UNIVERSITAT DE BARCELONA

Fatty Acids in Obese Pregnancies:

Maternal and Child Outcomes

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Maternal and Child Outcomes

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To my family,

This story began almost 5 years ago when I started planning to study a master's degree in the University of Barcelona. Back in my hometown, I had everything sort of figured out so I remember I wasn't sure about leaving for 1 year that the master would last. My family always encouraged me to do so, so I did; I left Monterrey and came to Barcelona in September 2012. One year later, I completed my master's degree and suddenly it wasn't enough, I wanted more.

I decided to start my PhD in the same research department I did my master's internship. I've always specialized in child nutrition, so I chose this department because it was the only one I could found that was somehow related to it. I was very lucky, I really liked the people and the work there. My directors, tutors and co-workers, who all eventually became friends, were always very welcoming and supportive.

Before coming here, I was specialized in clinical nutrition, so the past few years I've had the chance to discover the research world. I had the chance to do so many things which I learned a lot from and made me eager to learn and continue to contribute to this beautiful profession.

Ever since I decided to start this experience, my family was my main support. So first of all, I thank my family for encouraging me to come here from the beginning, and even though it wasn't for just 1 year as initially planned, they have always been the most supportive. Thank you, ma, pa, gorda y gordo. I also thank all my extended family and friends back in México for always being present and pretend that I never left. My partner, my new family and Tao have always made me feel like home, so thank you Daniel for sharing this with me. Special thanks to my two directors, Carmen and Ana thank you for everything you've always done to make me feel welcome and the chances you have given me to learn and grow professionally. Rosa and Maribel, thank you for everything you have taught and helped me, I can say I learned a lot from two excellent persons and professionals. All of my co-workers: Leo, María, Marta, Isa, Mariana, Gemma, Leyre and Charlotte, thank you for making this work and stay very easygoing.

I conclude this experience very happy and satisfied with the work I accomplished, but most importantly, with all the wonderful people I got to know and the invaluable experiences I had the opportunity to live.

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ABBREVIATIONS

AA	Arachidonic Acid
BMI	Body Mass Index
CVD	Cardiovascular Disease
CLA	Conjugated Linoleic Acid
CHD	Coronary Heart Disease
CRP	C-Reactive Protein
DM2	Diabetes Mellitus type 2
DGLA	Dihomo-γ-Linoleic Acid
DHA	Docosahexaenoic Acid
DBS	Drop Blood Spots
EPA	Eicosapentaenoic Acid
ELA	Elaidic Acid
ELOVL	Elongation of Very Long-Chain
EL	Endothelial Lipase
FA	Fatty Acid
FADS	Fatty Acid Desaturase
FAME	Fatty Acid Methyl Esters
FAT	Fatty Acid Translocase
FATP	Fatty Acid Transport Proteins
FABP	Fatty Acid-Binding Protein
FDA	Food and Drug Administration
FFA	Free Fatty Acid
GPRs	G Protein-Coupled Receptors
GC	Gas Chromatography
GDM	Gestational Diabetes Mellitus
HDL	High Density Lipoprotein
HTA	Hypertension
IL-6	Interleukin 6
IBS	Irritable Bowel Syndrome
LA	Linoleic Acid
Lp a	Lipoprotein a

LPL	Lipoprotein Lipase
LXR	Liver X Receptor
LCFA	Long-Chain Fatty Acid
LC-PUFA	Long-Chain Polyunsaturated Fatty Acid
LDL	Low Density Lipoprotein
MFSD2a	Major Facilitator Super family Domain containing 2A
MCFA	Medium-Chain Fatty Acid
MUFA	Monounsaturated Fatty Acid
NO	Nitric Oxide
NF-kB	Nuclear Factor-Kb
PL	Phospholipids
PUFA	Polyunsaturated Fatty Acid
PGI2	Prostacyclin
RXR	Retinoic Acid X Receptor
SFA	Saturated Fatty Acid
SCFA	Short-Chain Fatty Acid
SNPs	Single Nucleotide Polymorphisms
SPE	Solid Phase Extraction
SREBP	Sterol Response Element Binding Protein
TLC	Thin-Layer Chromatography
TG	Triacylglycerol
TNF-α	Tumor Necrosis Factor
VAC	Vaccenic Acid
VLDL	Very Low Density Lipoprotein
WOG	Weeks of Gestation
WAT	White Adipose Tissue
WHO	World Health Organization
ALA	α-Linolenic Acid
GLA	γ-Linoleic Acid

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1. ABSTRACT

There are several factors that can influence health throughout life-span, ones we can modify and other we cannot. Having the tools and knowledge on the modifiable factors helps us to identify opportunity areas to act on our behalf and contribute to a better chance of a good and healthy life. Obesity is a well-known epidemic disease linked to many conditions with high mortality and morbidity. The last decades have shown increasing rates of obesity in women of reproductive age, and therefore more obese pregnancies occur as a result. Maternal obesity has become a big issue due its implications on the health of future generations; it is known to be heritable which makes the obesity cycle repeat. Moreover, an individual can be programmed during pregnancy by maternal nutritional status, among others. The mother is usually the main source of nutrients for the fetus and neonate, so consequently her nutritional status and nutrients' availability is crucial for the child. Fatty acids (FAs), especially long chain polyunsaturated fatty acids (LC-PUFAs), are nutrients that play a key role during pregnancy because they are involved in fetal growth and development. Since these nutrients are known to be altered by conditions such as obesity, maternal obesity could impair fetal/neonatal FA supply, and consequently child outcomes.

This thesis presents 4 manuscripts about the influence of maternal pre-pregnancy weight on fatty acid quality and concentrations, along with the implications on maternal and child outcomes. We studied mother and child pairs selected from the total participants in the observational PREOBE cohort study and divided them into 4 groups according to maternal pre-pregnancy body mass index (BMI) and gestational diabetes status (since it is highly associated to maternal obesity); 1) normal-weight (BMI=18.5-24.99), overweight (BMI=25-29.99), obese (BMI≥30) and gestational diabetic.

Given the role of the fatty acid desaturase (*FADS*) and elongation of very long (*ELOVL*) genes on LC-PUFA production, we investigated if genetic variants in the mentioned genes were associated with pre-pregnancy BMI or affected PUFA levels in plasma of pregnant women. We found that minor allele carriers of *FADS1* and *FADS2* SNPs had an increased risk for obesity and that the effects of genotype on plasma FA concentrations differed by maternal pre-pregnancy weight status. Enzymatic activity and FA levels were reduced in normal-weight women who were minor allele carriers of *FADS* SNPs; these reductions were not significant in overweight/obese participants. This suggests that women with a BMI>25 are less affected by *FADS* genetic variants in this regard. In the presence of *FADS2* and *ELOVL2* SNPs, overweight/obese women showed higher n-3 LC-PUFA production indexes in plasma than those women in the normal-weight group, but this was not enough to obtain a higher n3 LC-PUFA concentration.

We also analyzed the differences in colostrum PUFA composition according to maternal pre-gestational BMI and *FADS* genotype. We observed that a high maternal pre-pregnancy BMI was associated with altered FA levels in colostrum, but then again *FADS* genotypes modulated these results. Minor allele carriers resulted with decreased enzymatic activity and PUFA levels only in normal-weight individuals, making their FA levels similar to those of overweight/obese women. Therefore, *FADS* genetic variation in overweight/obese women had a different impact, possibly improving their FA status. We also found

that dietary intake of DHA in late pregnancy influenced colostrum levels of DHA, so possibly a high intake of this FA could be a recommendation to improve breast milk composition.

Prior to FA analysis in the infants, we evaluated cheek cells and capillary blood as less invasive alternatives to traditional plasma sampling for FA analysis from the phospholipid fraction. After standardizing the experimental procedures, we found that the modifications applied to sampling and sample preparation methodology of both matrices, resulted in a positive correlation for most of the FAs, especially LC-PUFAs, when compared with plasma samples. Therefore, cheek cells and capillary blood are less invasive and reliable methods and can be used as alternatives to traditional plasma sampling for analysis of these FAs.

Once we established the method to analyze FAs in infants, we determined the impact of maternal BMI and/or infant feeding practice in infant FA concentrations, and analyze if these FAs associate with cognitive performance. Maternal pre-pregnancy BMI altered the infant FA behavior in evolution, feeding practice and cognition. In general, FA concentrations decreased towards the 3 years of life, except for the SFAs, n6:n3 and LC-n6:n3 ratios which were increased. Exclusive breastfeeding seems to increase crucial FAs (e.g. DHA) in infants at 6 months of age, which is a critical period for development. Cognitive performance was found improved in infants with high levels of PUFAs (e.g. DHA, AA) at 6 months of age, with the positive effect of the n3 FAs still present at 1.5 years of life.

These studies are a contribution to the scientific evidence of the importance of a healthy pre-pregnancy weight, and identify groups of women who could benefit from a high intake of n3 FAs and controlled n6:n3 ratio to achieve an improved FA status that fulfills fetal/neonatal requirements. Maternal obesity is known to propagate obesity through generations, so and interruption of the obesity cycle could help to lessen the increasing incidence of obesity and its related comorbidities. We therefore should promote a healthy weight and diet in women before, during and after pregnancy to have beneficial effect on child outcome and consequently prevent some nutrition-related issues through their life.

Key words

Maternal obesity, programming, genetics, fatty acids, maternal weight, omega 3, DHA, AA, pregnancy, breast milk, colostrum, breastfeeding, growth, development.

2. BACKGROUND

BACKGROUND

2.1. FATTY ACIDS

2.1.1. STRUCTURE & NOMENCLATURE

Fatty acids (FAs) are hydrocarbon chains with a methyl group and a carboxyl group each at one end of the molecule. The carbon right next to the carboxyl group is called the *α* carbon, and the subsequent is the *β* carbon. The FAs are classified according to their length, saturation, and amount and position of double bonds. Saturated FAs (SFAs) have no double bonds and they are further classified according to their length in short-chain (3-7 carbons), medium-chain (8-13 carbons), long-chain (14-20 carbons) and very-long-chain FAs (≥21 carbons).

Stearic acid: C18:0

FIGURE 1. STRUCTURE OF A SFA.

On the other hand, monounsaturated FAs (MUFAs) have 1 double bond and polyunsaturated FAs (PUFAs) contain ≥2 double bounds.

Oleic acid: C18:1,n-9

FIGURE 2. STRUCTURE OF A MUFA.

These unsaturated FAs are also classified by their length into short-chain (≤19 carbons), long-chain (20-24 carbons) and very-long-chain unsaturated FAs (≥25 carbons) (9). The letter *n*, the symbol *ω* or the symbol Δ are used to indicate the position of the double bond that is closest to the terminal methyl group. If the first double bound of a PUFA is found in the 3rd, 6th or 9th position, the FA would be defined as n3, n6 or n-9 FA, respectively (10).

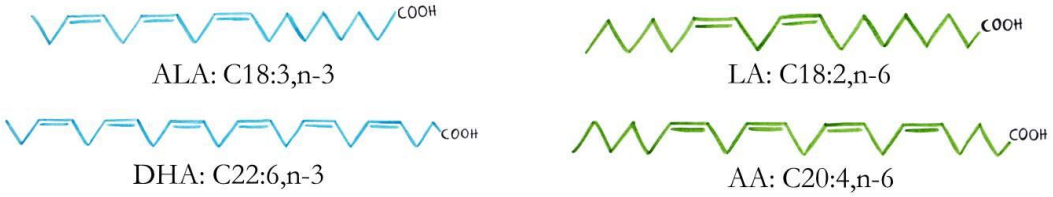


FIGURE 3. STRUCTURE OF N3 AND N6 PUFAS.

The configuration of a FA is called *cis* when the hydrogen atoms attached to the double bonds are on the same side, but if the hydrogen atoms are opposite, the FA is defined as *trans* (9). FAs have scientific names according to their nomenclatures, nevertheless trivial names are used more frequently for an easier identification (Table 1) (11).

TABLE 1. FATTY ACIDS ANALYZED IN THE PRESENT STUDY (9, 12, 13).

Fatty Acids	Series	Common Name	Scientific Name (IUPAC)
Saturated fatty acids (SFA)			
C6:0		Caproic acid	Hexanoic acid
C8:0		Caprylic acid	Octanoic acid
C10:0		Capric acid	Decanoic acid
C12:0		Lauric acid	Dodecanoic acid
C14:0		Myristic acid	Tetradecenoic acid
C15:0		Pentadecylic acid	Pentadecanoic acid
C16:0		Palmitic acid	Hexadecanoic acid
C17:0		Margaric acid	Heptadecanoic acid
C18:0		Stearic acid	Octadecanoic acid
C20:0		Arachidic acid	Eicosanoic acid
C22:0		Behenic acid	Docosanoic acid
C23:0		Tricosylic acid	Tricosanoic acid
C24:0		Lignoceric acid	Tetracosanoic acid
Monounsaturated fatty acids (MUFA)			
C14:1n-5	n5	Myristoleic acid	cis-9-Tetradecenoic acid
C16:1n-9	n9	Palmitoleic acid	cis-9-Hexadecenoic acid
C16:1n-7	n7	Palmitoleic acid	cis-9-Hexadecenoic acid
C16:1n-7*t*	n7	Palmitelaidic acid	trans-9-Hexadecenoic acid
C17:1n-7	n7	Heptadecenoic acid	cis-10-Heptadecenoic acid
C18:1n-7	n7	Vaccenic (VAC)	cis-11-Octadecenoic acid
C18:1n-7*t*	n7	*t*-Vaccenic (*t*-VAC)	trans-11-Ocatadecenoic acid
C18:1n-9	n9	Oleic acid	cis-9-Octadecenoic acid
C18:1n-9*t*	n9	Elaidic acid (ELA)	trans-9-Octadecenoic acid
C20:1n-9	n9	Gadoleic acid	cis-9-Eicosenoic acid
C22:1n-9	n9	Erucic acid	cis-13-Docosenoic acid
C24:1	n9	Nervonic acid	cis-15-Tetracosenoic acid
Polyunsaturated fatty acids (PUFA)			
C18:2n6	n6	Linoleic acid (LA)	all cis-9,12-Octadecadienoic
C18:2n-7*c*9*t*11	n9	Rumenic acid (c9, *t*11-CLA)	cis-9, trans-11-Octadecadienoic acid
C18:3n3	n3	α-Linolenic acid (ALA)	all cis-9, 12, 15-Octadecatrienoic acid
C18:3n6	n6	γ-Linolenic Acid (GLA)	all cis-6, 9, 12-Octadecatrienoic acid
C20:2n6	n6	Eicosadienoic acid (EDA)	all cis-11, 14-Eicosadienoic acid
C20:3n6	n6	Dihomo-γ-Linolenic Acid (DGLA)	all cis-8, 11, 14-Eicosatrienoic acid
C20:3n9	n9	Mead acid	all cis-5, 8, 11-Eicosatrienoic acid
C20:4n6	n6	Araquidonic acid (AA)	all cis-5, 8, 11, 14-Eicosatetraenoic acid
C20:5n3	n3	Eicosapentaenoic acid (EPA)	all cis-5, 8, 11, 14, 17- Eicosapentaenoic acid
C22:2n6	n6	Docosadienoic acid	all cis-13, 16 - Docosadienoic acid
C22:4n6	n6	Adrenic acid (AdA)	all cis-7, 10, 13, 16- Docosatetrtaenoic acid
C22:5n3	n3	Docosapentaenoic acid (DPAn3)	all cis-7, 10, 13, 16, 19- Docosapentaenoic acid
C22:5n6	n6	Docosapentaenoic acid (DPAn6)	all cis-4, 7, 10, 13, 16- Docosapentaenoic acid
C22:6n3	n3	Docosahexaenoic acid (DHA)	all cis-4, 7, 10, 13, 16, 19- Docosahexaenoic acid

FAs can be found in their free form or as a component of complex lipids, such as triacylglycerols (TGs) and phospholipids (PLs) (10). TGs are the main source of dietary fat and are composed of 1 glycerol and 3 FAs (Figure 4) (14).

