández, M.; Roca, A.; Monteagudo-Cascales, E.; et al. Histamine: A Bacterial Signal Molecule. *Int J Mol Sci* **2021**, *22*, 6312, doi:10.3390/ijms22126312.

159. Sudo, N. Biogenic Amines: Signals Between Commensal Microbiota and Gut Physiology. *Front Endocrinol (Lausanne)* **2019**, *10*, 504, doi:10.3389/fendo.2019.00504.

160. Ferstl, R.; Frei, R.; Schiavi, E.; Konieczcna, P.; Barcik, P.; Ziegler, M.; et al. Histamine Receptor 2 Is a Key Influence in Immune Responses to Microbial Ligands. *J Allergy Clin Immunol* **2014**, *134*, 741-744, doi:10.1016/j.jaci.2014.04.029.

161. O'Mahony, L.; Akdis, M.; Akdis, C.A. Regulation of the Immune Response and Inflammation by Histamine and Histamine Receptors. *J Allergy Clin Immunol* **2011**, *128*, 1153–1162, doi:10.1016/j.jaci.2011.06.051.

162. Mishima, Y.; Ishihara, S. Molecular Mechanisms of Microbiota-Mediated Pathology in Irritable Bowel Syndrome. *Int J Mol Sci* **2020**, *21*, 1–25, doi:10.3390/ijms21228664.

163. Frei, R.; Ferstl, R.; Konieczna, P.; Ziegler, M.; Simon, T.; Rugeles, T.M.; et al. Histamine Receptor 2 Modifies Dendritic Cell Responses to Microbial Ligands. *J Allergy Clin Immunol* **2013**, *132*, 194-204, doi:10.1016/j.jaci.2013.01.013.

164. Levy, M.; Thaiss, C.A.; Zeevi, D.; Dohnalová, L.; Zilberman-Schapira, G.; Mahdi, J.A.; et al. Microbiota-Modulated Metabolites Shape the Intestinal Microenvironment by Regulating NLRP6 Inflammasome Signaling. *Cell* **2015**, *163*, 1428–1443, doi:10.1016/j.cell.2015.10.048.

165. Jackson, M.A.; Verdi, S.; Maxan, M.E.; Shin, C.M.; Zierer, J.; Bowyer, R.C.E.; et al. Gut Microbiota Associations with Common Diseases and Prescription Medications in a Population-Based Cohort. *Nat Commun* **2018**, *9*, 2655, doi:10.1038/s41467-018-05184-7.

166. McMurtry, V.E.; Gupta, R.W.; Tran, L.; Blanchard, E.E.; Penn, D.; Taylor, C.M.; et al. Bacterial Diversity and Clostridia Abundance Decrease with Increasing Severity of Necrotizing Enterocolitis. *Microbiome* **2015**, *3*, 11, doi:10.1186/s40168-015-0075-8.

167. Chao, G.; Zhang, S. The Characteristics of Intestinal Flora of IBS-D with Different Syndromes. *Immun Inflamm Dis* **2020**, *8*, 615–628, doi:10.1002/iid3.348.

168. Das, A.; O'Herlihy, E.; Shanahan, F.; O'Toole, P.W.; Jeffery, I.B. The Fecal Mycobiome in Patients with Irritable Bowel Syndrome. *Sci Rep* **2021**, *11*, doi:10.1038/s41598-020-79478-6.

169. Wacklin, P.; Laurikka, P.; Lindfors, K.; Collin, P.; Salmi, T.; Lähdeaho, M.L.; et al. Altered Duodenal Microbiota Composition in Celiac Disease Patients Suffering from Persistent Symptoms on a Long-Term Gluten-Free Diet. *Am J Gastroenterol* **2014**, *109*, 1933–1941, doi:10.1038/ajg.2014.355.

170. Power, S.E.; O'Toole, P.W.; Stanton, C.; Ross, R.P.; Fitzgerald, G.F. Intestinal Microbiota, Diet and Health. *Br J Nutr* **2014**, *111*, 387–402, doi:10.1017/S0007114513002560.

171. Rinninella, E.; Cintoni, M.; Raoul, P.; Lopetuso, L.R.; Scaldaferri, F.; Pulcini, G.; et al. Food Components and Dietary Habits: Keys for a Healthy Gut Microbiota Composition. *Nutrients* **2019**, *11*, 2393, doi:10.3390/nu11102393.

172. Salonen, A.; de Vos, W.M. Impact of Diet on Human Intestinal Microbiota and Health. *Annu Rev Food Sci Technol* **2014**, *5*, 239–262, doi:10.1146/annurev-food-030212-182554.

173. Staudacher, H.M.; Whelan, K.; Irving, P.M.; Lomer, M.C.E. Comparison of Symptom Response Following Advice for a Diet Low in Fermentable Carbohydrates (FODMAPs) versus Standard Dietary Advice in Patients with Irritable Bowel Syndrome. *J Hum Nutr Diet* **2011**, *24*, 487–495, doi:10.1111/j.1365-277X.2011.01162.x.

174. Starz, E.; Wzorek, K.; Folwarski, M.; Kaźmierczak-Siedlecka, K.; Stachowska, L.; Przewłócka, K.; et al. The Modification of the Gut Microbiota via Selected Specific Diets in Patients with Crohn's Disease. *Nutrients* **2021**, *13*, 2125, doi:10.3390/nu13072125.

175. Lenhart, A.; Dong, T.; Joshi, S.; Jaffe, N.; Choo, C.; Liu, C.; et al. Effect of Exclusion Diets on Symptom Severity and the Gut Microbiota in Patients With Irritable Bowel Syndrome. *Clin Gastroenterol Hepatol* **2022**, *20*, 465–483, doi:10.1016/j.cgh.2021.05.027.

176. De Palma, G.; Nadal, I.; Collado, M.C.; Sanz, Y. Effects of a Gluten-Free Diet on Gut Microbiota and Immune Function in Healthy Adult Human Subjects. *Br J Nutr* **2009**, *102*, 1154–1160, doi:10.1017/S0007114509371767.

177. Gibson, P.R.; Halmos, E.P.; Muir, J.G. Review Article: FODMAPS, Prebiotics and Gut Health-the FODMAP Hypothesis Revisited. *Aliment Pharmacol Ther* **2020**, *52*, 233–246, doi:10.1111/apt.15818.

178. De Filippo, C.; Cavalieri, D.; di Paola, M.; Ramazzotti, M.; Poullet, J.B.; Massart, S.; et al. Impact of Diet in Shaping Gut Microbiota Revealed by a Comparative Study in Children from Europe and Rural Africa. *Proc Natl Acad Sci U S A* **2010**, *107*, 14691–14696, doi:10.1073/pnas.1005963107.

179. Levine, A.; Wine, E.; Assa, A.; Sigall-Boneh, R.; Shaoul, R.; Kori, M.; et al. Crohn's Disease Exclusion Diet Plus Partial Enteral Nutrition Induces Sustained Remission in a Randomized Controlled Trial. *Gastroenterology* **2019**, *157*, 440–450, doi:10.1053/j.gastro.2019.04.021.

180. Bjornsdottir-Butler, K.; Jones, J.L.; Benner, R.; Burkhardt, W. Development of a Real-Time PCR Assay with an Internal Amplification Control for Detection of Gram-Negative Histamine-Producing Bacteria in Fish. *Food Microbiol* **2011**, *28*, 356–363, doi:10.1016/j.fm.2010.06.013.

181. Mukhopadhya, I.; Hansen, R.; El-Omar, E.M.; Hold, G.L. IBD-What Role Do Proteobacteria Play? *Nat Rev Gastroenterol Hepatol* **2012**, *9*, 219–230, doi:10.1038/nrgastro.2012.14.

182. Wagner, J.; Short, K.; Catto-Smith, A.G.; Cameron, D.J.S.; Bishop, R.F.; Kirkwood, C.D. Identification and Characterisation of Pseudomonas 16S Ribosomal DNA from Ileal Biopsies of Children with Crohn's Disease. *PLoS One* **2008**, *3*, 3578, doi:10.1371/journal.pone.0003578.

183. Parent, K.; Mitchell, P. Pseudomonas-like Group Va Bacteria in Crohn's Disease. *Gastroenterology* **1978**, *75*, 765.

184. Parent, K.; Mitchell, P. Cell Wall-Defective Variants of Pseudomonas-like (Group Va) Bacteria in Crohn's Disease. *Gastroenterology* **1978**, *75*, 368–372, doi:10.1016/0016-5085(78)90834-x.

185. Wei, B.; Huang, T.; Dalwadi, H.; Sutton, C.L.; Bruckner, D.; Braun, J. Pseudomonas Fluorescens Encodes the Crohn's Disease-Associated I2 Sequence and T-Cell Superantigen. *Infect Immun* **2002**, *70*, 6567–6575, doi:10.1128/IAI.70.12.6567-6575.2002.

186. Sękowska, A. Raoultella Spp.—Clinical Significance, Infections and Susceptibility to Antibiotics. *Folia Microbiol (Praha)* **2017**, *62*, 221–227, doi:10.1007/s12223-016-0490-7.

187. Kanki, M.; Yoda, T.; Tsukamoto, T.; Shibata, T. Klebsiella Pneumoniae Produces No Histamine: Raoultella Planticola and Raoultella Omithinolytica Strains Are Histamine Producers. *Appl Environ Microbiol* **2002**, *68*, 3462–3466, doi:10.1128/AEM.68.7.3462-3466.2002.

188. Bjornsdottir-Butler, K.; Bolton, G.E.; Mcclellan-Green, P.D.; Jaykus, L.-A.; Green, D.P. Detection of Gram-Negative Histamine-Producing Bacteria in Fish: A Comparative Study. *J Food Prot* **2009**, *72*, 1987–1991, doi:10.4315/0362-028x-72.9.1987.

189. Tamanai-Shacoori, Z.; Smida, I.; Bousarghin, L.; Loreal, O.; Meuric, V.; Fong, S.B.; Bonnaure-Mallet, M.; Jolivet-Gougeon, A. Roseburia Spp.: A Marker of Health? *Future Microbiol* **2017**, *12*, 157–170, doi:10.2217/fmb-2016-0130.

190. Venegas, D.P.; de La Fuente, M.K.; Landskron, G.; González, M.J.; Quera, R.; Dijkstra, G.; Short Chain Fatty Acids (SCFAs)Mediated Gut Epithelial and Immune Regulation and Its Relevance for Inflammatory Bowel Diseases. *Front Immunol* **2019**, *10*, 277, doi:10.3389/fimmu.2019.00277.

191. Fernández, J.; Redondo-Blanco, S.; Gutiérrez-del-Río, I.; Miguélez, E.M.; Villar, C.J.; Lombó, F. Colon Microbiota Fermentation of Dietary Prebiotics towards Short-Chain Fatty Acids and Their Roles as Anti-Inflammatory and Antitumour Agents: A Review. *J Funct Foods* **2016**, *25*, 511–522, doi:10.1016/j.jff.2016.06.032.

192. David, L.A.; Maurice, C.F.; Carmody, R.N.; Gootenberg, D.B.; Button, J.E.; Wolfe, B.E.; et al. Diet Rapidly and Reproducibly Alters the Human Gut Microbiome. *Nature* **2014**, *505*, 559–563, doi:10.1038/nature12820.

193. Tomova, A.; Bukovsky, I.; Rembert, E.; Yonas, W.; Alwarith, J.; Barnard, N.D.; et al. The Effects of Vegetarian and Vegan Diets on Gut Microbiota. *Front Nutr* **2019**, *6*, 47, doi: 10.3389/fnut.2019.00047.

194. Dahl, W.J.; Rivero Mendoza, D.; Lambert, J.M. Diet, Nutrients and the Microbiome. *Prog Mol Biol Transl Sci* **2020**, *171*, 237–263, doi:10.1016/bs.pmbts.2020.04.006.

195. Oosting, E.; Keyzer, J.; Wolthers, B.G. Correlation between Urinary Levels of Histamine Metabolites in 24-Hour Urine and Morning Urine Samples of Man: Influence of Histamine-Rich Food. *Agents Actions* **1989**, *27*, 205–207, doi:10.1007/BF02222240.

196. Saito, K.; Horie, M.; Nakazawab, H. Determination of Urinary Excretion of Histamine and 1-Methylhistamine by Liquid Chromatography. *J Chromatogr B Biomed Appl* **1994**, *654*, 270–275, doi:10.1016/0378-4347(94)00056-5.

197. Oosting, E.; Keyzer, J.; Wolthers, B.G.; Scholtis, R.J.H. Age Dependent Normal Values of Histamine and Histamine Metabolites in Human Urine. *Agents Actions* **1988**, *23*, 307–310, doi:10.1007/BF02142572.

198. Butterfield, J.H.; Pongdee, T.; Ravi, A. Urinary Markers of Mast Cell Disease and Their Role in Diagnosis and Management; In *Mastocytosis*; Akin, C., Ed.; Springer International Publishing: Cham, 2020; pp. 55–67, doi:10.1007/978-3-030-27820-5.

199. Keyzer, J.J.; de Monchy, J.G.R.; van Doormaal, J.J.; van Voorst Vader, P.C. Improved Diagnosis of Mastocytosis by Measurement of Urinary Hist Amine Metabolites. *N Engl J Med* **1983**, *309*, 1603–1605, doi:10.1056/NEJM198312293092603.

200. Lamale, L.M.; Lutgendorf, S.K.; Zimmerman, M.B.; Kreder, K.J. Interleukin-6, Histamine, and Methylhistamine as Diagnostic Markers for Interstitial Cystitis. *Urology* **2006**, *68*, 702–706, doi:10.1016/j.urology.2006.04.033.

201. Van Toorenenbergen, A.W.; Oranje, A.P. Comparison of Serum Tryptase and Urine N-Methylhistamine in Patients with Suspected Mastocytosis. *Clin Chim Acta* **2005**, *359*, 72–77, doi:10.1016/j.cccn.2005.03.041.

202. He, G.H.; Cai, W.K.; Zhang, J. B; Ma, C.Y.; Yan, F.; Lu, J.; et al. Associations of Polymorphisms in HRH2, HRH3, DAO, and HNMT Genes with Risk of Chronic Heart Failure. *Biomed Res Int* **2016**, *2016*, 1208476, doi:10.1155/2016/1208476.

203. Granerus, G. Effects of Oral Histamine, Histidine and Diet on Urinary Excretion of Histamine, Methylhistamine and 1-Methyl-4-Imidazoleacetic Acid in Man. *Scand J Clin Lab Invest* **1968**, *22*, 49–58, doi: 10.1080/00365516809168040.

204. Keyzer, J.; Breukelman, H.; Wolthers, B.; van den Heuvel, M.; Kromme, N.; Berg, W. Urinary Excretion of Histamine and Some of Its Metabolites in Man: Influence of the Diet. *Agents Actions* **1984**, *15*, 189–194, doi:10.1007/BF01972348.

205. Evans E; Nicholls J A Method for the Gas Chromatographic Determination of Urinary 1,4-Methyl-Imidazoleacetic. *J Chromatogr* **1973**, *82*, 394–397, doi: 10.1007/BF01716947.

206. Mitchell R.G; Code, C.F. Effect of Diet on Urinary Excretion of Histamine. *J Appl Physiol* **1954**, *6*, 387–392, doi:10.1152/jappl.1954.6.7.387.

7. ANNEXES

7. ANNEXES

7.1. Contribucions científiques derivades de la tesi doctoral

7.1.1. Publicacions

Publicació 1

Oriol Comas-Basté, Sònia Sánchez-Pérez, M. Teresa Veciana-Nogués, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou. Concept, aetiology and current diagnostic and treatment approaches of histamine intolerance: A review. Vide Leaf, 2021, Prime Achieves in Nutrition, Editor: Eloy A Zepeda-Carrillo.

Publicació 2

Sònia Sánchez-Pérez, Oriol Comas-Basté, Judit Rabell-González, M. Teresa Veciana-Nogués, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou. Biogenic amines in plant-origin foods: Are they frequently underestimated in low-histamine diets? Foods, 2018, 7, 205.

Publicació 3

Sònia Sánchez-Pérez, Oriol Comas-Basté, M. Teresa Veciana-Nogués, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou. Low-histamine diets: Is the exclusion of foods justified by their histamine content? Nutrients, 2021, 13, 1395.

Publicació 4

Sònia Sánchez-Pérez, Oriol Comas-Basté, Judit Costa-Catala, Irache Iduriaga-Platero, M. Teresa Veciana-Nogués, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou. The rate of histamine degradation by diamine oxidase is compromised by other biogenic amines. Frontiers in Nutrition, 2022, 9, 897028.

Publicació 5

Sònia Sánchez-Pérez, Oriol Comas-Basté, Adriana Duelo, M. Teresa Veciana-Nogués, Mercedes Berlanga, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou. Intestinal dysbiosis in patients with histamine intolerance. Nutrients, 2022, 14, 1774.

Publicació 6

Sònia Sánchez-Pérez, Oriol Comas-Basté, Adriana Duelo, M. Teresa Veciana-Nogués, Mercedes Berlanga, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou. The effect of dietary treatment on the intestinal microbiota of women with histamine intolerance. Acceptada en la revista Frontiers in Nutrition.

Publicació 7

Sònia Sánchez-Pérez, Ricard Celorio-Sardà, Oriol Comas-Basté, M. Teresa Veciana-Nogués, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou. 1-methylhistamine as urinary biomarker of histamine intolerance. Frontiers in Nutrition, 2022, 9, 973682.

7.1.2. Comunicacions orals

Comunicació oral 1 | Premi a la millor comunicació oral

Sònia Sánchez-Pérez, Oriol Comas-Basté, Judit Costa-Catala, M. Teresa Veciana-Nogués, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou. Dietary diamines significantly reduce the degradation rate of histamine by DAO enzyme. VI Workshop de l'Institut de Recerca en Nutrició i Seguretat Alimentària (INSA-UB), 2022, Barcelona.

Comunicació oral 2

Sònia Sánchez-Pérez, Oriol Comas-Basté, Judit Rabell-González, M. Teresa Veciana-Nogués, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou. Incertidumbres de la intoxicación histamínica. 14ª Reunión anual de la Sociedad Española de Seguridad y Calidad Alimentaria (SESAL), 2018, Salamanca.

Comunicació oral 3

Sònia Sánchez-Pérez, Oriol Comas-Basté, Adriana Duelo, Judit Costa-Catala, M. Teresa Veciana-Nogués, Mercedes Berlanga, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou. Is there a different intestinal microbial pattern for histamine intolerants? IX Reunión de Jóvenes Investigadores de la Sociedad Española de Nutrición (SEÑ), 2022, Cartagena.