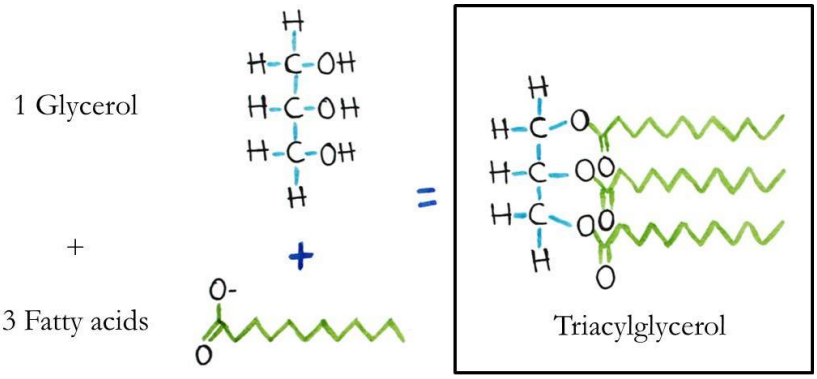


FIGURE 4. TRIACYLGLYCEROL COMPOSITION.

On the other hand, PLs are the major components of the cell membranes and they have a polar head group and 2 hydrophobic hydrocarbon tails (usually contain FAs of 14-24 carbons) (Figure 5). The FAs included as tails are very important because their length and level of saturation will define the membrane's properties. Usually, one of the tails is a SFA and the other one a MUFA or PUFA (15).

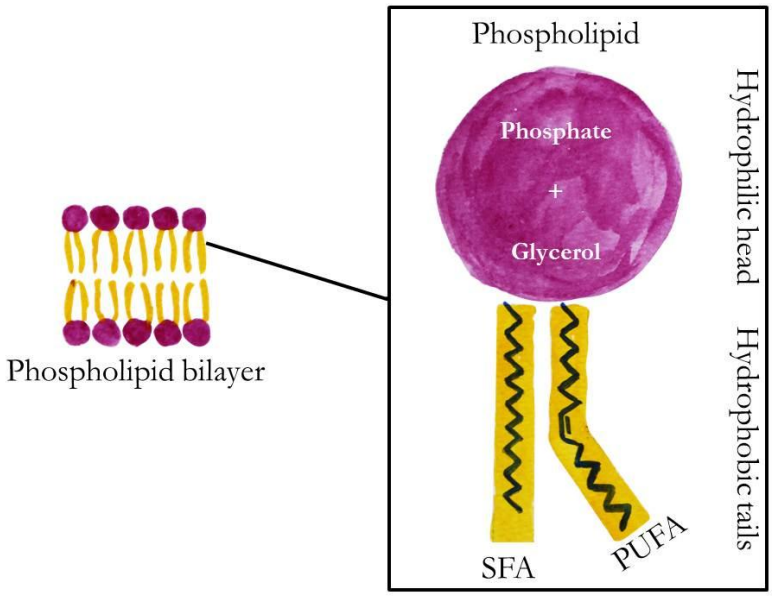


FIGURE 5. PHOSPHOLIPID COMPOSITION.

2.1.2. BIOLOGICAL FUNCTIONS IN GROWTH AND DEVELOPMENT

FAs are physiological important molecules with many biological activities and are obtained through the diet or endogenous production (16). FAs constitute the main part of TGs, PL and other complex lipids (10, 14). Transportation in the bloodstream occurs either as free FAs (FFAs) or within lipoproteins as part of a complex lipid (e.g. TGs and PLs) (16). There are 7 types of plasma lipoproteins: chylomicrons, chylomicron remnants, lipoprotein a (Lp a), and very low (VLDL), intermediate (IDL), low (LDL) and high (HDL) density lipoproteins. All these, except for Lp a and HDL, are atherogenic (17).

FAs influence health and disease risk because they play key roles in metabolism; FAs can be used as energy, cell membrane components and cell signaling. Their main roles in human health are explained below:

- **Energy:** TGs usually accumulate in the cytoplasm of adipocytes from where FAs are oxidized and used as fuel. This source of energy can be used by aerobic tissues (except the brain) and it becomes useful when glucose is limited.
- **Cell membrane components:** FAs function as part of cell membrane PLs, and therefore they contribute to the membrane's properties and influence its function impacting on cell and tissue metabolism, function and responsiveness to signals.
- **Cell signaling:** Membrane lipids (FAs included) are precursors if signaling molecules, and therefore FAs can influence the membrane's activity. FAs that are released from membrane lipids can have different roles in metabolism, signaling or function and they can control gene expression and protein production. This permits FAs to regulate FA synthesis and oxidation, lipoproteins, insulin sensitivity, inflammation, among others.

2.1.2.1. SATURATED FATTY ACIDS

C16:0 is the SFA most commonly found in dietary fats, followed by C14:0 and C18:0. SFA are part of structures according to their function and location. C16:0 and C18:0 are found in high proportions as part of cell membrane PLs, C18:0 is also found in gangliosides and SFA in general are highly present in ceramides and sphingophospholipids. SFA are known to modulate cholesterol, FAs, TGs, lipoproteins and inflammation by influencing cell signaling/gene expression through different mechanisms:

- Lipid rafts (cell signaling platforms) contain high amounts of PLs and sphingolipids rich in SFAs, such as C14:0 and C16:0 which can modify proteins involved in cell signaling.
- SFA of 10-18 carbon atoms elevate levels of messenger RNA for PGC-1β (which is part of a family of transcription factors called sterol response element binding protein (SREBP)). SREBP regulate genes involved with cholesterol, FAs, TGs, PLs, LDL, etc.
- SFAs also activate liver X receptor (LXR) and retinoic acid X receptor (RXR) that bind to hepatocyte nuclear factor 4 and increase VLDL hepatic secretion.
- SFAs upregulate genes involved in inflammation (e.g. pro-inflammatory cytokines). For example, C12:0 and C16:0 enhance the inflammatory nuclear factor-kB (NF-kB). Another inflammatory

cytokine upregulated by SFAs is the tumor necrosis factor (TNF-α) that affects the insulin receptor substrate, and therefore compromises insulin sensitivity.

SFAs are known to induce inflammation since they have also been positively associated with inflammation markers, such as C-reactive protein (CRP) and interleukin 6 (IL-6). SFAs in diet and plasma are associated to insulin resistance, and replacing dietary SFA with unsaturated FAs can improve this condition. It has been observed that SFA increase total and LDL cholesterol, but not all SFAs have the same power; C18:0<MCFA<C16:0<C12:0<C14:0. The last 3 SFAs are associated to increase total and LDL cholesterol, influence FA, TG and lipoprotein metabolism, increase inflammation, insulin resistance, coagulation, and risk for cardiovascular disease (CVD), coronary heart disease (CHD) and diabetes mellitus type 2 (DM2). On the other hand, C15:0 and C17:0 are associated to a lower risk of CVD, CHD and DM2.

2.1.2.2. MONOUNSATURATED FATTY ACIDS

The 2 major MUFA components in diet seem to have biological importance. C18:1n-9 is the most common MUFA (or FA in general) in dietary fats, while C16:1n-7 is in second place, and both are highly found in cell membrane PLs. When C18:1n-9 replaces SFA, it has been associated to partially lower total and LDL cholesterol and limit LDL oxidization. As a result, C18:1n-9 is thought to lower inflammation, however results are still unclear. C18:1n-9 is also associated to decrease blood pressure, improve glucose control and insulin sensitivity. In any case, results are still unclear on whether the beneficial effects are attributed to the removal of SFA or to the increased dietary intake of C18:1n-9. Although not all authors agree, it has also been observed that C18:1n-9 may lower the risk for CHD, cardiovascular events and cardiovascular mortality. Lately, C16:1n-7 has gained attention since its increased intake has been associated to lower total and LDL cholesterol and TGs, increase insulin sensitivity and lower the risk for DM2.

2.1.2.3. N6 POLYUNSATURATED FATTY ACIDS

In regards of n6 PUFAs, the 2 major components in the diet are the linoleic (LA: C18:2n6) and arachidonic acids (AA: C20:4n6), and they have different biological roles. LA is an essential FA to produce n6 LC-PUFAs, such as γ-Linoleic (GLA), dihomo-γ-linoleic (DGLA) and AA. Membrane phospholipids contain high amounts of LA and AA, and some DGLA. For example, LA is important in skin barrier function because it is highly present in skin ceramides, hence an LA deficiency would compromise skin integrity. However very low LA intake is needed and usually most diets provide excess of this FA. When LA replaces SFAs, it has been associated to lower total and LDL cholesterol, but the effect is not linear and the benefits are only seen with a low-moderate LA intake. The mechanisms of this effect could be:

- Through SREBPs, LA reduces cholesterol and promotes hepatic LDL receptor gene and protein expression. Consequently, circulating LDL decreases.
- Through LXR and gene expression, LA increases the bile acid production which decreases cholesterol in the liver, and therefore it helps to promote hepatic LDL receptor expression.

Some have observed that LA can partially lower blood pressure and increase insulin sensitivity. On the other hand, when LA increases in a LDL molecule, it increases its atherogenic effect because it is more likely to oxidize. This FA also can activate the inflammatory factor NF-kB and it can be metabolized into inflammation and cell injury derivatives.

The AA has a structural role in the brain, and therefore it is important in brain development and function. The AA, along with the docosahexaenoic acid (DHA: C22:6n3), has been associated to improve cognition in infants. Free AA can also promote inflammation by signaling through the NF-kB. In cell membrane, AA is a substrate to eicosanoids (prostaglandins, thromboxanes, and leukotrienes). These eicosanoids influence pain, inflammation, immune response, bone turnover, coagulation, muscle contraction, renal function, tumor cells and cancer. However, eicosanoid mediators can have opposing effects, thereby resulting in a controlled biological response. Cell membrane AA is influenced by its dietary intake, but apparently not by LA intake. Some have considered that a high AA level in cell membranes is associated to increase coagulation, inflammation, allergies and tumors, while some others have found protective effects at higher AA levels (16).

It has been observed that replacing SFA intake with PUFAs (mainly LA), results in a lower risk for CHD and CHD mortality, but results are not yet conclusive. In general, increased n6 PUFAs in the diet are related to increased inflammatory factors (PGE2, LTB4, TXA2, IL-1β, IL-6, and TNF-α) that are associated to chronic diseases such as CVD, diabetes, obesity, rheumatoid arthritis and irritable bowel syndrome (IBS) (18).

2.1.2.4. N3 POLYUNSATURATED FATTY ACIDS

Among the n3 PUFAs, the α-linolenic acid (**ALA**: C18:3n3) is the **essential FA** in this series and it is the precursor to other n3 LC-PUFAs, such as eicosapentaenoic acid (EPA: C20:5n3), docosapentaenoic acid (DPAn3: C22:5n3) and DHA. Cell membranes contain ALA<EPA<DPA<DHA, and consequently cell membrane properties and functions are highly influenced by the presence of n3 PUFAs, especially DHA. Dietary intakes of these n3 PUFAs correlate to the amounts present in cell membranes; if n3 PUFAs increase in the diet they will also increase in the cell membranes, but only dose and time dependent.

Some of the functional effects of EPA and DHA depend on their incorporation into cell membrane PLs. For instance, brain and eye membranes are very rich in DHA levels so it influences development and function. Therefore, supply of n3 PUFAs, especially **DHA**, in early life is vital for appropriate visual, neural and behavioral development. Regarding **visual function**, PLs containing DHA optimize the function of rhodopsin. EPA and DHA also seem to influence **brain function** (neurological, psychological and psychiatric aspects) through life span by positively affecting childhood attention, learning/behavioral disorders, depression, bipolar manic depression, unipolar depressive disorder, schizophrenia, aggression, etc. EPA and DHA in cell membranes also influence **transcription factor activation** (NF-κB, PPAR, –α and γ, SREBPs) and, consequently, **gene expression**. These effects play an important role on controlling inflammation, FA and TG metabolism, and adipocyte differentiation.

EPA and DHA also have functional effects involved on inflammation, insulin resistance, blood lipids, blood pressure, atherosclerosis and cancer and they could be used as prevention or as part of the treatment of an existing disease. Some of these functional effects and mechanisms are the following:

- **Reduce inflammation and insulin resistance:** G protein-coupled receptors (GPRs) have a high specificity for n3 PUFAs. For example, GPR120 is highly found in inflammatory macrophages and adipocytes, and by binding DHA, GPR120 influences anti-inflammatory effects on macrophages and insulin sensitivity on adipocytes.
- **Reduce blood pressure:** These 2 FAs also lower blood pressure by producing eicosanoids with vasoactive effects, aldosterone, nitric oxide (NO), vascular reactivity and cardiac hemodynamics (16).
- **Prevent atherosclerosis:** EPA and DHA also reduce cholesterol plaques by producing the anti-inflammatory NO and prostacyclin (PGI_2) (19).
- **Reduce inflammation:** Replacing AA with EPA and DHA decreases the production of inflammatory eicosanoids, and increases the production of resolvins and protectins. As a result, there is a consequent decrease of inflammation markers such as cytokines, adhesion molecules and acute phase proteins. EPA and DHA therefore decrease risk for CVD, metabolic and inflammatory diseases, and have beneficial effects over immunity, allergies, bone turn over, coagulation, vasoconstriction, cancer, etc.
- **Influence tumor cell proliferation and viability:** DHA induces tumor cell apoptosis and EPA+DHA reduce prostaglandin E2 (which promotes tumor growth and proliferation). These 2 FAs have been associated to prevent and slower prostate, colorectal and breast cancers, and to improve the quality of life and physical function of cancer patients.
- **Reduce blood lipids:** N3 PUFAs also modify blood lipids. EPA and DHA have shown to lower TG concentrations possibly by 1) lowering hepatic synthesis and secretion of very low density lipoproteins (VLDLs) (main lipoproteins carrying TGs), 2) upregulating the lipoprotein lipase (LPL) in adipose tissue and therefore promoting TG clearance, 3) decreasing the release (from adipocytes) of FFAs that function as substrates to synthesize TGs in the liver, 4) increasing β-oxidation in skeletal and cardiac muscle and hence limiting FFAs for the liver. EPA and DHA also increase levels of HDL and LDL. This last molecule (LDL) is also increased in size lessening its atherogenic effect (16).
- **Weight loss and maintenance:** Since n3 PUFAs stimulate lipid oxidation they also contribute to reduce fat mass. Moreover, they are associated to regulating appetite and satiety, possibly by lowering leptin levels, which is known as the satiety hormone (20, 21).