Comunicació oral 4

Sònia Sánchez-Pérez, Ricard Celorio-Sardà, Oriol Comas-Basté, Salvador Hernández-Macias, Judit Costa-Catala, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou. Alteración en la diversidad de la microbiota intestinal en individuos intolerantes a la histamina. 16ª Reunión anual de la Sociedad Española de Seguridad y Calidad Alimentaria (SESAL), 2020, virtual, Barcelona.

7.1.3. Comunicacions escrites

Comunicació escrita 1

Sònia Sánchez-Pérez, Oriol Comas-Basté, Nelly C. Muñoz-Esparza, M. Teresa Veciana-Nogués, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou. Aminas biógenas en alimentos de origen vegetal: ¿Estan frecuentemente subestimadas en las dietas bajas en histamina?. 15ª Reunión Anual de la Sociedad Española de Seguridad Alimentaria (SESAL), 2019, Alicante.

Comunicació escrita 2

Sònia Sánchez-Pérez, Oriol Comas-Basté, Salvador Hernández-Macias, Nelly C. Muñoz-Esparza, M. Teresa Veciana-Nogués, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou. Histamine-free diets: What about legumes?. XII Conferència Internacional de Dieta Mediterrània, 2018, Barcelona.

Comunicació escrita 3

Oriol Comas-Basté, Sònia Sánchez-Pérez, Nelly C. Muñoz-Esparza, Salvador Hernández-Macias, M. Teresa Veciana-Nogués, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou. Dietary management of histamine intolerance: Establishing the biogenic amine profile of foods excluded in low-histamine diets. Conferencia de la Federación Iberoamericana de Nutrición (FINUT), 2020, Ciudad de México.

Comunicació escrita 4

Sònia Sánchez-Pérez, Oriol Comas-Basté, Salvador Hernández-Macias, M. Luz Latorre-Moratalla, M. Teresa Veciana-Nogués i M. Carmen Vidal-Carou. Estimación de la ingesta de histamina para validar la exclusión de alimentos en el tratamiento dietético de la intolerància a la histamina. 13ª Reunión Anual de la Sociedad Española de Seguridad Alimentaria (SESAL), 2017, Girona.

Comunicació escrita 5

Sònia Sánchez-Pérez, Giulietta Solari, Max Carreras-Leal de Ibarra, Judit Costa-Catala, Oriol Comas-Basté, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou. Dietes bajas en histamina: el porqué de la exclusión de algunos alimentos sin histamina. XIX Congreso de la Sociedad Latinoamericana de Nutrición (SLAN), 2021, virtual, Paraguai.

Comunicació escrita 6

Sònia Sánchez-Pérez, Oriol Comas-Basté, Adriana Duelo, Judit Costa-Catala, M. Teresa Veciana-Nogués, Mercedes Berlanga, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou. Intestinal dysbiosis in patients with histamine intolerance. XIII Conferència Internacional de Dieta Mediterrània, 2022, Barcelona.

7.2. Altres contribucions científiques

Les següents comunicacions escrites, encara que no es troben directament vinculades amb els objectius d'aquesta tesi doctoral, s'han produït durant el període de realització de la tesi fruit de la participació de la doctoranda en altres línies d'investigació del grup de recerca.

7.2.1. Publicacions

Publicació 8

Oriol Comas-Basté, Sònia Sánchez-Pérez, M. Teresa Veciana-Nogués, M. Luz Latorre-Moratalla, M. Carmen Vidal-Carou (2020). Histamine intolerance: The current state of the art. Biomolecules, 10, 1181.

Publicació 9

Oriol Comas-Basté, M. Luz Latorre-Moratalla, Sònia Sánchez-Pérez, M. Teresa Veciana-Nogués, M. Carmen Vidal-Carou (2019). In vitro determination of diamine oxidase activity in food matrices by an enzymatic assay coupled to UHPLC-FL. Analytical and Bioanalytical Chemistry, 411, 7595-7602.

Publicació 10

Oriol Comas-Basté, M. Luz Latorre-Moratalla, Sònia Sánchez-Pérez, M. Teresa Veciana-Nogués, M. Carmen Vidal-Carou (2019). Histamine and other biogenic amines in foods. From scrombroid poisoning to histamine intolerance. Biogenic Amines. Editor: Charalampos Proestos. IntechOpen.

7.2.2. Comunicacions orals

Comunicació oral 5 | Premi a la millor comunicació oral

Oriol Comas-Basté, Judit Rabell-González, Sònia Sánchez-Pérez, M. Luz Latorre-Moratalla, M. Teresa Veciana-Nogués, M. Carmen Vidal-Carou. Nuevas fuentes de

enzima DAO: del riñón de cerdo a los brotes de legumbre. II Congreso de Jóvenes Investigadores en Ciencias Agroalimentarias, 2019, Almeria.

Comunicació oral 6 | Premi a la millor comunicació oral

Nelly C. Muñoz-Esparza, Oriol Comas-Basté, Sònia Sánchez-Pérez, Salvador Hernández-Macías, M. Luz Latorre-Moratalla, M. Teresa Veciana-Nogués, M. Carmen Vidal-Carou. Polyamines in breast milk and infant formulas. V Workshop de l'Institut de Recerca en Nutrició i Seguretat Alimentària de la Universitat de Barcelona (INSA-UB), 2019, Santa Coloma de Gramenet, España.

Comunicació oral 7

Salvador Hernández-Macias, Sergio González-Pastor, Oriol Comas-Basté, Sònia Sánchez-Pérez, Nelly C. Muñoz-Esparza, M. Teresa Veciana-Nogués, M. Luz Latorre-Moratalla, M. Carmen Vidal-Carou. Histaminase activity of Lactobacillus sakei and the promoting effect of cava lees on its growth. V Workshop de l'Institut de Recerca en Nutrició i Seguretat Alimentària de la Universitat de Barcelona (INSA-UB), 2019, Santa Coloma de Gramenet, España.

Comunicació oral 8

Sònia Sánchez-Pérez, Oriol Comas-Basté, Salvador Hernández-Macias, Nelly C. Muñoz-Esparza, M. Luz Latorre-Moratalla, M. Teresa Veciana-Nogués, M. Carmen Vidal-Carou. Should fresh meat and meat derivatives placed at diferent levels of the nutritional pyramid?. V Reunión de Jóvenes Investigadores de la Sociedad Española de Nutrición (SEÑ), 2018, Barcelona.

Comunicació oral 9 | Premi a la millor comunicació oral

Oriol Comas-Basté, Sònia Sánchez-Pérez, Roberto Garza-Guajardo, M. Luz Latorre-Moratalla, M. Teresa Veciana-Nogués, M. Carmen Vidal-Carou. What about legumes as a plant source of the DAO enzyme? XVII Congreso de la Sociedad Española de Nutrición (SEÑ) y X Jornada de l'Associació Catalana de Ciències de l'Alimentació (ACCA), 2018, Barcelona.

Comunicació oral 10

Oriol Comas-Basté, Sònia Sánchez-Pérez, M. Luz Latorre-Moratalla, M. Teresa Veciana-Nogués, M. Carmen Vidal-Carou. Noves eines en el diagnòstic i control de la intolerància a la histamina. XI Jornada de Recerca de la Facultat de Farmàcia i Ciències de l'Alimentació, 2018, Barcelona.

7.2.3. Comunicacions escrites

Comunicació escrita 7

Nelly C. Muñoz-Esparza, Oriol Comas-Basté, Sònia Sánchez-Pérez, Salvador Hernández-Macías, M. Teresa Veciana-Nogués, M. Luz Latorre-Moratalla, M. Carmen Vidal-Carou. ¿Es comparable el contenido y perfil de poliaminas en la leche materna y fórmulas infantiles? IV Congreso de la Federación Española de Sociedades de Nutrición, Alimentación y Dietética (FESNAD), 2020, Virtual, Zaragoza.

Comunicació escrita 8

Oriol Comas-Basté, M. Luz Latorre-Moratalla, Salvador Hernández-Macias, Sònia Sánchez-Pérez, M. Teresa Veciana-Nogués, M. Carmen Vidal-Carou. Determination of histamine-degrading activity in food matrices by an enzymatic assay coupled to UHPLC-FL. XV Reunión Anual de la Sociedad Española de Seguridad y Calidad Alimentarias (SESAL), 2019, Alicante.

Comunicació escrita 9

Oriol Comas-Basté, Sònia Sánchez-Pérez, Roberto Garza-Guajardo, M. Luz Latorre-Moratalla, M. Teresa Veciana-Nogués, M. Carmen Vidal-Carou. In vitro DAO activity of porcine kidney extracts and DAO supplements. XII Conferència Internacional de Dieta Mediterrànea, 2018, Barcelona.

Comunicació escrita 10

Oriol Comas-Basté, Judit Rabell-González, Federica Pederzani, Sònia Sánchez-Pérez, Nelly C. Muñoz-Esparza, M. Luz Latorre-Moratalla, M. Teresa Veciana-Nogués, M. Carmen Vidal-Carou. Histamine-degrading capacity of lyophilised legume sprouts. XVIII Reunión de la Sociedad Española de Nutrición (SEÑ), 2019, Soria.