ALA, EPA, DPA and DHA have proven to possess biological related functions, and even though they all contribute to lowering the risk of coronary outcomes, each FA has its own function. EPA and DPA production increases when replacing dietary LA with ALA, and yet DPA to DHA conversion is poor. Regardless of this, increasing ALA can mimic the beneficial effects of EPA, possibly by its consequent increased production.

2.1.2.5. *TRANS* UNSATURATED FATTY ACIDS

Trans unsaturated FAs can result from metabolic, industrial and cooking processes. Apparently, natural *trans* FAs have different properties than the ones industrially produced. There is a wide variety of *trans* FAs because all *cis* unsaturated FAs can result in multiple *trans* isomers. The most studied *trans* FAs in human health are the elaidic (ELA), vaccenic (VAC) and conjugated linoleic acids (CLA).
Properties of *trans* double bonds change the fluidity of the membranes and therefore influence the membrane's function affecting cell signaling. *Trans* properties are more like those of SFA. It has been observed that *trans* FAs increase the risk for CVD by increasing LDL cholesterol and decreasing HDL cholesterol (*trans* are the only FAs to lower HDL). For this reason, *trans* FAs are more unfavorable than FAs. *Trans* FAs are also associated to influence blood pressure, coagulation and inflammation, but results are not clear.

2.1.3. DIETARY SOURCES & RECOMMENDATIONS

2.1.3.1. DIETARY SOURCES

SFA are mainly provided by animal fats and some tropical oils, such as palm, coconut and peanut oil. The most predominant SFA in the diet is C16:0, followed by C18:0 and C14:0 (16). The MUFA dietary fats (C18:1n9 is the most common MUFA in diet) mainly derive from both animal and vegetable oils. Regarding PUFAs, LA and ALA are defined as essential FAs because mammals do not have the capacity to synthesize them, so their inclusion in the diet is crucial. LA and ALA are present in almost all dietary fats (vegetable oils have them in big proportions). LA is highly available in dietary foods compared to ALA availability (9). LA (produced in plants and found in seeds, nuts and plant oils) (16) and the AA (found in animal foods such as meat, eggs, fish and some aquatic plants) are the most important and predominant n6 PUFAs (9, 22). ALA is commonly the most predominant n3 PUFA in the diet, unless EPA and DHA supplements are being consumed (16). Some food sources of ALA are: seeds and nuts, plants and vegetable oils (9). In the n3 PUFA series, while the ALA derives from plant oils, EPA, DPA and DHA derive from marine lipids. EPA, and especially DHA, are the most important n3 PUFAs and they are found in mackerel fish, salmon, sardine, herring and smelt (9) and fish oil supplements (16). The *trans* FAs are also available in dietary sources and they derive mainly from partially hydrogenated oils (e.g. margarine) (9).

2.1.3.2. DIETARY RECOMMENDATIONS

TGs, and therefore FAs, are the main sources of dietary fat (16). Fat dietary intake should be equivalent to 25-35% of total energy intake (9) and it refers to all plants or animals that are included in the diet and contain lipids. Dietary fats may come as solids or liquids and, once they are in the body, TGs are

hydrolyzed by lipases to liberate FFAs for their absorption (10, 14). Thus consumed FAs can be found in the bloodstream of healthy people after digestion and absorption (16). Some of the dietary recommendations in regards of FA intake include:

- ↓ SFA intake (maximum 10% of total energy intake)
- ↑ MUFA intake
- ↑ PUFA intake to minimum 6-11% of total energy
- ↑ n3 PUFA intake
- Assure LA and ALA in diet (of total energy intake, LA a minimum of 2.5% and 0.5% ALA) (9)
- ↓ n6:n3 dietary ratio (18)
- Avoid *trans* FA intake

These recommendations could result in 1) ↓ LDL and total/HDL cholesterol ratio, risk of chronic inflammatory diseases, such as obesity, diabetes, CHD, metabolic syndrome, IBD, fatty liver, rheumatoid arthritis, Alzheimer's, and 2) ↑ HDL cholesterol and insulin sensitivity(9).

Limiting n6:n3 ratio of dietary intake seems important because LC-PUFAs from both series use the same enzymes, and so an increased n6:n3 ratio could result in negative consequences by restricting n3 LC-PUFA production (23). Current dietary habits vary widely, but they may correspond to a 10-20:1 n6:n3 ratio, which is a lot higher than 1-4:1 that has been mentioned as recommended (10, 18). However, there is no agreed recommendation for dietary n6:n3 ratio (9). Moreover, it has been suggested we need to consume 0.5 g/day of EPA+DHA, and while it is possible to observe beneficial effects with 1 or 2 fish meals per week, the beneficial effects on risk factors are observed with an intake of 1-2 g/day (10).

2.1.4. ENDOGENOUS SYNTHESIS

Apart from diet, FAs can be obtained through endogenous synthesis from acetyl-CoA (intermediate in lipid metabolism) ending with the C18:0. Afterwards, elongase enzymes are in charge of adding carbons and increase the length of the molecule up to 24 carbon atoms (24). To complete cell membrane requirements, the δ-9-desaturase enzyme begins the process of desaturation with C18:0 by introducing 1 double bond and creating C18:1n9. The δ-12 and δ-15 desaturases are enzymes that continue the process of desaturation in order to obtain the first PUFAs; linoleic acid (LA: C18:2n6) and α-linolenic acid (ALA: C18:3n3). However, δ-12 and δ-15 desaturases are not present in mammals, only in plants. Therefore, LA and ALA are defined as essential FAs for mammals and they need to be included in the diet (10). Once LA and ALA are consumed, they function as precursors to produce n3 and n6 LC-PUFAs (25) (Figure 6). The production requires desaturation reactions catalyzed by δ-5 and δ-6 fatty acid desaturases (FADS), and elongation reactions catalyzed by elongase enzymes (25-27). The n3 and n6 series compete for these enzymes since they participate in both pathways.

2.1.5. FADS & ELOVL POLYMORPHISMS

Single nucleotide polymorphisms (SNPs) are genetic variations that represent an alteration in a nucleotide of the DNA. The DNA is formed by nucleotides that are composed by 2 out of 4 possible nitrogen bases;

cytosine, guanine, adenine and thymine. If, for example, a thymine replaces a cytosine, that would be defined as a SNP. These genetic variations are common, they occur around every 300 nucleotides and if the SNPs occur within a gene, they may influence the genes activity (28).

The δ-5 and δ-6 fatty acid desaturases are encoded by the *FADS*1 and *FADS*2 genes, respectively, while elongases are encoded by the *ELOVL* gene (elongation of very long chain fatty acids) (25-27). Therefore, SNPs in the *FADS* and *ELOVL* genes may affect LC-PUFA production, and consequently alter FA levels (27). For instance, minor allele carriers of the *FADS1, FADS2* and *ELOVL2* genes, have been linked to lower LC-PUFA production, and consequently they show increased concentrations of substrates and decreased levels of products in the LC-PUFA metabolic pathway (25, 27, 29-31).

Certain SNPs in the *FADS* gene have been linked to diseases such as coronary artery disease (32) and DM2 (33). Obesity is related to these conditions and is already known to affect lipid metabolism during pregnancy (34); however, to the best of our knowledge, maternal obesity has not yet been directly linked to SNPs in the *FADS* and *ELOVL* genes. Studies have demonstrated separately that obesity and *FADS* and *ELOVL* SNPs affect FA levels of biological samples, such as blood and breast milk (25, 27, 29, 35-39). However, the results are not yet conclusive. Since FA composition is influenced by both maternal obesity and SNPs, it is of interest to determine whether FA levels vary according to both variables and to explore any association between obesity and SNPs in the *FADS* and *ELOVL* genes.

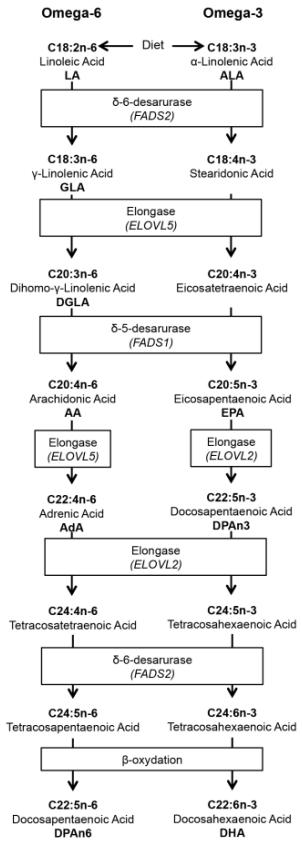
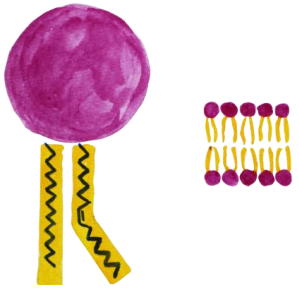


FIGURE 6. METABOLISM PATHWAYS OF OMEGA-6 AND OMEGA-3 PUFAS

2.1.6. ANALYSIS TECHNIQUES



FAs can be analyzed from food samples and different biological compartments, such as whole blood, red blood cells, capillary blood, plasma, adipose tissue and cheek cells. Of these, plasma is most commonly used to determine an individual's FA status (40). FAs can be analyzed from different lipid fractions (e.g. TGs, PLs, total lipids)(9), which have different FA composition due to dietary intake and metabolism (16). Separation of the phospholipid fraction is the best way to assess FAs since other lipid fractions, such as triglycerides or total lipids, are more influenced by recent diet. Depending on the matrix and lipid fraction studied, experimental procedures can involve all or some of the next steps: 1) **lipid extraction**, 2) **separation of lipid fraction** studied, 3) fatty acid methyl esters **(FAME) conversion**, and 4) FAME analysis using **gas-liquid chromatography** (GC) to determine FA profile.

Although **plasma** remains the **most common matrix** for FA analysis, it is considered **invasive** and is not recommended in studies involving infants and young children, or subjects with inaccessible veins. This presents an issue when recruiting volunteers for studies, especially if multiple blood sampling is necessary (40). When the analysis is considered invasive, studies on FAs often rely on food intake questionnaires which do not allow accurate estimation of FA status, since they merely provide data about the type of diet consumed and therefore dietary fat intake (40, 41).

Since the 1980s several studies have proposed the **cheek cells** as an alternative matrix for evaluating FA status. However, the sampling and analytical methods applied in these studies have differed widely, thus hindering the comparison of results. A wide variety of materials has been used for sample collection, such as distilled water (42, 43), plastic spoons (44), sterile cotton swabs (45), sterile wooden spatulas (46), sterile gauzes (47) and endocervical brushes (41, 48-50). Furthermore, the lipid extraction methods proposed by Bligh & Dyer (51), Folch et al. (52) and Hoffman & Uauy (53), have been previously used as references (41-50, 54). Regarding the separation of phospholipids, two methods have commonly been used: thin-layer chromatography (TLC) (43-48, 54) or solid phase extraction (SPE) with cartridges (42, 50), the latter of which limits the quantity of samples that can be processed simultaneously (40). Meanwhile, **capillary blood**, commonly called drop blood spots (DBS), has also been proposed as an alternative to plasma analysis (55-61). Normally, the process consists of direct methylation of whole capillary blood from fingertips absorbed on a chromatographic paper (55-57, 59, 61). This method is much simpler and reduces the amount of analytical time required when compared with plasma samples (60).

As previously shown, different attempts have been made to compare plasma sampling with other less invasive techniques, such as cheek cells and capillary blood (40), and yet there is no agreed standard procedure (See Table 2).

TABLE 2. STUDIES OF FA ANALYSIS FROM CHEEK CELL AND CAPILLARY BLOOD SAMPLES.

Author and year	Sampling	Lipid fraction	Lipid isolation and analysis
CHEEK CELL SAMPLING			
McMurchie et al. 1985 (54)	Pellet	Phospholipids	Lipid extraction according to Bligh and Dyer's method (51). Separation of phospholipids by TLC. Methylation in 1% H_2SO^4 in methanol. Analysis by GC.
Sampugna et al. 1988 (44)	Plastic spoon	Total lipids Phospholipids	Lipid isolation by Folch et al (52). Separation of phospholipids by TLC. Methylation using acidic catalyst. Analysis by GC.
Koletzko et al. 1999 (45)	Sterile cotton swab	Phospholipids	Lipid isolation with chloroform/methanol (1:1) (52). Separation of phospholipids by TLC. Methylation in methanolic hydrochloric acid. Analysis by GC.
Hoffman et al. 1999 (46)	Sterile wooden spatula	Phospholipids	According to Hoffman, 1992 (53). Separation of phospholipids by TLC. Analysis by GC.
Connor et al. 2000 (47)	Sterile gauze	Phospholipids Cholesterol Fatty acids Triacylglycerol Cholesteryl esters	Lipids isolation with chroloform:methanol (2:1) (52). Separation of phospholipids by TLC. Methylation with boron trifluoride. Analysis by GC.
Laitinen et al. 2006 (50)	Circular-shaped Gynobrush (Heintz Herenz)	Phospholipids	Lipid extraction with chloroform/methanol (2:1) (52). Phospholipids isolation by Solid-Phase Extraction (SPE). Methylation with boron trifluoride. Analysis by GC.
Kirby et al. 2010 (49)	Soft endocervical brush	Phospholipids	Lipid extraction by method Folch et al (52). Methylation with acetyl chloride-methanol solution. Analysis by GC.
Klingler et al. 2011 (41)	Gynobrush Plus	Glycerophospholipids Triacylglycerols Phospholipids Total lipids	Lipid extraction with chloroform/methanol. Separation of phospholipids by TLC. Methylation with methanolic HCL. Analysis by GC.
CAPILLARY BLOOD SAMPLING			
Marangoni et al. 2004 (58)	Drop of blood on a chromatography paper.	Total lipids Phospholipids	Lipid extraction with chloroform/methanol (2:1). Separation of phospholipids by TLC. Methylation in methanolic hydrochloric acid. Analysis by GC.
Ryan et al. 2008 (61)	Drop of blood on a chromatography paper (Whatmann Inc).	Total lipids	Saponification with sodium hydroxide. Direct methylation with boron trifluoride in methanol. Analysis by GC.
Armstrong et al. 2008 (55)	Drop of blood on a chromatography paper.	Total lipids	Direct methylation with boron trifluoride in methanol with hexane. Analysis by GC.
Bailey-Hall et al. 2008 (56)	Drop of blood on chromatography paper (Whatmann Inc).	Total lipids	Saponification with sodium hydroxide. Direct methylation with boron trifluoride in methanol. Analysis by GC.
Bilcalho et al. 2008 (57)	Drop of blood.	Total lipids	Direct methylation with acetyl chloride in methanol. Analysis by GC.
Min et al. 2011 (60)	Drop of blood on a dipstick from Fluka Blood Collection Kit and Whatman Standard Grade 3.	Phospholipids Total lipids Triacylglycerides	Lipid extraction with chloroform/methanol (2:1). Separation of phospholipids by TLC. Methylation with acetyl chloride in methanol. Analysis by GC.