Comunicació escrita 11

M. Luz Latorre-Moratalla, Silva M. Benine-Ramos, Sònia Sánchez-Pérez, Oriol Comas-Basté, M. Teresa Veciana-Nogués, M. Carmen Vidal-Carou. Aminas biógenas en sashimi de atún: la importancia de mantenir la cadena de frío. XV Reunión Anual de la Sociedad Española de Seguridad y Calidad Alimentarias (SESAL), 2019, Alicante.

Comunicació escrita 12

Salvador Hernández-Macias, Gemma Rius-Olivella, Oriol Comas-Basté, Sònia Sánchez-Pérez, Nelly C. Muñoz-Esparza, M. Teresa Veciana-Nogués, Sara Bover-Cid, M. Luz Latorre-Moratalla, M. Carmen Vidal-Carou. Estudio de la actividad histaminasa de *Lactobacillus sakei* y del efecto promotor de las lías del cava en su crecimiento. V Congreso Internacional de Calidad y Seguridad Alimentaria (ACOFESAL), 2019, Barcelona.

Comunicacio escrita 13

Sònia Sánchez-Pérez, Oriol Comas-Basté, Salvador Hernández-Macias, Nelly C. Muñoz-Esparza, M. Luz Latorre-Moratalla, M. Teresa Veciana-Nogués, M. Carmen Vidal-Carou. Is there correspondence between compositional vàlues of tables and those in labels of commercial meat products? XVII Congreso de la Scoiedad Española de Nutrición (SEÑ) y X Jornada de l'Associació Catalana de Ciències de l'Alimentació (ACCA), 2018, Barcelona.

Comunicacio escrita 14

Sònia Sánchez-Pérez, Oriol Comas-Basté, Judit Rabell-González, M. Teresa Veciana-Nogués, M. Luz Latorre-Moratalla i M. Carmen Vidal-Carou. Incertidumbres de la intoxicación histamínica. 14ª Reunión anual de la Sociedad Española de Seguridad y Calidad Alimentaria (SESAL), 2018, Salamanca.

Comunicacio escrita 15

Sònia Sánchez-Pérez, Oriol Comas-Basté, Salvador Hernández-Macias, Nelly C. Muñoz-Esparza, M. Luz Latorre-Moratalla, M. Teresa Veciana-Nogués, M. Carmen Vidal-Carou. Should fresh meat and meat derivatives placed at different levels of the nutritional pyramid? XVII Congreso de la Scoiedad Española de Nutrición (SEÑ) y X Jornada de l'Associació Catalana de Ciències de l'Alimentació (ACCA), 2018, Barcelona.

Comunicació escrita 16

Oriol Comas-Basté, Sònia Sánchez-Pérez, Roberto Garza-Guajardo, M. Luz Latorre-Moratalla, M. Teresa Veciana-Nogués, M. Carmen Vidal-Carou. What about legumes as a plant source of the DAO enzyme? XVII Congreso de la Sociedad Española de Nutrición (SEÑ) y X Jornada de l'Associació Catalana de Ciències de l'Alimentació (ACCA), 2018, Barcelona.

Comunicació escrita 17

Nelly C. Muñoz-Esparza, Oriol Comas-Basté, Sònia Sánchez-Pérez, Salvador Hernández-Macias, M. Luz Latorre-Moratalla, M. Teresa Veciana-Nogués, M. Carmen Vidal-Carou. Are poliamines underestimated antioxidants in food? IV Workshop de l'Institut de recerca en Nutrició i Seguretat Alimentària (INSA-UB), 2018, Santa Coloma de Gramenet.

Comunicació escrita 18

Salvador Hernández-Macias, M. Luz Latorre-Moratalla, Oriol Comas-Basté, Sònia Sánchez-Pérez, Montserrat Riu-Aumatell, M. Teresa Veciana-Nogués, Elvira López-Tamames, M. Carmen Vidal-Carou. Cava lees as promoters of the growth of fermentative and probiòtic bacteria. XVII Congreso de la Sociedad Española de Nutrición (SEÑ) y X Jornada de l'Associació Catalana de Ciències de l'Alimentació (ACCA), 2018, Barcelona.

Comunicació escrita 19 | Premi a la millor infografia

Sònia Sánchez-Pérez, Oriol Comas-Basté i Roberto Garza-Guajardo. Noves aplicacions dels llegums: una possible font vegetal de l'enzim DAO. l'Institut de Recerca en Nutrició i Seguretat Alimentària (INSA) i Federación Española de Sociedades de Nutrición, Alimentación y Dietética (FESNAD), Barcelona, 2018.

Comunicació escrita 20

Oriol Comas-Basté, Sònia Sánchez-Pérez, Salvador Hernández-Macias, M. Luz Latorre-Moratalla, M. Teresa Veciana-Nogués, Montserrat Rivero-Urgell, M. Carmen Vidal-Carou. Estimation of the current polyamine intake in the Spanish population. IUNS 21st International Congress of Nutrition, 2017, Buenos Aires (Argentina).

Comunicació escrita 21

Salvador Hernández-Macias, Oriol Comas-Basté, Sònia Sánchez-Pérez, M. Luz Latorre-Moratalla, M. Teresa Veciana-Nogués, M. Carmen Vidal-Carou. ¿Pueden las lías del cava ser promotoras del crecimiento de microorganismos fermentadores y probióticos? XIII Reunión Anual de la Sociedad Española de Seguridad Alimentaria (SESAL), 2017, Girona.

7.3. Evidències de les comunicacions orals i escrites derivades de la tesi doctoral

VI Workshop Anual INSA·UB

Revalorització i reaprofitament alimentari: la ciència darrere d'una alimentació i gastronomia sostenibles

Sònia Sánchez Pérez

ha rebut el premi

Millor Comunicació Oral

al VI Workshop de l'Institut de Recerca en Nutrició i Seguretat Alimentària de la Universitat de Barcelona (INSA·UB)

que ha tingut lloc el 9 de febrer de 2022
al Campus de l'Alimentació de Torribera

Rosa Mª

Dra. Rosa Lamuela Raventós
Directora de l'INSA·UB

INSA Institut de Recerca en Nutrició i Seguretat Alimentària UNIVERSITAT DE BARCELONA

UNIVERSITAT DE BARCELONA

Campus de l'Alimentació Universitat de Barcelona

sesal

- SOCIEDAD ESPAÑOLA DE SEGURIDAD Y CALIDAD ALIMENTARIAS -

La Sociedad Española de Seguridad y Calidad Alimentarias
certifica que

Sònia Sánchez Pérez

ha presentado una comunicación científica en la
16ª REUNIÓN ANUAL DE LA SESAL. Más allá de la crisis de la COVID-19,
celebrada de forma online los días 28 y 29 de octubre de 2020, titulada:

¿Existe una alteración de la microbiota intestinal en individuos intolerantes a la histamina?

Sánchez-Pérez S., Celorio-Cerdà R., Comas-Basté O., Hernández-Macias S., Latorre-Moratalla M.L., Vidal-Carou M.C.

Barcelona, 14 de enero de 2021

Sra. Cristina Díez
Presidenta de la SESAL

sesal
- SOCIEDAD ESPAÑOLA DE SEGURIDAD Y CALIDAD ALIMENTARIAS -

La Sociedad Española de Seguridad y Calidad Alimentarias
certifica que

Sònia Sánchez

ha presentado una comunicación oral en la **14ª REUNIÓN ANUAL DE LA SESAL**,
celebrada en Salamanca los días 4 y 5 de octubre de 2018, titulada:

Incertidumbres sobre la intoxicación histamínica

Sánchez-Pérez, S.; Comas-Basté, O.; Rabell-González, J.; Latorre-Moratalla, M.L.; Veciana-Nogués, M.T.; Vidal-Carou, M.C.

Salamanca, 5 de octubre de 2018

Sra. Cristina Díez
Presidenta de la SESAL

Aminas biógenas en alimentos de origen vegetal:
¿Estan frecuentemente subestimadas en las dietas bajas en histamina?

S. Sánchez-Pérez; O. Comas-Basté; N.C. Muñoz-Esparza; M.T. Veciana-Nogués; M.L. Latorre-Moratalla; M.C. Vidal-Carou.

Departament de Nutrició, Ciències de l'Alimentació i Gastronomia, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona (UB), Av. Prat de la Riba 171, Santa Coloma de Gramenet (Spain); Institut de Recerca en Nutrició i Seguretat Alimentària (INSA-UB), Xarxa de Referència en Tecnologia dels Aliments de la Generalitat de Catalunya (XaRTA). mcvidal@ub.edu

Introducción - Objetivo

Las dietas bajas en histamina se usan frecuentemente para reducir la sintomatología de la intolerancia a la histamina, un desorden en la homeostasis de la histamina que incrementa los niveles plasmáticos de esta amina y principalmente producido por un déficit en la actividad de la enzima Diamino-Oxidasa (DAO)[1]. Este tipo de dietas excluyen una amplia variedad de alimentos, muchos de ellos de origen vegetal, debido a que los pacientes los relacionan con la aparición de la sintomatología (Tabla 1).

El **objetivo** de este trabajo fue estudiar si la presencia de histamina y otras aminas biógenas en alimentos de origen vegetal podría explicar su adecuación o no a las dietas bajas en histamina.