2.2. OVERWEIGHT & OBESITY

2.2.1. DEFINITION & CLASSIFICATION

According to the World Health Organization (WHO), overweight and obesity are conditions were there is an excessive fat accumulation (62). It is an inflammatory disease characterized by an increased dietary intake of fat and n6 PUFAs along with a decreased intake of n3 PUFAs (20). To date, the most commonly used tool to identify these conditions in adults is the Body Mass Index (BMI). It is calculated by a person's weight divided by the square of his height (kg/m^2). An adult is overweight when his BMI is in between 25 and 29.99 and obese when his BMI is $\geq$30. This tool is very simple to use, can be used in both sexes and all adult ages, but it should be used carefully because it may not always correspond to the person's degree of fatness, since it only considers weight and height and, for instance, there is no knowledge of other conditions such as muscle mass.

In children, overweight and obesity are identified using Child Growth Charts that measure if their weight is appropriate for their height or if their BMI is appropriate for their age. According to the WHO standards, if the children are spotted on the chart between an 85 and 96.7 percentile it would mean they are overweight and $\geq$97 obese. Besides percentiles, z-scores can estimate overweight and obesity and they can be determined on a chart by spotting for example height and weight. For children under 5, if the z-score is greater than 2 standard deviations (SD) the child would be overweight, and if it is greater than 3 SD it would be obese. For children between 5 and 19 yo, if the z-score is greater than 1 SD the child would be overweight, and if it is greater than 2 SD it would be obese (62).

2.2.2. EPIDEMIOLOGY

Obesity is a non-communicable epidemic disease that has more than doubled since 1980. It was previously considered to be a high-income country disease, but overweight and obesity are expanding in low and middle-income countries as well. It is estimated that 2.8 million people die each year as a result of being overweight or obese, this means that currently more people is dying by these conditions than underweight (63).

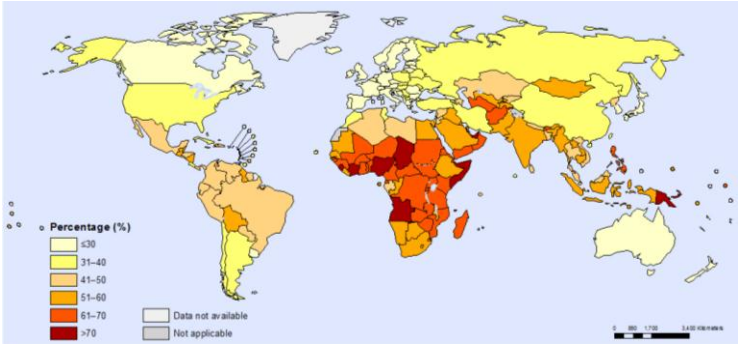


FIGURE 7. PERCENTAGE OF DEATHS DUE TO NONCOMMUNICABLE DISEASES OCCURRING UNDER AGE OF 70, BOTH SEXES, 2015 (1).

It has been observed that women are more likely to be obese than men. By 2014, 39% of men and 40% of women (≥18 years) were overweight and 11% of men and 15% of women were obese. Obesity rates have risen both for men and women in the past 4 decades. In 1975, 3% of men were obese and 21% were overweight, whereas 6% of women were obese and 23% overweight (64). The following table presents the overweight and obesity global estimates of both males and females from 1975 to 2014, and it demonstrates how women have a higher prevalence than men (Table 3).

TABLE 3. PERCENTAGE OF ADULT POPULATION WITH OVERWEIGHT OR OBESITY (7).

	Global Estimates % [SD]			
	BMI ≥ 25		BMI ≥ 30	
Year	Male	Female	Male	Female
2014	38.3 [36.3-40.4]	39.0 [37.0-41.0]	10.8 [9.7-12.0]	14.9 [13.6-16.1]
2013	37.7 [35.7-39.6]	38.5 [36.6-40.4]	10.4 [9.5-11.5]	14.5 [13.4-15.7]
2012	37.0 [35.2-38.8]	37.9 [36.2-39.7]	10.1 [9.2-11.0]	14.2 [13.1-15.2]
2011	36.3 [34.7-38.0]	37.4 [35.8-39.0]	9.7 [8.9-10.6]	13.8 [12.9-14.8]
2010	35.7 [34.1-37.2]	36.8 [35.4-38.4]	9.4 [8.7-10.2]	13.5 [12.6-14.4]
2009	35.0 [33.5-36.4]	36.3 [35.0-37.7]	9.1 [8.4-9.8]	13.2 [12.4-14.0]
2008	34.3 [33.0-35.7]	35.8 [34.5-37.1]	8.8 [8.1-9.4]	12.9 [12.1-13.6]
2007	33.6 [32.3-34.9]	35.2 [34.0-36.5]	8.4 [7.9-9.0]	12.5 [11.8-13.3]
2006	33.0 [31.7-34.1]	34.6 [33.5-35.9]	8.1 [7.6-8.7]	12.2 [11.5-12.9]
2005	32.3 [31.1-33.4]	34.1 [33.0-35.2]	7.9 [7.3-8.4]	11.9 [11.3-12.5]
2004	31.7 [30.6-32.8]	33.6 [32.5-34.7]	7.6 [7.1-8.1]	11.6 [11.0-12.2]
2003	31.1 [30.0-32.2]	33.1 [32.0-34.1]	7.3 [6.9-7.8]	11.3 [10.7-11.9]
2002	30.5 [29.5-31.6]	32.6 [31.6-33.6]	7.1 [6.6-7.6]	11.0 [10.5-11.6]
2001	30.0 [29.0-31.0]	32.1 [31.1-33.2]	6.9 [6.4-7.3]	10.8 [10.2-11.4]
2000	29.5 [28.4-30.5]	31.6 [30.6-32.7]	6.6 [6.2-7.1]	10.5 [10.0-11.1]
1999	28.9 [27.9-30.0]	31.2 [30.1-32.2]	6.4 [6.0-6.9]	10.3 [9.7-10.8]
1998	28.5 [27.4-29.6]	30.7 [29.7-31.8]	6.2 [5.8-6.7]	10.1 [9.5-10.6]
1997	28.0 [27.0-29.2]	30.3 [29.2-31.4]	6.1 [5.6-6.5]	9.8 [9.3-10.4]
1996	27.6 [26.5-28.8]	29.9 [28.8-31.0]	5.9 [5.4-6.4]	9.6 [9.1-10.2]
1995	27.2 [26.1-28.4]	29.5 [28.3-30.7]	5.7 [5.2-6.2]	9.4 [8.8-10.0]
1994	26.8 [25.6-28.1]	29.1 [27.9-30.3]	5.6 [5.1-6.1]	9.3 [8.7-9.9]
1993	26.4 [25.2-27.8]	28.8 [27.5-30.0]	5.4 [4.9-6.0]	9.1 [8.5-9.8]
1992	26.1 [24.8-27.5]	28.4 [27.1-29.7]	5.3 [4.8-5.8]	8.9 [8.3-9.6]
1991	25.8 [24.4-27.2]	28.1 [26.7-29.5]	5.1 [4.6-5.7]	8.8 [8.1-9.5]
1990	25.4 [24.0-26.9]	27.8 [26.3-29.2]	5.0 [4.5-5.6]	8.6 [7.9-9.4]
1989	25.1 [23.6-26.7]	27.4 [25.9-28.9]	4.8 [4.3-5.5]	8.4 [7.7-9.2]
1988	24.7 [23.2-26.4]	27.0 [25.5-28.6]	4.7 [4.1-5.3]	8.3 [7.5-9.1]
1987	24.4 [22.8-26.1]	26.7 [25.1-28.3]	4.6 [4.0-5.2]	8.1 [7.3-8.9]
1986	24.0 [22.4-25.9]	26.3 [24.7-28.1]	4.4 [3.8-5.1]	7.9 [7.1-8.8]
1985	23.7 [21.9-25.6]	26.0 [24.2-27.8]	4.3 [3.7-5.0]	7.8 [6.9-8.7]
1984	23.4 [21.5-25.4]	25.6 [23.8-27.5]	4.2 [3.5-4.9]	7.6 [6.7-8.6]
1983	23.0 [21.1-25.2]	25.3 [23.4-27.3]	4.0 [3.4-4.8]	7.5 [6.5-8.5]
1982	22.7 [20.7-25.0]	25.0 [23.0-27.1]	3.9 [3.3-4.7]	7.3 [6.4-8.4]
1981	22.4 [20.3-24.8]	24.7 [22.6-26.9]	3.8 [3.1-4.6]	7.2 [6.2-8.3]
1980	22.1 [19.9-24.6]	24.3 [22.2-26.7]	3.7 [3.0-4.5]	7.1 [6.0-8.2]
1979	21.8 [19.4-24.4]	24.0 [21.8-26.5]	3.6 [2.9-4.4]	6.9 [5.8-8.1]
1978	21.5 [19.0-24.3]	23.7 [21.4-26.3]	3.5 [2.8-4.3]	6.8 [5.6-8.0]
1977	21.2 [18.6-24.1]	23.4 [20.9-26.1]	3.4 [2.6-4.3]	6.6 [5.5-7.9]
1976	20.9 [18.2-24.0]	23.0 [20.5-25.9]	3.3 [2.5-4.2]	6.5 [5.3-7.9]
1975	20.7 [17.8-23.9]	22.7 [20.1-25.7]	3.2 [2.4-4.1]	6.4 [5.1-7.8]

2.2.2.1. EPIDEMIOLOGY IN WOMEN

Obesity in women and maternal obesity have gained a lot of attention due to its increasing rates and relationship with health of future generations. The WHO has divided countries into different regions to observe health trends. Those regions are Africa (24 countries), Asia-Pacific (6 countries), Latin America and Carribean (5 countries) and Eastern Europe (4 countries). Figure 8 shows that the likelihood of being overweight or obese increases with wealth levels, except in Europe (2).

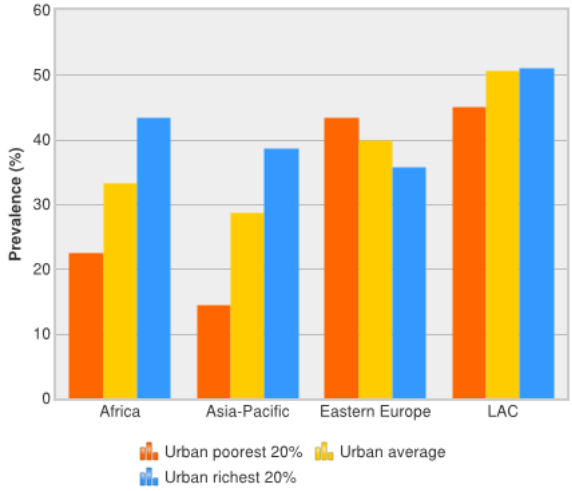


FIGURE 8. PREVALENCE OF OBESITY IN WOMEN (15-49 YEARS) IN URBAN AREAS, 2005-2013 (59).

According to observatory data of 2013, more than 50% of women in Latin America and Carribean regions were either overweight or obese, while in Europe around 35% of women were (2). The next figures show the prevalence in women over 18 yo by the year of 2014. Figure 9a shows that overweight in women prevails in many countries of America and Europe, among others, and figures 9b and 9c show that countries such as Mexico and the United States of America (USA) have $\geq$60% and $\geq$30% prevalence of overweight and obesity in women, respectively. In European countries, adult women have an overweight prevalence of 40-59.99% and an obesity prevalence of20-29.9% prevalence of obesity.

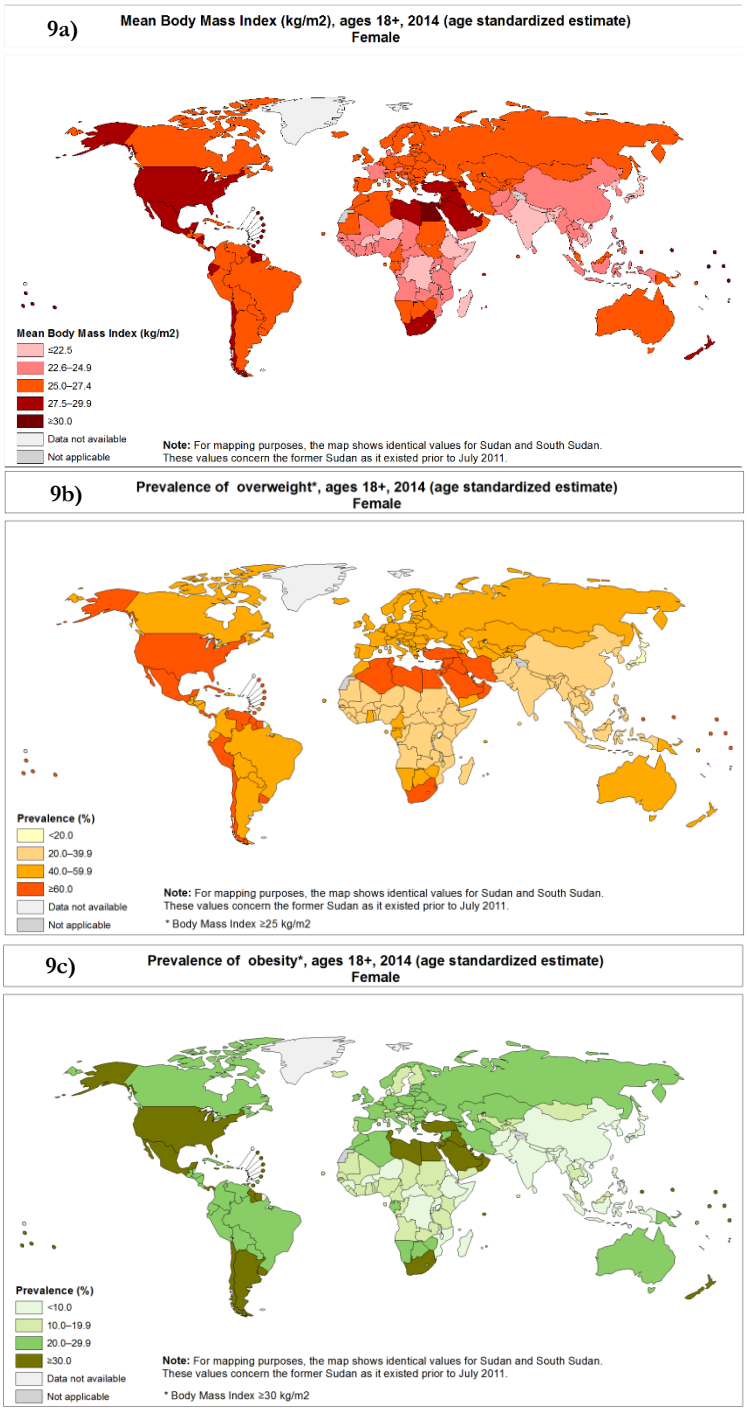


FIGURE 9. PREVALENCE IN WOMEN OVER 18 YEARS OLD, 2014 (1).