Alimentos eliminados de las dietas bajas en histamina
Lácteos: Quesos (a excepción del fresco)
Verduras: Espinacas, tomate, berenjena, judías, lentejas, guisantes, pimiento, lechuga y champiñón.
Frutas: Cítrico, kiwi, fresas, piña, papaya, plátano, frambuesas, aguacate, ciruela, uva y frutos rojos.
Cereales y derivados
Carnes y derivados: Derivados curados y fermentados curados
Pescados y derivados: Pescado azul, marisco, conservas y semiconservas (atún, anchoas, etc.)
Huevos y derivados
Frutos secos: nueces, almendras, cacahuetes, avellanas
Vegetales fermentados: Chucrut, derivados de la soja
Chocolate y vainilla
Bebidas alcohólicas: vino, cerveza y destilados

Tabla 1. Alimentos que deben evitarse de acuerdo con las propuestas dietéticas encontradas en la literatura[1-8]

Metodología

Se determinaron diferentes aminas biógenas (histamina, putrescina, tiramina, cadaverina, espermidina y espermina) en un total de 450 alimentos de origen vegetal (no fermentados) pertenecientes a las categorías de vegetales (20), frutas (19), cereales (8) y legumbres (5). Todas las muestras fueron analizadas por duplicado mediante cromatografía líquida de ultra alta eficacia (UHPLC) acoplada a detección fluorimétrica[9].

Resultados

Los únicos alimentos de origen vegetal (no fermentados) con niveles significativos de histamina fueron berenjena (39,4±30,7 mg/kg), espinacas (31,8±17,0 mg/kg) y tomate (2,5±4,1 mg/kg), mostrando una gran variabilidad en sus contenidos (Figura 1).

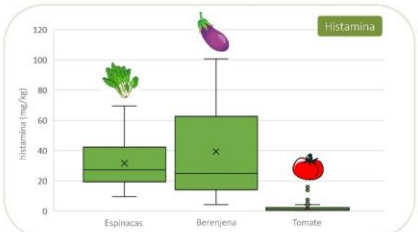


Figura 1. Distribución de los contenidos de histamina (mg/kg) en alimentos de origen vegetal no fermentados

Por el contrario, las aminas putrescina, espermidina y espermina se encontraron en prácticamente todos los productos de origen vegetal. Entre estos, los alimentos con mayores contenidos de putrescina fueron los cítricos (91,2±41,7 mg/kg), el pimiento verde (90,0±41,7 mg/kg) y el pomelo (55,6±12,8 mg/kg) (Figura 2). En cuanto a la espermidina, destacaron el germen de trigo (325,5±3,0 mg/kg), las pipas (131,2± 13,3 mg/kg) y el champiñón (128,5±14,6 mg/kg) (Figura 3).

Destacar que las espinacas y la berenjena, además de ser alimentos ricos en histamina, también contenían niveles importantes de putrescina y espermidina (Figuras 1-3). Por lo que respecta a los niveles de cadaverina y tiramina, éstos no se encontraron en cantidades significativas en ninguno de los alimentos analizados.

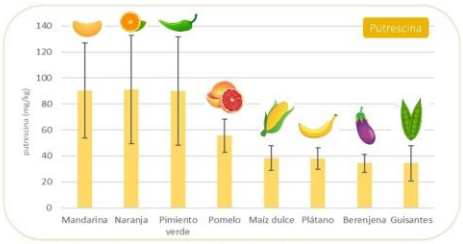


Figura 2. Alimentos de origen vegetal (no fermentados) que destacan por sus contenidos en putrescina

Figura 3. Alimentos de origen vegetal (no fermentados) que destacan por sus contenidos en espermidina

Conclusiones

La presencia de histamina explicaría la exclusión de tan sólo tres alimentos de origen vegetal (berenjena, espinacas y tomate) de las dietas bajas en histamina. Por lo tanto, la eliminación de otros alimentos (sin histamina o con contenidos muy bajos) de estas dietas podría justificarse, en parte, por la presencia de altos contenidos de otras aminas, como putrescina y espermidina. Estas podrían ser corresponsables de la aparición de los efectos adversos de la intolerancia a la histamina ya que al ser también sustratos de la DAO, competirían con la histamina por esta enzima. No obstante, son necesarios más estudios para conocer hasta que punto la putrescina y la espermidina interfieren en el metabolismo de la histamina.

References: [1]Maintz, L., y col. 2007. Am. J. Clin. Nutr.85:1185.; [2]Böhn, L., y col. 2013. Am. J. Gastroenterol. 108:634; [3]Rosell-Camps, A., y col. 2013. Rev. Esp. Enferm. Dig.105: 201; [4]Lefèvre, S., y col. 2016. Rev.Fr.Allergol. 57:24; [5]Swiss Interest Group Histamine Intolerance (SIGHI). 2016. Disponible online: www.histaminintoleranz.ch; [6]Wagner, N., y col. 2017. J. Eur. Acad. Dermatol. Venereol. 31:650; [7]Ede, G. 2017. J. Evol. Health. 2:11; [8]Son y col. 2018, Ann dermatol. 30:164.[9]Latorre-Moratalla, y col. 2019. J. Cromath A., 45 :7715-20.

Acknowledgements: This work was supported by Direcció General de Recerca of the Generalitat de Catalunya (2017 SGR-1376). Sònia Sánchez Pérez is a recipient of a doctoral fellowship from the University of Barcelona (APIF 2018).

HISTAMINE FREE DIETS: WHAT ABOUT LEGUMES?

Sánchez-Pérez, S., Comas-Basté, O., Hernández-Macias, S., Muñoz-Esparza, N.C., Latorre-Moratalla, M.L., Veciana-Nogués, M.T. & Vidal-Carou, M.C.

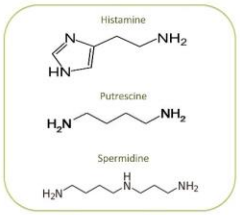
Departament de Nutrició, Ciències de l'Alimentació i Gastronomia, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona (UB). Av. Prat de la Riba 171, 08921 Santa Coloma de Gramenet (Spain); Institut de Recerca en Nutrició i Seguretat Alimentària (INSA-UB), Universitat de Barcelona (UB); Xarxa de Referència en Tecnologia dels Aliments de la Generalitat de Catalunya (XaRTA). mcvidal@ub.edu

INTRODUCTION

Histamine intolerance is a disorder in the homeostasis of histamine due to a reduced intestinal degradation of this amine by diamine-oxidase (DAO) enzyme. This fact entails an increased histamine absorption, its accumulation in plasma and the appearance of multi-faced clinical symptoms[1]. The dietary management of histamine intolerance is mainly based on the exclusion of histamine-containing foods. Histamine is widely distributed in foods and with variable contents, even within the same type of product. Histamine and other bioactive amines can be found in foods with presence of aminoacid-descarboxilase activity of microbial origin. This could be in both, fermented products (cheese, dry-fermented sausage...) and fresh foods (meat, fish...) under poor hygienic conditions. On the other hand, some products without or with low levels of histamine are also related with the onset of symptoms and consequently excluded from this type of diet[1-6] .

This fact could be explained by:

a) The presence of other bioactive amines, such as putrescine and spermidine, that could be co-responsible of the triggering of adverse effects by competing with histamine for DAO enzyme.
b) Some foods could release endogenous histamine.



LEGUMES, a key component of the Mediterranean Diet, are one of the food categories without general consensus about its exclusion in histamine-free diets. Some authors allowed legumes and their derivates but others avoided them[1,3,4,6]. Likewise, other authors only avoided the fermented soy-products[2,6].

OBJECTIVE

The **aim** of this work was to study if the exclusion of legumes in a histamine-free diet could be justified by histamine, putrescine or spermidine contents.

MATERIAL AND METHODS

Samples: The foods considered were lentils (n=7), white beans (n=6), chickpeas (n=4), peas (n=9), peanuts (n=7), soy sprouts (n=3), soy beverage (n=3), tofu(n=8) and fermented legumes (tamari, miso, tempeh, soy sauce, natto and soy paste; n=21).

Assay: Histamine, putrescine and spermidine contents were determined by duplicate through an ultra-high performance liquid chromatography (UHPLC) method with fluorimetric detection[7].

RESULTS AND DISCUSSION

Figure 1 shows histamine, putrescine and spermidine contents in fermented legumes mostly excluded in histamine-free diets. Although only some of the fermented legumes analysed (sufu, tamari and soy-paste) contain histamine, all legumes fermented products are susceptible to accumulate this amine due to their production process. Then, their exclusion from this type of diets could be justified by their potential histamine content.

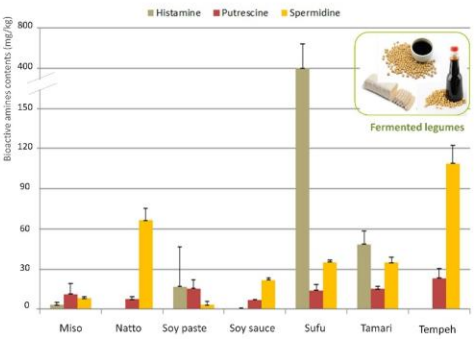


Figure 1. Histamine, putrescine and spermidine contents in fermented legumes.

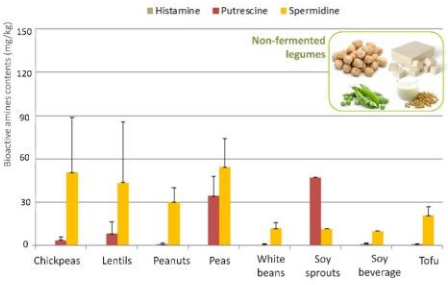


Figure 2. Putrescine and spermidine contents in non-fermented legumes.

Figure 2 shows that histamine was not found in non-fermented legumes. Both, fermented and non fermented legumes contain putrescine, being peas and soy sprouts the products with highest contents. According to putrescine data, only peas and soy sprouts could be excluded from histamine-free diets. The spermidine levels determined in chickpeas, lentils and peanuts may be considered high in comparison with the contents of this polyamine generally found in foods. This could potentially explain their exclusion from some histamine-free diets.

CONCLUSIONS

In light of these results, the onset of symptoms associated to histamine intolerance that some patients link to legumes intake could eventually be related with their histamine, putrescine and spermidine contents. However, more studies are needed to confirm these previous data and also to know until what extend putrescine and spermidine interfere in histamine metabolism by DAO enzyme.

[1]Maintz et al., (2007) Am J Clin Nutr. 85:1185-96; [2]Rosell-Camps et al., (2013) Rev. Esp. Enf. Digest. 105: 261-7; [3]Wagner et al., (2016) Eur. Acad. Dermatol. Venereol. 31: 650-656; [4]Novakova-Hanuskova et al., (2015) Allergol Immunopathol. 43: 498-506. [5]Vleg-Boerstra et al., (2005) Neth. J. Med. 63: 244-9. [6]Swiss Interest Group Histamine Intolerance (SIGHI) (2015) Schweizer Interessengemeinschaft; [7]Latorre-Moratalla, et al., (2009) J. Chromatogr. A 1216: 7715-7720.

Acknowledgements: This work was supported by Direcció General de Recerca of the Generalitat de Catalunya (2017 SGR-1376). Sònia Sánchez Pérez is a recipient of a doctoral fellowship from the University of Barcelona (APIF 2018).

CONFERENCIA FINUT 2020
11-13 OCTUBRE VIRTUAL
Trabajando todos unidos por una nutrición mejor

FINUT
Fundación Iberoamericana de Nutrición

Ref: P006

DIETARY MANAGEMENT OF HISTAMINE INTOLERANCE:
Establishing the biogenic amine profile of foods excluded in low-histamine diets

O. Comas-Basté, S. Sánchez-Pérez, N.C. Muñoz-Esparza, S. Hernández-Macias, M.T. Veciana-Nogués, M.L. Latorre-Moratalla, M.C. Vidal-Carou.

Departament de Nutrició, Ciències de l'Alimentació i Gastronomia, Campus de l'Alimentació de Torribera, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona – INSA·UB – XaRTA. Av. Prat de la Riba 171, Santa Coloma de Gramenet (Spain). mcvidal@ub.edu

BRACKGROUND & AIM

Low-histamine diets are currently advised to reduce symptoms of histamine intolerance, a disorder in histamine homeostasis that increases plasmatic levels histamine, mainly due to a reduced diamine oxidase (DAO) activity[1]. These diets should exclude foods susceptible to content high amounts of histamine, but current recommendations also advise the exclusion of other foods that patients have related to the onset of the symptomatology and, a priori, do not contain histamine.

Therefore, in order to evaluate the adequacy of dietary recommendations of low-histamine diets reported in the literature, the aim of this work was to study the qualitative and quantitative biogenic amine profile of frequently excluded foods.

METHODOLOGY

A selective search of scientific literature dealing with low-histamine diets was performed in PubMed database.

Biogenic amine content (histamine, tyramine, putrescine, cadaverine, spermidine and spermine) was determined in food samples through UHPLC coupled with fluorometric detection[2].

RESULTS

Foods excluded in low-histamine diets

Figure 1 summarises the list of foods that should be avoided on a low-histamine-diet according to ten different studies available in the literature[3-12]. While the majority of reviewed works coincide in the exclusion of fermented foods and beverages, oily fish and its derivatives and few specific vegetables; no general consensus was found regarding the avoidance of milk, eggs and a range of fruits and vegetables.

Figure 1. Summary of foods excluded in different low-histamine diets.

Dairy: cured and semi-cured cheese, grated cheese and milk
Fish: oily fish, its canned and semi-preserved derivatives and shellfish
Meat: dry-fermented meat products
Eggs
Legumes: lentils, chickpeas, soybeans and fermented soy derivatives
Vegetables: spinach, tomatoes, fermented cabbage, eggplant, avocado and mushrooms
Fruits: citrus, strawberries, banana, kiwi, pineapple, plum and nuts
Chocolate
Alcoholic beverages: wine and beer

>60% 20-60% <20% Percentage of low-histamine diets that report the exclusion

Biogenic amine profile

Foods with histamine occurrence are listed in table 1.A. Overall, mean histamine content was usually low, but some samples achieved extremely high levels above 400 mg/kg in fermented products and up to 650 mg/kg for seafoods. A significant occurrence of putrescine and cadaverine was also found in practically all analysed foods, although with high variability. Tyramine was also specifically found in fermented products, even in many cases with higher levels than histamine (data not shown).

On the other hand, histamine was not detected in certain plant-origin foods excluded from low-histamine diets, in which high levels of putrescine were found (table 1.B). Finally, in few food products excluded by low-histamine diets, such as milk, avocado and plums, any biogenic amine was detected.

Table 1. Histamine, putrescine and cadaverine content (min – max) in different foodstuffs (mg/Kg or mg/L). A: left; B: right.

FOOD	HISTAMINE	PUTRESCINE	CADAVERINE
Fruits, vegetables and legumes			
Spinach	9,5 - 69,7	ND - 9,2	ND - 0,1
Tomato	ND - 17,1	6,3 - 35,6	ND - 2,3
Eggplant	4,2 - 100,6	24,1 - 48,6	ND
Ferm. soy derivatives	ND - 730,1	2,7 - 31,1	ND - 36,9
Meat and meat products			
Fresh meat	ND - 77,1	ND - 258,6	ND - 376,5
Cured meat	ND - 150,0	ND - 17,4	ND - 305,0
Dry-ferm. sausages	ND - 474,8	ND - 537,1	ND - 658,1
Fish and seafood products			
Fresh oily fish	ND - 111,3	ND - 39,9	ND - 400,2
Semi-preserved fish	ND - 34,9	0,4 - 21,2	ND - 55,8
Canned fish	ND - 657,1	ND - 2,2	ND - 12,0
Dairy products			
Cheese	ND - 389,9	ND - 610,5	ND - 355,9
Alcoholic beverages			
Cerveza	ND - 21,6	ND - 14,5	ND - 31,4
Wine	0,1 - 55,0	ND	ND

FOOD	HISTAMINE	PUTRESCINE	CADAVERINE
Fruits, vegetables and legumes			
Citrus	ND	ND - 151,0	ND
Banana	ND	25,5 - 49,5	ND
Kiwi	ND	0,5 - 15,6	ND
Pineapple	ND	1,4 - 7,9	ND
Plum	nd	ND	ND
Strawberry	ND	2,0 - 6,4	ND
Avocado	ND	ND	ND
Mushrooms	ND	0,1 - 3,7	ND - 1,6
Lentils	ND	2,0 - 21,8	ND
Chickpeas	ND	0,9 - 6,4	ND
Soybeans	ND	41,1 - 47,5	ND
Nuts	ND	ND - 39,5	ND
Chocolate	ND	ND	ND - 2,8
Fish and seafood products			
Shellfish	ND	1,4 - 9,8	ND
Dairy products			
Milk	ND	ND	ND
Egg and derivatives			
Fresh egg	ND	ND	ND

CONCLUSIONS

Apart from those foods avoided due to their histamine content, the exclusion of certain foods may be justified by the occurrence of other biogenic amines, mainly putrescine. The fact that other amines are also substrate of DAO enzyme could explain their co-responsibility in the triggering of histamine intolerance symptoms by competing with histamine for the same catabolic enzyme. Further studies are needed to assess until what extend the presence of other amines interferes in the intestinal degradation of histamine.

References

[1]Comas-Basté et al. [2020]; [2]Latorre-Moratalla et al. [2009]; [3]Maintz & Novak [2007]; [4]Wagner et al. [2017]; [5]Rosell-Camps et al. [2013]; [6]Son et al. [2018]; [7]Joneja & Carmona-Silva [2001]; [8]Pediatril et al. [2016]; [9]Böhn et al. [2013]; [10]Lefèvre et al. [2017]; [11]Ede [2017]; [12]Swiss Interest Group Histamine Intolerance (SIGHI).

ESTIMACIÓN DE LA INGESTA DE HISTAMINA PARA VALIDAR LA EXCLUSIÓN DE ALIMENTOS EN EL TRATAMIENTO DIETÉTICO DE LA INTOLERANCIA A LA HISTAMINA

Sánchez-Pérez, S., Comas-Basté, O., Hernández-Macias, S., Latorre-Moratalla, M.L., Veciana-Nogués, M.T. & Vidal-Carou, M.C

Departament de Nutrició, Ciències de l'Alimentació i Gastronomia, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona (UB). Av. Prat de la Riba 171, 08921 Santa Coloma de Gramenet (Spain); Institut de Recerca en Nutrició i Seguretat Alimentària (INSA), Universitat de Barcelona (UB). mcvidal@ub.edu

INTRODUCCIÓN

La intolerancia a la histamina (IH) es un trastorno en la homeostasis de la histamina debido a una degradación intestinal reducida, causada principalmente por la deficiencia del enzima diamino oxidasa (DAO)[1]. A diferencia de la intoxicación histamínica, los síntomas pueden aparecer tras el consumo de alimentos con cantidades bajas de histamina. La actual gestión dietética de esta intolerancia resulta compleja, ya que se excluye un elevado número de alimentos que se relacionan a nivel clínico con la aparición de síntomas. No existe consenso sobre qué alimentos deben excluirse e incluso habitualmente se eliminan alimentos sin histamina[2,3].