2.2.2.2. EPIDEMIOLOGY IN CHILDREN

Prevalence of childhood overweight and obesity is increasing in alarming rates and has become one of the most serious public health challenges (65). In 2015, nearly 42 million of children under 5 years old were estimated to be already overweight or obese compared to the 32 million in 1990 (65, 66). If the current trends continue, it is estimated that by 2025 there will be 70 million of infants and young children with overweight or obesity (66). The children, who suffer this disease before puberty, are more likely to continue to be obese during childhood, adolescence and adulthood (65).

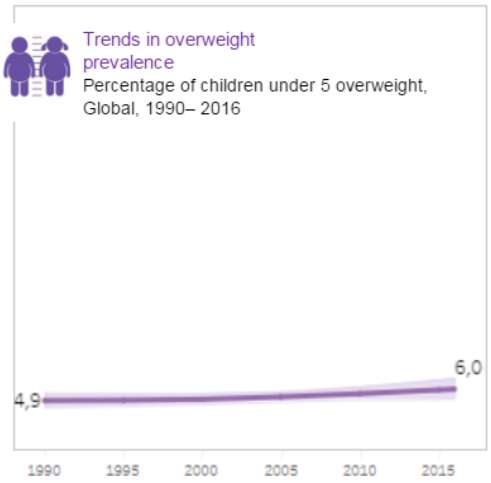


FIGURE 10. CHILD OVERWEIGHT GLOBAL ESTIMATES (3).

2.2.3. PATHOPHYSIOLOGY & COMPLICATIONS

Adipose tissue, and particularly abdominal fat, plays a central and key role in the development of and inflammatory state and chronic diseases. The storage of FA TGs within adipocytes has been considered to be protective because if prevents FAs to circulate in the blood and produce oxidative stress (lipotoxicity). However, when there is an excessive storage of fat (e.g. obesity), then the lipolysis increases leading to the release of FFAs into the bloodstream causing lipotoxicity. This release also inhibits lipogenesis causing an accumulation of blood TGs. When the FAs are released by the LPL within β lipoproteins, it results in malfunction of β receptor and consequently in insulin resistance. The liver tries to compensate and increases gluconeogenesis, which accentuates the hyperglycemia by glucose production. Moreover, FFA inhibits the utilization of muscle glucose, accentuating even more the hyperglycemic state. Lipotoxicity caused by FFAs also decreases (until exhaustion) insulin secretion from β cells in pancreas, leaving a permanent hyperglycemic state.

Adipocytes constitute the fat mass which is considered to be a large endocrine organ that releases FAs, lectin, adiponectin, visfatin, resistin and inflammatory adipokines, such as TNF-α, IL-6 and CRP. The

inflammatory substances derive mainly from the white adipose tissue (WAT) and they contribute to higher risk for chronic diseases, such as insulin resistance, diabetes, fatty liver, atherosclerosis, dyslipidemia, sleep apnea and hypertension. Obesity plays a central role in the actions of these substances and diseases.

- **Inflammatory state**: Adipocytes secrete inflammatory markers, such as leptin, resistin, interleukins (IL-1, IL-6, IL-8), TNF-α, fat-associated macrophages, acute phase reactants, among others.
- **DM2 and fatty liver:** CRP, an acute-phase reactant, is further increased in insulin resistance and associated to DM2 and fatty liver.
- **Fatty liver and fat deposition:** TNF-α increases proportionally with fat mass and it is related to fatty liver and fat deposition in organs, such as pancreas and gut.
- **Insulin resistance:** Adipocytes increase insulin resistance by secreting TNF-α, IL-6, resistin and fat-associated macrophages that, for example, induce the NF-kB (related to insulin resistance).
- **Dyslipidemia:** Inflammatory cytokines inhibit lipolysis thereby increasing concentration of TGs.
- **Hypertension (HTA):** Some adipokines are also related to hypertension by secreting renin, angiotensinogen, and angiotensin II from the adipocytes.
- **Atherogenesis:** Foam cells formation results from increased endothelial uptake of oxidized LDL and FFAs that accumulate from FA peroxidation. As atherogenesis progresses, more cytokines are released and these increase inflammatory lesions of atherosclerotic plaques in the vascular wall, such as thrombosis, and they also increase angiogenesis in the WAT (67).
- **Sleep apnea:** While obesity is considered a risk factor for developing sleep apnea, it has been suggested that sleep apnea itself promotes overweight by affecting energy metabolism and daytime activities (68).

Lipotoxicity and related chronic diseases promote atherogenesis and CHD. Adipocytes also secrete anti-inflammatory, and thus anti-atherogenetic, substances and they confer protective effects against the pro-inflammatory markers. It is clear that adiponectin and visfatin can improve insulin sensitivity and vascular endothelium dysfunction, but it is not quite clear as for resistin and leptin because they may act as both anti and pro-inflammatory markers. For example, while leptin can improve insulin sensitivity, it can also enhance inflammation and angiogenesis (67).

Obesity is a chronic disease that is immersed in a vicious cycle. As noted, obesity is characterized by an accumulation of fat, partially derived from an excessive energy intake. The accumulation of fat causes inflammation by the production cytokines. This inflammation state causes insulin and leptin resistance which promotes obesity and diabetes; and the same cycle starts again.

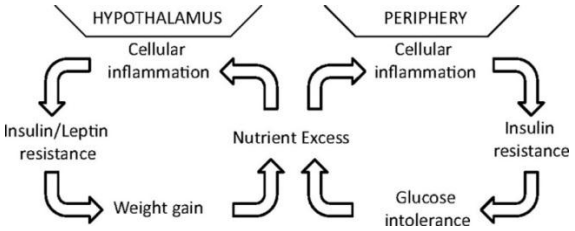


FIGURE 11. THE VICIOUS CYCLE OF OBESITY (4).

2.2.4. RISK FACTORS

Obesity is a multifactorial disease and therefore can be attributed to diverse causes. Risk factors such as age, family history, genetics, race, ethnicity, medical issues and sex, cannot be changed. On the other hand, some other risk factors are modifiable since they are related to unhealthy lifestyles and environments (69). Among the modifiable risk factors are the lack of physical activity, short sleep duration, high amounts of stress, lack of information and unhealthy dietary habits (unhealthy fats and sugar, fast food, large portions, sugary beverages, etc.), (69-71).

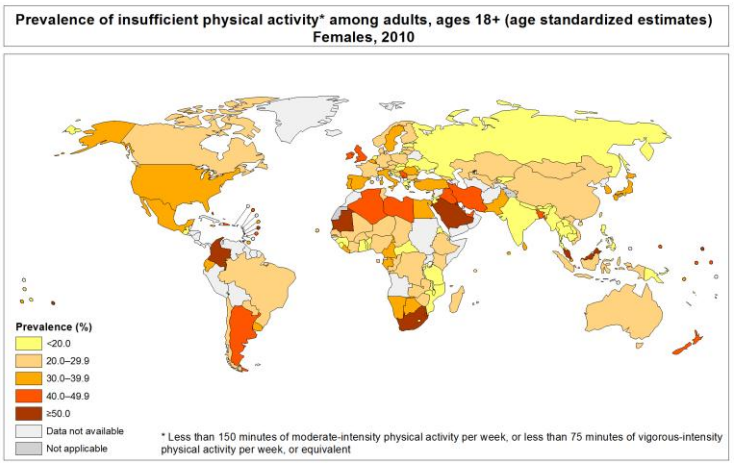


FIGURE 12. PREVALENCE OF INSUFFICIENT PHYSICAL ACTIVITY AMONG ADULT WOMEN, 2010 (1).

It has been observed that obesity and obesity related issues in women around pregnancy period are also modifiable risk factors for persistent obesity in women and children. A high pre-pregnancy weight, excessive gestational weight gain and gestational diabetes mellitus (GDM) increase the likelihood of obesity in both women and child (72, 73). The lack of maternal weight loss after pregnancy is also considered to contribute to the development of obesity in women (73).

Studies have found that overweight and obesity can run in families possibly because of different factors such as genetics, environments and lifestyle habits (71). These conditions can be largely preventable focusing on lifestyle habits and environments (65). In order to prevent obesity in future generations, special attention and action should be paid in women/maternal and childhood obesity because obese adult women have a high risk of having obese children. These obese children are more likely stay obese into adulthood when they could eventually become parents, and therefore continue the obesity cycle once more.

2.3. FATTY ACIDS & PERINATAL OUTCOMES

2.3.1. LIPID METABOLISM IN PREGNANCY

Maternal fat depots and hyperlipidemia develop during the first 2 trimesters in normal pregnancies (74). The function of this lipid metabolism is a key determinant to fetal growth and development (75). Maternal fat mass increases around 3.5kg between the 10-30th week of gestation (WOG). By the 30th WOG, fat mass reaches its highest peak and by this last trimester is when maternal lipolysis begins causing an increment in FFAs (76).

When maternal fat mass increases during the first 2 trimesters of pregnancy, it can be attributed to maternal hyperphagia, lipogenesis and, possibly, an increase of LPL in the adipose tissue. Pancreatic β-cells are increased in early pregnancy, without changing or increasing insulin sensitivity. This can increase fat deposition because insulin sensitivity influences glycerolgenesis and lipogenesis. Moreover, LPL controls fat deposition, because it hydrolyzes circulating TGs in the form of VLDL and its products enter the adipose tissue. Eventually, fat mass reaches its highest peak at the 30th WOG. In this last trimester of pregnancy, fat breakdown occurs by the process of lipolysis and releases FFAs and glycerol causing an accumulation in maternal circulation. Lipolysis can be enhanced by different mechanisms, such as fasting and insulin resistance. Insulin resistance develops throughout the pregnancy and 1) prevents fat deposition, 2) promotes lipolysis, and 3) possibly decreases adipose tissue LPL, decreasing VLDL removal from maternal circulation and fat deposition in adipose tissue. Then, maternal circulation accumulates FFAs and glycerol causing hyperlipidemia. The products travel to the liver where they synthesize TGs and mostly form VLDL. Estrogen has been shown to promote liver production of VLDL and decrease hepatic lipase function. Since estrogen increases during pregnancy, it is considered to be highly responsible for maternal hyperlipidemia. LDL and HDL are also synthesized but in smaller proportion, their synthesis could be also promoted by changes in mid and late pregnancy; 1) the increased activity of the cholesteryl ester transfer protein, and 2) the decreased activity of the hepatic lipase. Therefore, maternal hyperlipidemia benefits the fetus by 2 different paths. Even though TGs cannot cross the placenta, the placental membrane is capable of degrading TGs into FAs to deliver them to the fetus. Glycerol can also be used for gluconeogenesis and FFAs for β-oxidation to acetyl-CoA and ketones synthesis. This seems to have a beneficial effect in the fetus, especially under maternal fasting conditions, because fetal supply of glucose and ketones (used to synthesize brain lipids) increase.

The maternal lipolysis, or catabolic state, coincides with the phase of maximum fetal growth, when the mother must increase the supply of nutrients. Therefore, lipolysis plays an important role in fetal development because, as mentioned above, the released FFAs increase maternal TGs and fetal fat mass (75). Contrary to the adipose tissue, TGs (hyperlipidemia) never stop increasing throughout pregnancy, and thus FAs in TGs have the highest levels in the last trimester (76). It has been observed that the enzymes involved in LC-PUFA production (elongases and desaturases) are more active in pregnant than non-pregnant women (77). Accordingly, DHA, among other FAs, increase in maternal plasma during pregnancy (78). Estrogen could also influence these results, since it is a hormone that facilitates LC-PUFA

synthesis (79, 80) (probably by regulating delta-6 desaturase) and increases during a regular pregnancy (79). These could be alternative factors for the increased maternal LC-PUFAs levels.

2.3.2. PERINATAL PHYSIOLOGICAL IMPORTANCE OF FATTY ACIDS

During and after pregnancy, the mother is usually the sole source of key nutrients for the fetus, and hence fetal and postnatal development depends on the nutrients available in maternal circulation (75). FAs, especially n3 and n6 LC-PUFAs, are known be crucial for an appropriate fetal and child development by influencing cell signaling, gene expression, cell fluidity, oxidative stress, and glucose (81). The availability of FAs in maternal circulation will define the supply of these key nutrients to the fetus and newborn (75) through placental transfer and breast milk, respectively. There is evidence that FAs of the neonate highly correlate with those of the mother at delivery, meaning the higher FA levels in the mother, the higher FA levels in the neonate. Therefore, since maternal FAs in blood and tissues correlate to FA dietary levels, maternal dietary intake of FAs becomes an important issue (76).

When the mother transfers FAs to the fetus/neonate through the placenta and breast milk, respectively, the supplied FAs go into the growing tissues of the fetus/neonate. Any condition altering maternal FA status (lipid metabolism altered, obesity, diabetes, etc.) (75) and fetal/neonatal FA delivery (FA placental transfer and breast milk FA composition), could affect fetal/neonatal growth and development through the systems where FAs intervene; autonomic nervous system (function of internal organs such as the eyes), immune system and brain.

LC-PUFAs have been mainly associated to infant growth and visual, cognitive and immune function (9). It has been shown that maternal LA intake is inversely associated with birth size, whereas AA has been positively associated with it (76). Studies have shown that AA, and particularly docosahexaenoic acid DHA, play an important role in many physiological conditions (38), such as neural and visual development (25, 82). This can be explained because DHA and AA are the main PUFAs present in the grey matter of the brain. Fetal brain growth accentuates during the last trimester of pregnancy and the first year of life (83), which is when DHA conveniently accumulates in the brain. After birth, this accumulation of DHA also serves as a pool for different growing organs and tissues (76).

Some of the perinatal health outcomes that LC-PUFAs, especially DHA, have been positively associated to are summarized below:

- Prevention and improvement of maternal depression
- Increased length of pregnancy
- Reduced preterm births
- Improvement of visual function
- Improvement of cognitive function (vocabulary, problem solving, coordination, etc.)
- Reduced admissions neonatal intensive care units
- Reduced neonatal deaths

- Reduced asthma and allergic asthma
- Increased immunity (9)
- Reduced food allergies
- Reduced eczema (83)

2.3.3. FATTY ACID DIETARY RECOMMENDATIONS FOR PREGNANT & LACTATING WOMEN

Maternal fat stores reflect long-term dietary intake so it is important to consume enough PUFA and LC-PUFAs not only during pregnancy, but all though life span (84). ALA dietary intake does not increase DHA concentrations in blood or breast milk, so specifically DHA dietary intake must be enhanced during pregnancy and lactation (9). In 2004, the Food and Drug Administration (FDA) stablished it was safe to limit seafood intake to 340 g per week to prevent fetal exposure to neurotoxins, such as mercury. This amount of seafood would provide around 200 mg/day of DHA. However, many women limit their intake to little or no fish at all as a precaution, thereby limiting the supply of this key nutrient to the fetus or neonate (83). Additionally, many women following Western diets would not be able to reach the minimum amounts of DHA to meet fetal/neonatal requirements (77). There are currently many fish oil supplements containing DHA in different amounts ranging from 150 to 1200 mg/day. These supplements can be used in addition or as an alternative to fish intake. Currently, many prenatal supplements already contain 200-300 mg of DHA (83), which is the minimum amount that experts recommend in pregnancy and lactation. An even though this dietary intake is recommended through the entire pregnancy, it is especially important during the last trimester when most of DHA placental transfer occurs. It is important to point out that DHA, EPA or AA+DHA intake are not associated to any toxicity in the mother or child.