La **tabla 1** muestra un listado de los alimentos permitidos y eliminados en el marco de una dieta libre de histamina considerando la información disponible en la literatura[3,4].

OBJETIVO

El **objetivo** de este estudio fue estimar la ingesta de histamina en la población española. Esta información puede ser también útil para validar si se justifica la exclusión de determinados alimentos teniendo en cuenta no solo su contenido en histamina sino también su consumo.

Tabla 1. Alimentos permitidos y eliminados de las dietas para el tratamiento de la intolerancia a la histamina[2,3,4]

PERMITIDOS	ELIMINADOS
Cereales y derivados	Lácteos: Quesos (a excepción del fresco)
Lácteos: Yogurt y queso fresco	Verduras: Espinacas, tomate, berenjena
Verduras/tubérculos: patatas, lechuga, coliflor, brócoli, maíz dulce, pepino, zanahoria, ajo, calabaza, pimiento, champiñón, rábano, espárrago, calabacín, cebolla, olivas	Frutas: Cítrico, kiwi, fresas, piña, papaya, plátano, frambuesas y aguacate
Frutas: Manzana, melocotón, pera, ciruela, cerezas, melón, arándanos	Carnes y derivados: Derivados curados y fermentado curados
Carne y derivados: Carne fresca, aves de corral, derivados cárnicos cocidos	Pescados y derivados: Pescado azul, marisco, conservas y semiconservas (atún, anchoas, etc.)
Pescados y derivados: Pescado blanco	Huevos y derivados
Legumbres: Judía blanca, garbanzo, lentejas y soja	Frutos secos: nueces, almendras, cacahuetes, avellanas
Aceites vegetales y vinagre	Fermentados: Chucrut, derivados de la soja
Condimentos y especias	Chocolate y vainilla
	Bebidas alcohólicas: vino, cerveza y destilados

METODOLOGÍA

Para el cálculo de la exposición se ha considerado el contenido de histamina de un total de 1800 alimentos clasificados según el sistema FoodEx2 propuesto por EFSA[5]. Los datos de consumo de la población española proceden de la encuesta ENALIA-2[6]. La exposición (mg/día) se calculó mediante un método determinista, considerando el percentil-95 del contenido en histamina (mg/kg) y de consumo (g/día).

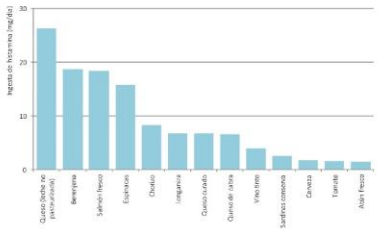
RESULTADOS

La **Fig. 1** muestra el aporte de histamina a la dieta por categorías de alimentos según la clasificación FoodEx2, observándose una amplia variabilidad dentro de cada grupo. Las categorías de alimentos que aportan un mayor nivel de histamina a la dieta fueron "leche y productos lácteos" y "carne y productos cárnicos". Los alimentos de las categorías "cereales y derivados", "huevos y derivados", "azúcares, dulces y confitería" y "aceites y grasas" no contribuyen al aporte de histamina.

Los alimentos responsables de la ingesta de histamina son queso (de leche no pasteurizada, curado y cabra), berenjena, salmón fresco, espinacas, embutidos crudos curados, vino tinto, sardinas en conserva, cerveza, tomate y atún fresco, aportando valores entre 2 y 26 mg/día (**Fig. 2**).

Figura 1. Aporte de histamina a la dieta por categorías de alimentos

Figura 2. Ingesta de histamina (percentil-95) en mg/día procedente del consumo de diferentes alimentos



Respecto a la validación de las dietas de exclusión para la IH se puede destacar:

A. Todos los alimentos que son fuente significativa de histamina (**Fig. 2**) son normalmente excluidos de estas dietas (**tabla 1**).

B. La exclusión de algunos de los alimentos como los cítricos y los plátanos se podría justificar por su aporte de putrescina. La putrescina, al ser también sustrato del enzima DAO, favorecería la absorción intestinal de histamina[7].

C. La exclusión de alimentos como el huevo, el aguacate, el chocolate y la leche no se puede explicar por su aporte de histamina ni putrescina. Aunque es un tema poco contrastado, una posible explicación sería que estos alimentos actuaran como liberadores de histamina endógena[8].

D. En la lista de alimentos permitidos se incluyen algunos como el pimiento, el maíz dulce, el calabacín, el pepino y los champiñones; que según nuestros datos aportan tanta o más putrescina que los alimentos considerados en el apartado B, por lo que aplicando el mismo criterio deberían ser excluidos.

CONCLUSIONES

- El uso de la clasificación FoodEx2 propuesta por EFSA no resulta adecuada para este tipo de peligro ya que dentro de una misma categoría se incluyen alimentos con concentraciones muy variables de histamina.
- Los alimentos que aportan más histamina a la dieta de los españoles son queso, berenjena, salmón fresco, espinacas, embutidos crudos-curados, vino tinto, sardinas en conserva, cerveza, tomate y atún fresco. Entre estos se incluyen alimentos que normalmente no se consideran fuente dietética de histamina como berenjena, espinacas y tomate.
- El aporte de histamina y putrescina solo valida la exclusión de algunos de los alimentos de la dieta para la IH, pero no todos.
- Son necesarios nuevos estudios para esclarecer el mecanismo por el que alimentos que no aportan histamina ni putrescina provocan la manifestación clínica de la IH.

Izquierdo-Casas et al., (2017). Low serum diamine oxidase (DAO) activity levels in patients with migraine. J. Physiol. Biochem. 1–7; Rosa-Campos et al., (2013). Histamine intolerance as a cause of chronic digestive complaints in pediatric patients. Rev. Esp. Enf. Digest. 105: 285–7; Maagaar et al., (2016). A peculiar myo-bloc histamine dec improves chronic spontaneous urticaria-fect or Factor?). Eur. Acad. Dermatol. Venereol. 31: 1654–60; Panel Interest Group Histamine Intolerance (SIGHI) (2015) Schweizer Interessengemeinschaft Hist. INSA (2015) The food classification and description system FoodEx2. Revision 2. EFSA Technical Report. TMCC05EN (2011) ENALIA 2 (Encuesta Nacional de Alimentación en Población Adulta, Infantil y Embarazadas). Reese et al., (2016) Control of biogenic amines in food existing and emerging approaches. J. Food Sci. 75: 139–153; Maga-Ramírez et al., (2005) Mastocytosis and adverse reactions to biogenic amines and histamine-releasing foods. What is the evidence? Nutr. J. Mast. 65: 744–9.

Acknowledgements: The authors would like to thank the Direcció General de Recerca of the Generalitat de Catalunya (2014 SGR 569) for their support and for a grant from the Universitat de Barcelona to the PhD student Oriol Comas Basté. This work was also supported by the Interministerial Commission for Science and Technology (CICYT) of the Ministerio de Educación, Cultura y Deporte through the Project AGL2012-39986.

INSA Institut de Recerca en Nutrició i Seguretat Alimentària

XaRTA Catalonian Reference Network on Food Technology

DIETAS BAJAS EN HISTAMINA: EL PORQUÉ DE LA EXCLUSIÓN DE ALGUNOS ALIMENTOS SIN HISTAMINA

S. Sánchez-Pérez, G. Solari, M. Carreras-Leal de Ibarra, J. Costa-Catala, O. Comas-Basté, M.L. Latorre-Moratalla, M.C. Vidal-Carou

Departament de Nutrició, Ciències de l'Alimentació i Gastronomia, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona (UB), Institut de Recerca en Nutrició i Seguretat Alimentària (INSA·UB), Av. Prat de la Riba 171, Santa Coloma de Gramenet, Spain.

Antecedentes y objetivo

La intolerancia a la histamina es un desorden de la homeostasis de la histamina debido a una deficiencia de la enzima Diamino-Oxidasa (DAO) a nivel intestinal[1]. Actualmente, su tratamiento se basa en el seguimiento de una dieta baja en histamina, que excluye alimentos que los pacientes relacionan con la aparición de la sintomatología[2,3]. En estas dietas, además de la exclusión de alimentos susceptibles de contener histamina, también se recomienda la eliminación de otros que, *a priori*, no contienen histamina, pero sí niveles significativos de otras aminas (Tabla 1). Se ha hipotizado que la presencia de otras aminas a nivel intestinal pueda retrasar la metabolización de la histamina por parte de la DAO, al competir todas ellas por esta enzima. En este caso, aumentaría la absorción intestinal de la histamina y, consecuentemente, la aparición de sintomatología[4].

Tabla 1. Alimentos excluidos de las dietas bajas en histamina[4].

Alimentos susceptibles de contener histamina y otras aminas biógenas (putrescina, cadaverina, tiramina, espermidina y/o espermina)	Embutidos crudos-curados, pescado y derivados, quesos curados y semicurados, derivados fermentados de la soja, bebidas fermentadas, chucrut, berenjena, espinacas y tomate.
Alimentos que no contienen histamina pero sí otras aminas biógenas (putrescina, cadaverina, tiramina, espermidina y/o espermina)	Calabaza, soja, albaricoque, aguacate, cereza, ciruela, cítricos, kiwi, papaya, piña, plátano, fresas, frutos rojos, uvas, olivas, frutos secos, chocolate y té.