Trans FAs are also transferred to the fetus/neonate and they have been associated to negative perinatal health outcomes, such as conception, fetal loss and growth. Therefore, *trans* FAs should be as low as possible in diets of pregnant and lactating women. Regarding total fat, MUFA or SFA, dietary recommendations for pregnant and lactating women are no different than the general population (9).

2.3.4. FATTY ACID PLACENTAL TRANSFERENCE

The mother is the sole source of nutrients during pregnancy. They supply the fetus with nutrients through placental transference. Thus, fetal growth and development depends on 1) maternal nutrients availability and 2) functioning of the placenta. As mentioned before, FAs, especially LC-PUFAs such as AA and DHA, are crucial for the fetus especially during the last trimester, and consequently their transport suffers adaptations throughout pregnancy to meet the increasing fetal demands. LC-PUFAs, especially AA and DHA, in fetal tissues and blood have been found in higher concentrations than other FAs. This preference for LC-PUFAs has been defined as biomagnification and occurs during the last trimester of pregnancy when the fetal demands increase (77). DHA is preferentially accumulated than any other FA by the fetus (76).

The placenta uptakes FAs in their free form, thus TGs and PLs must be degraded first by the lipases (LPL and endothelial lipase (EL)) to release FFAs (85). It has been suggested that LPL increases at the term of pregnancy (86). The FA transference is performed by crossing the syncytiotrophoblast layer (a membrane facing maternal and fetal blood) by passive diffusion or active transport. The bound proteins that promote the active transport are:

- **Fatty acid transport proteins (FATP's):** They include 6 integral transport proteins and they function as FA transporters or translocases. A preference for DHA transfer has been suggested.
- **Plasma membrane fatty acid-binding protein (FABPpm**): It functions as an extracellular FA receptor and therefor increases the transference. A preference for LC-PUFA transfer has been suggested.
- **Fatty acid translocase (FAT/CD36):** Like FATP's, it is a transport protein that functions as FA transporters or translocases. The largest portion of this protein are extracellular hydrophobic pockets, thus it promotes FA uptake. It has no preference for particular LC-PUFAs (77).
- **Major Facilitator Super family Domain containing 2A (MFSD2a):** This protein is highly expressed in the placenta and is a primary transporter for DHA and other LC-PUFAs (87).

Once the FAs are in the placental cytosol, the cytoplasmic FABPs can take them through different pathways; 1) transport them to the sites where FAs are then transferred to the fetus, or 2) used for metabolism, β-oxidation, signal transduction or ligand activation (85).

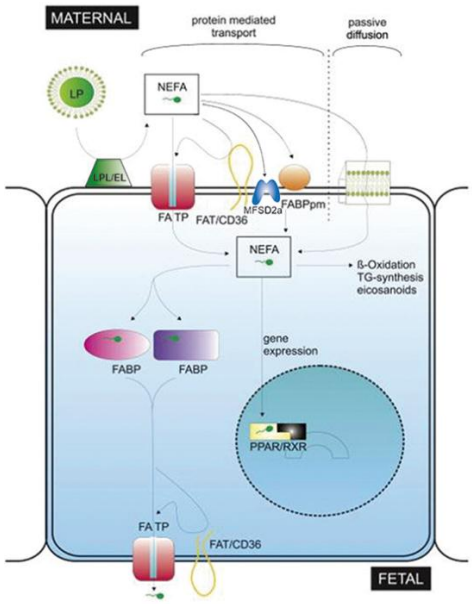


FIGURE 13. FATTY ACID PLACENTAL TRANSFER (5).

Placental transfer is the main source of FAs for the fetus, however 18 carbon PUFAs in the placenta and fetus could function as precursors to produce little amounts of LC-PUFAs. The desaturase enzymes that

catalyze LC-PUFA production have been found in the placenta and fetal liver, but in low concentrations thus their activity is low (88). For instance, maternal supplementation of 18 carbon PUFAs have shown no effect in maternal or offspring DHA status, while DHA supplementation has (88).

Any condition, such as GDM, altering the placental functioning, compromises LC-PUFA delivery to the fetus (6).

2.3.5. FATTY ACIDS IN BREAST MILK

2.3.5.1. BREAST MILK

Once the baby is born, breast milk is usually the main source of nutrients to the neonate so its composition is crucial for the neonate's health outcome. Breast milk is associated to have antimicrobial and immunomodulatory activity so it compensates the deficient neonatal immune system. It also influences gene expression in the neonatal gastrointestinal tract, thereby regulating intestinal cell proliferation, differentiation and barrier function. Breast milk also has an anti-inflammatory activity since it contains factors that inhibit inflammation and promotes antibody production.

Breast milk has many different components and its composition is believed to be adapted by each mother to meet the requirements of the neonate. Breast milk composition is very complex and it can vary by many factors, such as time of the day, nursing process, maternal dietary fat and maternal BMI. Among the different breast milk compartments, the lipid fraction is the most variable component (89).

2.3.5.2. FATTY ACID UPTAKE & FAT SYNTHESIS BY MAMMARY GLAND

Breast milk fat is synthesized by the mammary gland in the lactocytes using precursors present in the cell or obtained from maternal circulation. FAs from maternal circulation derive from LPLs, which have to be degraded by lipases, or LDL. Short-chain (SCFAs) and medium-chain FAs (MCFAs) can be synthesized de novo within the lactocytes. Like mentioned before, LC-PUFA endogenous synthesis is not possible, therefore LC-PUFAs or their precursors must be obtained through from maternal stores or diet (90). Maternal fat stores are therefore crucial for breast milk lipid composition and since fat stores reflect long-term dietary intake, it is important to consume enough PUFA and LC-PUFAs not only during pregnancy, but all though life span (84). The mechanisms of FFAs uptake and esterification are not very clear, but FAT and FABP analogues may play a role. Although there is no clear specificity, it has been suggested that n3 FAs are preferentially transferred to breast milk; higher levels of ALA and lower of LA have been found in breast milk than maternal adipose tissue and plasma. However, higher concentrations of LC-PUFAs and lower of the essential FAs were found in maternal plasma than in breast milk (76).

Once the FAs are obtained through maternal circulation or synthesized de novo, they are esterified (90) into TGs (98% of fat), diacylglycerides, monoacylglycerides, PLs, cholesterol or kept as FFAs. These components are packed into fat lipid globules; TGs are found in the core and PLs in the membrane of the globules (89). This amphipathic membrane allows the dispersion of the lipid globules in the watery breast milk and it also can bind lipase, thus facilitating the neonate's digestion (90).

2.3.5.3. LIPID & FATTY ACID COMPOSITION OF BREAST MILK

Lipids contribute 40-55% of the total energy of breast milk. Breast milk contains around 200 FAs, but some of them in very low concentrations. These FAs can be obtained from maternal plasma or can be synthesized by the mammary gland (89). SFAs predominate in breast milk over MUFAs and PUFAs (84).

The C18:1n-9 is the highest FA present in breast milk and it accounts for 30-40 g/100 g of fat. Other FAs present in high levels are C16:0 and C18:2n6. LC-PUFAs constitute around 2% of the total breast milk FAs (89) and DHA and AA are the major LC-PUFA components (9), AA having the highest concentration of all (84). SCFAs contribute an important amount of energy too and are thought to be crucial for development of the gastrointestinal tract. Sphingomyelins promote nervous system myelinization and neurobehavioral development. Lipids, especially MCFAs, in breast milk are known to inactivate pathogens and thereby protect from infections at the mucosal surface (89).
Fat composition of breast milk can be altered by:

- **Maternal plasma lipids:** Breast milk FA composition reflects the availability of FA in maternal plasma (76).
- **Time since last feed:** It is one of the most significant factors to alter breast milk fat concentration, the shorter the time, the higher concentration of fat.
- **Time of the day:** Fat concentrations in breast milk vary during the day from 5 g/100 ml to 3 g/100 ml. Midmorning has the highest peak of fat content and the lowest overnight.
- **Diet:** Variation in lipid concentrations occurs independently of the diet. Nevertheless, FAs specifically are sensitive to maternal diet (89). Dietary fat has its highest effect on breast milk fat concentration approximately 10 hours after consumption (76). Especially dietary n3 and n6 FAs are transferred very rapidly to breast milk and changes in diet can be perceived in breast milk within 2-3 days. A high-carbohydrate and low-fat diet can increase the production of MCFAs (89). Fish and fish oil supplements are shown to alter DHA and n3 FAs in breast milk for 1-2 days (76).
- **Ethnicity:** Fat content is the most affected breast milk component by ethnicity.
- **Weight gain during pregnancy:** It is not clear, but there could be a correlation between maternal weight gain and breast milk fat content.
- **Birth weight:** It has been shown that breast milk fat content increases when birth weight is not normal (89).
- **Genes** encoding for key lipogenic regulatory factors (76).

2.3.5.4. BREAST MILK TRANSITION

Breast milk is classified into colostrum (1-5 days of lactation), transitional milk (6-15 days of lactation) and mature milk (16-35 days of lactation) and they all contain different levels of SFAs, MUFAs and PUFAs (84). Colostrum differs from mature milk by having lower concentration of fat (among others) and higher concentrations of immunoglobulin, which suggests that main role of colostrum is immunologic, not nutritional. Colostrum also promotes growth; it contains more growth factors than mature milk (89). As lactation continues, fat concentration increases and the ratio of PL:TG decreases, perhaps fat globules

increase in size. However, LC-PUFA composition decays as lactation continues and it could be related to the decline in DHA of maternal plasma (76).

2.3.6. MATERNAL COMPLICATIONS ALTERING FATTY ACIDS

2.3.6.1. MATERNAL OBESITY

An altered nutritional status, such as obesity, in the mother would compromise her FA status and hence the fetal/neonatal delivery through the placenta and breast milk. As explained in chapter 2.2.3, obesity promotes an inflammatory state that leads to insulin resistance and influences FA (also associated to inflammation) (91). Since pregnant women already have a particular lipid metabolism, an inflammatory state and develop insulin resistance, obesity in pregnant women would increase inflammation, insulin resistance (92) and alter their FA status (93, 94); and consequently the FA supply to the fetus and neonate through the placenta and breast milk would be compromised.

Some of the associations studied between FAs and obesity are listed below:

- FA composition in diet and plasma is related to features of the metabolic syndrome, such as obesity.
- Excessive dietary fat intake is associated to obesity risk (9).
- The sum of SFAs in plasma has been found to be positively associated to weight status (93).
- LC-PUFAs (93), DHA and n3 PUFAs (94) are negatively associated to weight status.
- Overweight participants have higher SFAs and lower n3 PUFAs dietary intakes (93).
- The consumption of n3 PUFAs contributes to weight loss; influences the FA composition, which modulates metabolic processes within the adipocyte; and influences the development of obesity related diseases (94).
- EPA+DHA supplementation has been associated to reduce insulin resistance.
- Dietary n3 PUFAs in overweight and obese women have been shown to reduce inflammation in maternal adipose and placental tissue (91).
- Dietary n3 PUFAs are associated to weight loss and maintenance by regulating appetite and satiety and reducing fat mass (20, 21).
- Obesity is associated to an increased dietary intake of n6 PUFAs (18).

These results suggest that obese pregnant women could have increased concentrations of total fat, SFA and decreased concentrations of LC-PUFAs, DHA and n3 PUFAs. Therefore, children from obese pregnant women could be affected since LC-PUFAs, especially DHA, play a key role in fetal/neonatal development (See chapter 2.12.3-4).

Additionally, overweight and obesity in women before and during pregnancy have many consequences for both the mother and the child. Some of them are presented in Table 4.

TABLE 4. SHORT AND LONG-TERM CONSEQUENCES OF MATERNAL OBESITY (8).

Consequences of Maternal Obesity	
Before pregnancy	- **Infertility:** Obese women intending to conceive, take longer to become pregnant than normal-weight women. Time to pregnancy increases with the degree of obesity.
During pregnancy	- **Pregnancy loss:** Risk of fetal death and still death is raised in obese women. The risk of miscarriage is estimated to be 30% higher in pregnant women with obesity. - **Congenital anomalies:** Maternal obesity seems to increase the risk of some fetal defects and congenital anomalies such as neural tube defects, hydrocephaly, orofacial and limb reduction anomalies and anorectal malformations. - **High birthweight and large for gestational age infants:** Obese women have doubled the risk of having a large for gestational age infant than normal-weight women. They also have increased risk of having newborns with macrosomia or high birthweight. On the other hand, it has been suggested that there could be intrauterine growth restriction and small for gestational age delivery, possibly because of the pre-eclampsia in obese women. - **Pre-eclampsia:** The risk for pre-eclampsia is increased 3-10 times by maternal obesity. - **Thromboembolism:** The risk increases up to 4-5 times in obese women compared to those who are normal-weight. - **Mental health:** Obese women have a higher risk of suffering depression symptoms during pregnancy. - **Labor and delivery:** Obese women are more likely to deliver by cesarean section, require induction of labor and experience post-partum hemorrhage. - **Premature birth:** It is associated with maternal obesity. - **Gestational diabetes mellitus:** The risk of presenting GDM increases along with maternal BMI and the risk could be up to 4-9 times greater. Its prevalence is increasing globally. - **Fatty acids:** Maternal availability (93, 94), placental transfer (secondary to GDM)(6), thereby affecting delivery to the fetus.
Post-partum	- **Breastfeeding:** Obesity has been associated to failure to breastfeed and to decrease breastfeeding duration. - **Breastmilk composition:** FA concentrations (95-97) and other nutrients. - **Mental health:** Obese women have a higher risk of suffering depression symptoms after pregnancy. - **Post-partum weight retention:** Pregnancy comes with a normal weight gain but many women find it hard to lose the excess after the baby is born. Around 13- 20% of pregnant women keep at least 5kg above their pre-pregnancy weight after 1 year postpartum (73). In the mother this increases risks in following pregnancies and contributes to new or persistent obesity.
Life span	- **Obese women and child:** New or persistent obesity in the mother. Children with obesity are more likely to stay obese all through lifespan. - **Obesity related diseases:** General population with overweight and obesity, including mother and child, have an increased risk of presenting obesity related conditions (e.g. hypertension, dyslipidemia, DM2, coronary heart disease, stroke, gallbladder disease, osteoarthritis, sleep apnea and other breathing problems, cancer, mental illness (e.g. depression, anxiety), low quality of life, body pain and difficulty physical functioning (71)). Obese children are also related to underachievement in school and lower self-esteem (98). - **Increased mortality rate:** Related to obesity related diseases.