El **objetivo** de este estudio fue evaluar *in vitro* si la presencia de otras aminas biógenas influye en la velocidad de degradación de la histamina por parte de la enzima DAO.

Material y métodos

Se determinó *in vitro* la degradación de la histamina en presencia de otras aminas (putrescina, cadaverina, tiramina, espermidina o espermina) a diferentes proporciones (Figura 1) mediante un ensayo enzimático acoplado a UHPLC-FL. La actividad DAO se expresó en mU (nmol de histamina degrada)[5].

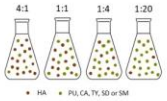


Figura 1. Esquema de las diferentes aminas (histamina, HA; putrescina, PU; cadaverina, CA; espermidina, SD; espermina, SM) y las diferentes proporciones estudiadas.

Resultados

La presencia de putrescina y cadaverina retrasó significativamente la velocidad de degradación de la histamina a todas las concentraciones ensayadas. El mayor efecto se observó cuando estas diaminas se encontraban en concentraciones superiores a las de la histamina ($p < 0,001$) (Fig. 2).

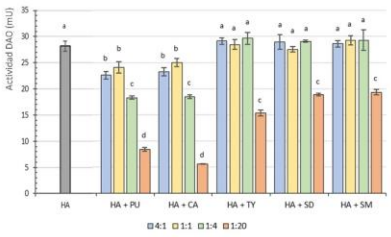
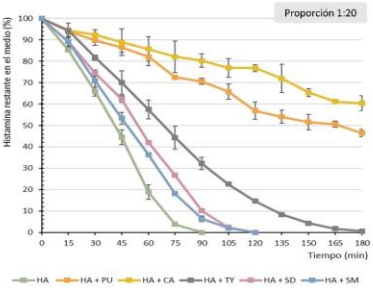


Figura 2. Actividad DAO (mU) para la histamina en presencia de las diferentes aminas consideradas (histamina, HA; putrescina, PU; cadaverina, CA; espermidina, SD; espermina, SM) y a las proporciones 4:1, 1:1, 1:4 i 1:20. Las diferentes letras denotan diferencias estadísticamente significativas ($p<0,05$).

La actividad de la enzima DAO cuando la histamina se encontraba sola en el medio fue de 28,16 ± 1 mU, mientras que, por ejemplo, la presencia de concentraciones 20 veces superiores de putrescina o cadaverina provocó una disminución de la actividad del 70 y el 80%, respectivamente (Figura 2).

En el caso de la tiramina, la espermidina y la espermina, únicamente concentraciones 20 veces superiores a las de histamina causaron un retraso significativo en la degradación de la histamina ($p < 0,001$), aunque más moderado que el observado por la putrescina y la cadaverina.

Figura 3. Cinética de degradación de la histamina en presencia de las diferentes aminas (histamina, HA; putrescina, PU; cadaverina, CA; espermidina, SD; espermina, SM) a proporción 1:20.



La Figura 3 muestra la cinética de degradación de la histamina en presencia de las diferentes aminas en una proporción 1:20. Cuando la histamina se encontraba sola en el medio, se degradó completamente a los 90 min de ensayo, mientras que a este mismo tiempo, la degradación de histamina fue aproximadamente del 90% en presencia de espermidina y espermina, del 70% en presencia de tiramina y, tan solo de entre un 20% y un 30% en presencia de putrescina o cadaverina.

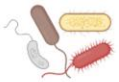
Conclusiones

Los resultados de este estudio explicarían que alimentos sin o con contenidos muy bajos de histamina, pero relevantes de putrescina o cadaverina, se relacionen con la aparición de síntomas en individuos con intolerancia a la histamina, y por lo tanto, se excluyan de las dietas bajas en histamina. En el caso de la presencia de tiramina, espermidina o espermina, serían necesarios niveles más elevados para retrasar la metabolización de la histamina.

Referencias: [1]Maintz, y col.; 2007. Am. J. Clin. Nutr. 85: 1185-96. [2]Rosell-Camps, y col.; 2013. Rev. Esp. Enferm. Dig., 105: 201-6. [3]Wagner, y col.; 2017. J. Eur. Acad. Dermatol. Venereol. 31: 650-5. [4]Sánchez-Pérez, y col.; 2021. Nutrients. 13: 1395 [5]Comas-Basté, y col.; 2019. Anal. Biol. Chem. 411: 7595-602.

INSA Institut de Recerca en Nutrició i Seguretat Alimentària
Campus de l'Alimentació
Universitat de Barcelona

Intestinal dysbiosis in patients with histamine intolerance



Sònia Sánchez-Pérez, Oriol Comas-Basté, Adriana Duelo, Judit Costa-Catala, M. Teresa Veciana-Nogués, Mercedes Berlanga, M. Luz Latorre-Moratalla and M. Carmen Vidal-Carou

Departament de Nutrició, Ciències de l'Alimentació i Gastronomia, Campus Alimentació, Universitat de Barcelona (UB). Av. Prat de la Riba 171, 08921 Santa Coloma de Gramenet (Spain); Institut de Recerca en Nutrició i Seguretat Alimentària (INSA-UB), Universitat de Barcelona (UB); Xarxa d'Innovació Alimentària (XIA). mcvidal@ub.edu

INTRODUCTION

Histamine intolerance is a disorder in histamine homeostasis, mainly provoked by a deficiency in the key enzyme responsible for histamine degradation at intestinal level, diamino oxidase (DAO), leading to a greater absorption of this amine, and the onset of the symptomatology[1,2]. This DAO deficiency could have a genetic, pharmacological or pathological origin[3]. Recently, it has been postulated that an alteration on the gut microbiota composition could also be a possible cause for DAO deficiency, though only a single study has challenged this hypothesis so far[4]. A major presence of histamine-secreting bacteria in the gut of histamine intolerance patients could also influence in the development of this intolerance[5].

The aim of this study was to characterize the composition of the intestinal microbiota of patients with histamine intolerance symptoms and to compare it with that of healthy individuals.

METHODOLOGY

A histamine intolerance group (HIT) was formed by 12 individuals from a centre specialized in histamine intolerance due to DAO deficiency and a control group with 14 healthy individuals. The analysis of the intestinal microbiota has been carried out from DNA isolated from stool samples (Qiagen) by sequencing of bacterial 16S rRNA genes (V3-V4 region) (MiSeq-Ilumina). The data analysis was performed by EzBioCloud Database.

RESULTS

As it can be seen in Figure 1, HIT group showed a significantly higher abundance of histamine-secreting bacteria in comparison with healthy group, including the genera *Staphylococcus, Proteus*, several unidentified genera belonging to the family *Enterobacteriaceae* and the species *Clostridium perfringens* and *Enterococcus faecalis* (p<0.05).

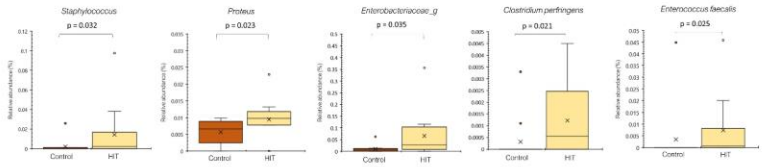


Figure 1. Relative abundance (%) of different histamine-secreting bacteria found in both study groups

A significantly low abundance of different bacteria associated with a healthy gut (*Ruminococcus*, *Faecalibacterium* and *Faecalibacterium prausnitzii*) was also found in HIT group (p<0.05) (Figure 2). *F. prausnitzii*, proposed as a marker of gut health is one of the most prevalent and abundant producers of butyrate in the human gut[6].

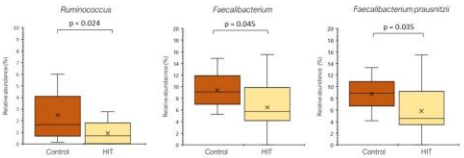


Figure 2. Relative abundance (%) of different healthy gut bacteria found in both study groups

CONCLUSIONS

A dysbiosis involving a greater abundance of histaminogenic bacteria would contribute to accumulate high levels of histamine in the gut, leading to a greater absorption of this amine in plasma and therefore, the appearance of adverse effects. The ability to degrade this histamine resulting from an intestinal dysbiosis would be easily overwhelmed in the individuals with DAO deficiency. Moreover, this dysbiosis could contribute to the mucosal inflammation which would ultimately affect the functionality of the DAO enzyme. An imbalance in the histamine-forming microbiota could account for another possible origin of histamine intolerance.

[1]Comas-Basté, et al. (2020) *Biomolecules*, 10,1-26. [2]EFSA (2011) *EFSA J*, 9,2393. [3]Maintz, et al. (2007) *Am J Clin Nutr*, 85, 1185-1196. [4]Schink, et al. (2018) *J Physiol Pharmacol*, 69, 579-793. [5]Pugin, et al. (2017) *Microb Ecol Health Dis*, 18, 1353881. [6]Martin, et al. (2017) *Front Microbiol*, 8, 1-13.

Referència bibliogràfica suggerida

Sánchez-Pérez, S (2022). Noves evidències de la intolerància a la histamina. Tesi doctoral. Universitat de Barcelona, Barcelona.

Crèdits

Disseny gràfic i il·lustracions: Aída Morales Michel
@aidamoralesdiseno