2.3.6.2. GESTATIONAL DIABETES MELLITUS

GDM, the most common pregnancy complication, is a hyperglycemia that occurs during pregnancy and characterizes by glucose values above normal but below a diabetes diagnosis. This condition is normally detected through prenatal screening (99) around the 24th WOG (100).

Since obesity promotes insulin resistance and pregnancy already involves a physiological insulin resistance during the 3rd trimester, obesity in pregnant women will increase the risk for GDM. The risk for this condition is increasing (56% increase since 2000 (101)) because the prevalence of obesity among women of reproductive age is on the rise (6). Besides obesity, other risk factors are associated to developing GDM, such as a high parity, advanced maternal age, family history of DM, nonwhite race, overweight, etc. (102).

The maternal hyperglycemia causes an extra glucose supply to the fetus, which consequently promotes extra insulin secretion in the fetus. By this mechanism the fetus receives extra energy and increases fetal adiposity possibly leading to macrosomia (100). GDM is related to adverse effects on both the mother and the child; some of them are listed below:

- **Neonatal adiposity and macrosomia:** This increases the chance of needing a cesarean section or induced labor.
- **Polyhydramnios:** Excessive amniotic fluid which can cause premature labor or problems at delivery.
- **Premature birth:** Birth before the 37th WOG.
- **Pre-eclampsia:** High blood pressure during pregnancy which has complications for mother and child.
- **DM2:** GDM increases the risk of developing long term DM2 in the mother.
- **GDM in future pregnancies** (103).
- **Increased maternal FFAs**, especially in obese individuals.
- **Reduced FA placental transfer** (e.g. DHA) (6)
- **Decrease FA cord blood concentrations** (e.g. DHA) (104)
- **Behavioral and intellectual development of the offspring**
- **Adverse effects on attention and motor functions** (105)
- **Perinatal mortality**
- **Offspring consequences can extend through life span**
- **Increased mead acid (C20:3n-9):** It is produced in PUFA (e.g. DHA) deficiency and serves as a deficiency biomarker.
- **Language impairment (101)**

As mentioned in chapter 2.2.3, lipid metabolism is altered by insulin resistance, so pregnant women with GDM can develop dyslipidemia and they also are more likely to develop DM2 later in life. Some have observed normal or even higher circulating DHA levels in pregnant women with GDM, and yet the cord

blood of GDM neonates presents lower levels of DHA (and other LC-PUFAs). This suggests an altered transfer of theses FAs to the fetus (105). As also mentioned in chapter 2.3.4, an appropriate functioning of the placenta is determining for fetal FAs supply. GDM may modify the delivery of these nutrients to the fetus because the placental structure is altered (increased in size and thickness) in the presence of this condition. Placental size increases possibly because its tissue has insulin receptors that may lead to regulation of cell proliferation and growth, so increased concentrations of maternal insulin could intensify these activities. It has been observed that fetal sex might play a role in the placental functioning because female fetuses, not males, were associated to placental weight (6). The mechanism by which placental transfer is altered under GDM is still uncertain, but some pathways could be:

- Decreased placental DHA utilization as FFA
- Decreased fetal mobilization of DHA as a component of an esterified lipid
- Increased maternal β-oxidation promotes DHA usage as energy, and therefore lowers DHA concentration and placental uptake
- Alterations in FABPs and lipases by regulation of PPARs
- Epigenetic placental changes affecting placental endocytosis, DNA signaling pathways, metabolism of lipids, leptin, adiponectin and LPL (101).

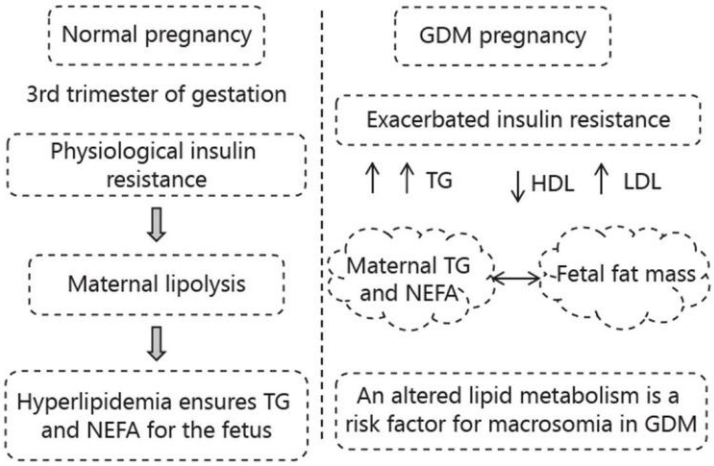


FIGURE 14. LIPID METABOLISM IN THE PRESENCE OF GDM (6).

3. HYPOTHESIS & AIMS

HYPOTHESIS & AIMS

Nutritional studies involving FAs and pregnant women are substantial since this period is characterized by alterations in lipid metabolism and induced synthesis of LC-PUFAs to secure fetal/neonatal supply. We hypothesized that a high maternal pre-pregnancy BMI could influence FA status of the mother and child, which would compromise growth and development of the latter.

The following aims were stablished to address our hypothesis:

- Determine if maternal *FADS* and *ELOVL* genetic variants are associated with pre-pregnancy BMI or affect PUFA levels in pregnant women.

- Analyze the differences in breast milk PUFA composition according to maternal pre-gestational BMI and *FADS* genotype.

- Evaluate cheek cells and capillary blood as less invasive alternatives to traditional plasma sampling for FA analysis and (2) to contribute to standardization of sample preparation independently of the matrix. In addition, an assessment was conducted of correlations between FA status in these two biological matrices, cheek cells and capillary blood, versus plasma (as the standard matrix).

- Determine the impact of maternal BMI and/or infant feeding practice in infant FA concentrations, and analyze if these FAs associate with cognitive performance.

4. METHODOLOGY

METHODOLOGY

4.1. ETHICS STATEMENT

The project complies with the Declaration of Helsinki. The protocol was approved by the medical ethics committees of the Clinical University Hospital San Cecilio and the Mother–Infant Hospital in the city of Granada, Spain. Written informed consent was obtained from all the participants at the beginning of the study.

4.2. STUDY DESIGN & PARTICIPANTS

Mother and child pairs were selected from the total participants in the observational PREOBE cohort study (study of maternal nutrition and genetics on fetal adiposity programming). Pregnant women were recruited at the Clinical University Hospital San Cecilio and the Mother–Infant Hospital in the city of Granada, Spain, where samples and information were also collected. Study design and information on PREOBE participants are exhibited.

Pregnant women were enrolled in the study between the 12th and 20th WOG. The inclusion criteria were singleton pregnancy and intention to deliver at the centers involved. Women were excluded if they were participating in other research studies, if they had been receiving drug treatment or supplements of either DHA or folate for more than the first 3 months of pregnancy, if they were suffering from disorders such as hypertension, pre-eclampsia, fetal intrauterine growth retardation, infections, hypo- or hyperthyroidism or hepatic renal diseases, or if they were following an extravagant or vegan diet.

Initially, 474 pregnant women were assessed for eligibility and 331 were finally included in the study. Mother and child pairs were divided into 4 groups according to maternal pre-pregnancy BMI and GDM status; 1) normal-weight (BMI=18.5-24.99), overweight (BMI=25-29.99), obese (BMI≥30) and gestational diabetic (Figure 15).

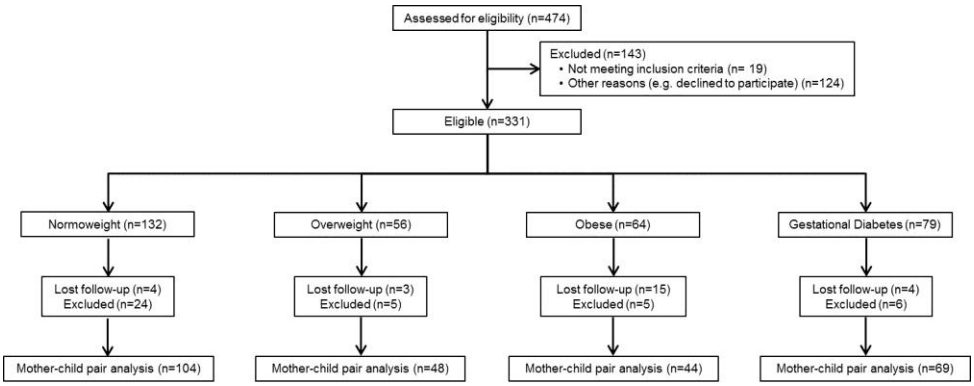


FIGURE 15. PARTICIPANTS IN THE PREOBE STUDY AND CLASSIFICATION FOLLOWING BMI AND GDM CRITERIA.

4.3. SAMPLE COLLECTION & ANALYSIS

All sample and data collection began at the 24th WOG and ended when the children were 3 years old. Using questionnaires and medical records, baseline and background characteristics were recorded such as maternal age, pre-pregnancy BMI, parity, smoking status, diet, alcohol drinking habits, socio-demographic information, educational level, and weight gain during pregnancy. Biological samples, such as plasma, breast milk, umbilical cord and cheek cells, were used for different analyses. The following diagram presents the timeline of sample and data collection throughout the study.

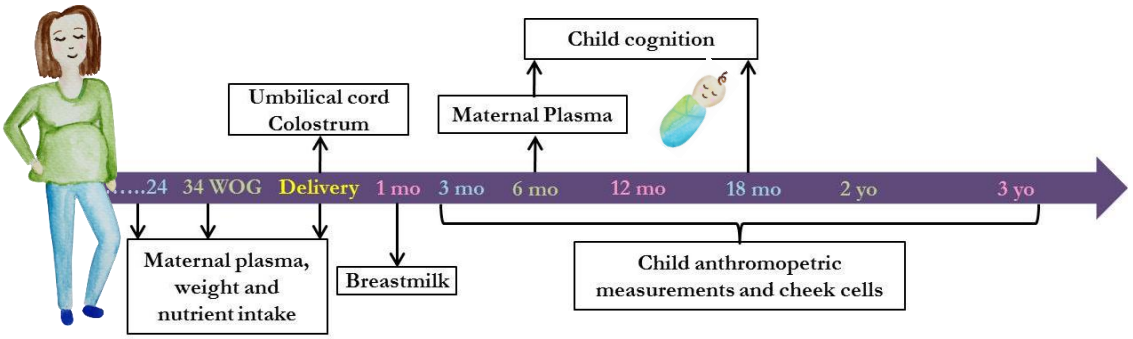


FIGURE 16. TIMING OF SAMPLE AND DATA COLLECTION. WOG: WEEKS OF GESTATION, MO: MONTHS OLD, YO: YEARS OLD.

4.4. STATISTICAL ANALYSIS

Statistical analysis was performed using the SPSS software (version 20.0; SPSS Inc., Chicago, IL, USA). The Kolmogorov–Smirnov test was used to study the normal distribution of data; non-normal data were natural-log transformed. The relative amount of each FA was quantified and expressed as the percentage of the total amount of FAs. FA percentages were expressed as mean ± standard deviation. Total FA levels were derived by adding the single FAs to n6 PUFAs, n3 PUFAs, n6 LC-PUFAs, n3 LC-PUFAs, n6:n3 PUFAs and n6:n3 LC-PUFAs. The confidence level was established at 95% for all the tests. Thus, results obtaining a P value of below 0.05 were considered statistically significant.

5. RESULTS

5.1. ASSOCIATION OF MATERNAL WEIGHT WITH *FADS* AND *ELOVL* GENETIC VARIANTS AND FATTY ACID LEVELS- THE PREOBE FOLLOW-UP

Maternal weight and *FADS* and *ELOVL* genetic variants on fatty acid levels

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Abstract

Single nucleotide polymorphisms (SNPs) in the genes encoding the fatty acid desaturase (*FADS*) and elongase (*ELOVL*) enzymes affect long-chain polyunsaturated fatty acid (LC-PUFA) production. We aimed to determine if these SNPs are associated with body mass index (BMI) or affect fatty acids (FAs) in pregnant women. Participants (n=180) from the PREOBE cohort were grouped according to pre-pregnancy BMI: normal-weight (BMI=18.5-24.9, n=88) and overweight/obese (BMI≥25, n=92). Plasma samples were analyzed at 24 weeks of gestation to measure FA levels in the phospholipid fraction. Selected SNPs were genotyped (7 in *FADS1*, 5 in *FADS2*, 3 in *ELOVL2* and 2 in *ELOVL5*). Minor allele carriers of rs174545, rs174546, rs174548 and rs174553 (*FADS1*), and rs1535 and rs174583 (*FADS2*) were nominally associated with an increased risk of having a BMI≥25. Only for the normal-weight group, minor allele carriers of rs174537, rs174545, rs174546, and rs174553 (*FADS1*) were negatively associated with AA:DGLA index. Normal-weight women who were minor allele carriers of *FADS* SNPs had lower levels of AA, AA:DGLA and AA:LA indexes, and higher levels of DGLA, compared to major homozygotes. Among minor allele carriers of *FADS2* and *ELOVL2* SNPs, overweight/obese women showed higher DHA:EPA index than the normal-weight group; however, they did not present higher DHA concentrations than the normal-weight women. In conclusion, minor allele carriers of *FADS* SNPs have an increased risk of obesity. Maternal weight changes the effect of genotype on FA levels. Only in the normal-weight group, minor allele carriers of *FADS* SNPs displayed reduced enzymatic activity and FA levels. This suggests that women with a BMI≥25 are less affected by *FADS* genetic variants in this regard. In the presence of *FADS2* and *ELOVL2* SNPs, overweight/obese women showed higher n-3 LC-PUFA production indexes than women with normal weight, but this was not enough to obtain a higher n-3 LC-PUFA concentration.

Introduction

During pregnancy, the mother is the sole source of key nutrients for the fetus, such as n-6 and n-3 long-chain (LC) polyunsaturated fatty acids (PUFAs) [1-3]. Studies have shown that arachidonic acid (AA; C20:4n−6) and particularly docosahexaenoic acid (DHA; C22:6n−3), play an important role in many physiological conditions [4], such as neural and visual development [1, 3, 5]. Hyperlipidemia develops in normal pregnancy; accordingly, it has been observed that levels of fatty acid (FA), such as DHA, increase in maternal plasma during pregnancy. Nevertheless, a higher activity of the enzymes encoded by the FADS and ELOVL genes could also be related to this hyperlipidemia [6]. Due to the beneficial effects of n-3 LC-PUFAs, it is recommended to increase their intake during pregnancy [4]. This is in contrast with the effect of excessive n-6 FA intake, which may lead to maternal obesity and obesity-related complications such as an increased risk of cardiovascular disease [7, 8]. Moreover, obese pregnancies lead to a higher risk of obesity in newborn babies, thereby increasing the likelihood of lifelong obesity and obesity-related complications [9, 10].

Apart from diet, LC-PUFAs can be obtained via endogenous synthesis from their essential n-6 and n-3 PUFA precursors: linoleic acid (LA) and α-linolenic acid (ALA), respectively [1] (S1 Fig). This process requires desaturation and elongation reactions, which are catalyzed by delta-5 and delta-6 fatty acid desaturases (encoded by FADS1 and FADS2 genes, respectively) and elongases (encoded by ELOVL gene (elongation of very long chain fatty acids)) [1, 11, 12]. The n-3 and n-6 series compete for these enzymes since they participate in both pathways. Single nucleotide polymorphisms (SNPs) in these genes may affect LC-PUFA production, and consequently alter FA levels [12]. For instance, minor allele carriers of the FADS1, FADS2 and ELOVL2 genes, have been linked to lower LC-PUFA production, and consequently they show increased concentrations of substrates and decreased levels of products in the LC-PUFA metabolic pathway [1, 12-15].

Certain SNPs in the FADS gene have been linked to diseases such as coronary artery disease [16] and type 2 diabetes [17]. Obesity is related to these conditions and is already known to affect lipid metabolism during pregnancy [18]; however, to the best of our knowledge, maternal obesity has not yet been directly linked to SNPs in the FADS and ELOVL genes.

Current studies analyzing the impact of *FADS* and *ELOVL* polymorphisms on FA levels are not properly comparable because their analyses include different tissue samples, FAs and SNPs. Given the impact of both obesity and SNPs on LC-PUFA production, the association between obesity and SNPs in *FADS* and *ELOVL* genes is of interest. These nutritional studies involving pregnant women are of great importance since the synthesis of LC-PUFA is induced in pregnancy and therefore the SNPs might have a different effect on pregnant women than on the general population. Thus, the aim of this study was to determine if *FADS* and *ELOVL* genetic variants are associated with body mass index (BMI) or affect PUFA levels in pregnant women.

Materials and Methods

Study design and participants

The study complies with the Declaration of Helsinki. The protocol was approved by the medical ethics committees of the Clinical University Hospital San Cecilio and the Mother–Infant Hospital in the city of Granada, Spain. Written informed consent was obtained from all the participants at the beginning of the study.

Pregnant women (n=180) were selected from the 331 individuals participating in the observational PREOBE cohort study (study of maternal nutrition and genetics on fetal adiposity programming) [19]. Participants were recruited at the Clinical University Hospital San Cecilio and the Mother–Infant Hospital in the city of Granada, Spain, where samples and information were also collected. Study design and information on PREOBE participants are exhibited in Fig 1.

The inclusion criteria were singleton pregnancy, gestation between 12 and 20 weeks at enrollment, and intention to deliver at the centers involved. Women were excluded if they were participating in other research studies, if they had been receiving drug treatment or supplements of either DHA or folate for more than the first 3 months of pregnancy, if they were suffering from disorders such as hypertension, pre-eclampsia, fetal intrauterine growth retardation, infections, hypo- or hyperthyroidism or hepatic renal diseases, or if they were following an extravagant or vegan diet. Using questionnaires and medical records, baseline and background characteristics were recorded such as maternal age, pre-pregnancy BMI, parity, smoking status, diet, alcohol drinking habits, socio-demographic information, educational level, and weight gain during pregnancy.

For this study, 180 pregnant women were divided into two groups according to their pre-pregnancy BMI, normal-weight (BMI=18.5-24.9, n=88) and overweight/obese (BMI$\geq$25, n=92). Plasma samples were collected at 24 weeks of gestation for FA analysis. Seventeen SNPs (7 in the *FADS1* gene, 5 in *FADS2*, 3 in *ELOVL2* and 2 in *ELOVL5*) out of the 32 initially selected were successfully genotyped and included in the analysis (S2 Table).

DNA analysis

Maternal material for DNA analysis was collected by scraping the inside of the cheek of the pregnant participants with a buccal swab. Thirty-two SNPs in genes involved in lipid metabolism (*FADS1*, *FADS2*, *FADS3*, *ELOVL2*, *ELOVL5*, *ELOVL6* and *FASN*) were initially genotyped from 5 μl of maternal DNA mixed with 5 μl of 2X TaqMan® OpenArray® Genotyping Master Mix. The analysis was then performed with 3 μl of the mixture in a microplate using Taqman® Open Array® genotyping technology. The OpenArray® instrument (which consists of a Dual Flat Block GeneAmp® PCR System 9700, Bio-Rad® thermal cycler with a Slide Chambers Dual-Block Alpha unit and Thermo Electron PX2 thermal cycler) and the corresponding OpenArray® SNP Genotyping Analysis software, located at the *Autonomous University of Barcelona* (UAB), were used for the analysis. The genotyping required two phases: a thermal

cycle (PCR amplification) and detection of the final fluorescence signal. The reagents used were supplied by Applied Biosystem (Foster City, CA, USA).

Fatty acid analysis

At 24 weeks of gestation, blood was obtained by arm venipuncture. Plasma was separated by centrifugation and immediately frozen and stored at -80°C until analysis. FAs were determined in the phospholipid fraction using the method developed by Chisaguano et al. [20]. Plasma lipids were extracted using 2:1 dichloromethane:methanol and phospholipids were isolated using solid-phase extraction (SPE). FA methyl esters from plasma phospholipids were analyzed using fast gas chromatography with a flame ionization detector. The results were expressed as percentages of the total FAs detected.

We analyzed the FAs involved in enzymatic reactions encoded by the FADS and ELOVL genes (substrates, products or indexes (product/substrates)). Furthermore, we studied the n3:n6 ratios (eicosapentaenoic acid (EPA):AA and DHA:AA), associated with obesity risk [7, 21-23].

Statistical analysis

Statistical analysis was performed using the SPSS software (version 20.0; SPSS Inc., Chicago, IL, USA). The Kolmogorov–Smirnov test was used to study the normal distribution of data; non-normal data were natural-log transformed. The agreement of genotype frequencies with Hardy-Weinberg equilibrium expectations was tested by the chi-square test. Due to the limited sample size, heterozygotes and homozygous for minor alleles, were analyzed as one group. SNPs were coded according to minor allele count (0 for major homozygotes and 1 for the carriers of at least one minor allele) and analyzed as a numerical variable. However, this codification implies an additive and dominant model. The associations between SNPs and FAs were analyzed using linear regression; while the associations between SNPs and obesity were analyzed by logistic regression. All associations were corrected for potential confounders such as age, education, smoking status and energy intake. FA levels were compared using univariate ANOVA. Since DHA supplementation was largely absent and it did not affect DHA levels in plasma, supplementation data were omitted for parsimony. The Bonferroni correction was applied to take multiple testing into account and p-value thresholds were set at 0.004, which was applied within each trait.

Results

Population characteristics

The characteristics of the groups are shown in Table 1. Normal-weight women were more likely to have a higher level of education and take DHA supplementation; while the overweight/obese group had a lower energy intake and weight gain during pregnancy. No differences were observed in dietary FA levels. S1 Table shows a comparison of plasma DHA concentrations at 24 weeks of gestation between pregnant women who took DHA supplementation and those who did not. After finding that the participants who reported taking DHA supplement (n= 17) did not have higher serum DHA concentrations (p= 0.636)

than the participants who did not take supplements (n= 93), we decided to include all the subjects in the analysis regardless of supplement use.

S2 Table presents the characteristics of the studied SNPs, including the distribution of participants in each allele group (major homozygotes, heterozygotes and minor homozygotes). Normal-weight women tended to be major homozygotes for all the *FADS1* and *FADS2* SNPs studied, while women in the overweight/obese group were mostly heterozygotes. Regarding *ELOVL2* SNPs, both groups presented mostly heterozygous alleles, and no pattern was observed for *ELOVL5* SNPs.

Association between SNPs and fatty acids

Table 2 shows nominal and significant associations between PUFA levels and *FADS* and *ELOVL* SNPs after adjusting for age, education, smoking status and energy intake (the complete analysis can be found in S3 Table). The most significant associations ($p \leq 0.004$) were only found in the normal-weight group, where minor allele carriers of rs174537, rs174545, rs174546 and rs174553 (*FADS1*) were negatively associated with the AA:dihomo-gamma-linolenic acid (DGLA) index.

Association of *FADS* SNPs with obesity risk

After adjusting for age, education, smoking status and energy intake, participants who were minor allele carriers of rs174545, rs174546, rs174548 and rs174553 (*FADS1*), and rs1535 and rs174583 (*FADS2*) were nominally associated ($p<0.05$) with an increased risk of having a BMI$\geq$25, compared with two major allele carriers (Table 3).

Fatty acid levels according to genotype and weight

Plasma LC-PUFA levels according to genotypes are presented in Tables 4 and 5 (the complete analysis can be found in S4 Table). Significant differences were found for normal-weight women: minor allele carriers of *FADS1* SNPs showed lower AA level and both AA:DGLA and AA:LA indexes than major homozygotes; while minor allele carriers of *FADS2* SNPs showed lower AA and higher DGLA levels ($p \leq 0.004$). Overweight/obese women presented the same tendencies, but without the results reaching statistical significance. No differences were found between genotypes of *ELOVL* SNPs.

Significant differences were also shown when comparing groups of weight ($p \leq 0.004$). Among the minor allele carriers of rs1535, rs174583 and rs99780 (*FADS2*), and rs2236212, rs3798713 and rs953413 (*ELOVL2*), overweight/obese women showed a higher DHA:EPA index than those in the normal-weight group (Table 4). Meanwhile, among minor alleles carriers of rs174537, rs174545, rs174546, rs174553 and rs174547 (*FADS1*), and rs1535, rs174575, rs174583 and rs99780 (*FADS2*), normal-weight women had higher levels of the substrate ALA than those in the overweight/obese group. In addition, among the major homozygotes of rs2397142 (*ELOVL5*), normal-weight women presented higher levels of EPA than women who were overweight/obese (Table 5).

Maternal plasma n3:n6 ratios, according to genotype, are presented in S5 Table. Among the minor allele carriers of rs3798713 (*ELOVL2*), normal-weight women had significantly higher EPA:AA ratio than those who were overweight/obese. This trend was also found in all gene clusters studied, even when comparing major homozygotes.

Discussion

The present study analyzed the effect of FADS and ELOVL genetic variants on a broad FA profile. To the best of our knowledge, this is the first study to explore associations between FADS and ELOVL SNPs, FA levels and maternal pre-pregnancy weight. Despite a lack of studies analyzing the association between obesity risk and FADS or ELOVL polymorphisms, some authors have observed that minor alleles of rs174547 (FADS1) confer a higher risk of obesity-related conditions, such as increased triglyceride levels and decreased high-density lipoprotein cholesterol concentrations [24-26], as well as an increased risk of coronary disease [26, 27]. In the present study, we found that women who carried at least one minor allele of the FADS1 and FADS2 SNPs, were associated with a higher risk of having a BMI>25 than homozygotes for the major allele. This association could explain why most of the overweight/obese women carried one minor allele copy and normal-weight women were mostly homozygous for the major alleles. Both weight groups showed very similar distributions within the allele groups of ELOVL SNPs; thus, we did not expect to find any associations between ELOVL genotypes and weight.

In line with other studies [5, 12], we found that the FADS1 and FADS2 SNPs were associated with FAs, mainly from the n-6 series, and less with those from the n-3 series. The only significant association (p-value <0.004) was found in the normal-weight group, where minor alleles of FADS1 were associated with a lower AA:DGLA index. Several nominal associations were also found; nevertheless, they were mainly in normal-weight women and the n-6 series. Regarding the overweight/obese group, minor allele carriers of rs2236212 and rs3798713 (ELOVL2) were nominally associated with a lower DHA:n-3 docosapentaenoic acid (DPAn3) index. Barman et al. observed results similar to this last association, but their significance did not persist after correction either [12].

We also observed that FADS genetic variants affected FA concentrations. Normal-weight women who were minor allele carriers of FADS1 SNPs had significantly lower levels of product (AA) and indexes (AA:LA and AA:DGLA) than major homozygotes. Consistently with this, nominal differences were also observed; minor allele carriers of FADS1 SNPs showed nominally lower substrates (DGLA and ALA). This was previously reported by other authors [1, 5, 12, 13, 28, 29], who observed that minor allele carriers in FADS displayed lower FA indexes and products, and increased amounts of substrates. Overweight/obese women with FADS1 SNPs showed the same trends and some nominal differences in DGLA and AA:DGLA, but none of them with a p-value <0.004. Other studies [1, 28, 29] have also found that FADS2 SNPs were related to lower levels of AA and lower AA:LA index. This supports our findings for the normal-weight group, where minor allele carriers of FADS2 SNPs had significantly lower levels of AA and higher DGLA. We also found nominal differences consistent with the previous results (lower AA:LA index and higher ALA and DPAn3). Similarly to other studies [30], the DGLA:LA index was nominally higher in minor allele carriers. This could be because the DGLA:LA index precedes the AA:LA index and, therefore, a lower AA:LA index would cause an accumulation of DGLA, thereby

"increasing" the DGLA:LA index. Overweight/obese women who were minor allele carriers of FADS2 SNPs only showed nominally higher levels of DGLA than major homozygotes.

As shown previously, the FADS1 and FADS2 genetic variants were found to affect mainly the n-6 FAs, even though desaturases and elongases work on both the n-6 and n-3 series. Several studies have observed that the DHA status, or that of the n-3 series, is less influenced by genetic variants in the FADS genes [1, 5, 12, 13, 28, 31]. One possible explanation for this is that the final conversion step from DPA to DHA requires translocation to the peroxisomes (which is not performed in the endoplasmic reticulum where the other reactions occur) [1], making DHA the least efficiently synthesized n-3 LC-PUFA. Therefore, the influence of SNPs might not ultimately affect DHA levels [1, 32]. It has also been postulated that DHA supplementation during pregnancy could reduce dependence on endogenous DHA synthesis [1]. However, previous studies have reported that increased dietary intake of EPA and DHA is linked to higher FADS1 and lower FADS2 activities, suggesting that endogenous LC-PUFA production remains active and is possibly enhanced, despite additional dietary intake of LC-PUFAs [1, 33]. Moreover, in our study, we observed no differences in dietary intake of EPA, DHA or AA between the two BMI groups; and although normal-weight pregnant women were more likely to take DHA supplementation, it was very unusual and this supplementation did not affect DHA level in plasma.

Our results suggest that BMI modifies genotype responses. According to FADS SNPs, n-6 FAs showed the same effects in the 2 groups studied, but significant effects were only found in the group of normal-weight women. This suggests that overweight/obese women are less affected. This could explain why women in the normal-weight group had significantly higher n-3 substrate (ALA) levels and nominally lower n-6 product (AA) levels than overweight/obese women, only when we compared minor allele carriers of SNPs in FADS1 and FADS2. Additionally, among the minor allele carriers of FADS2, overweight/obese women showed significantly higher DHA:EPA index than the normal-weight group (the DHA:DPAn3 index showed the same trend). This suggests that minor alleles of FADS2 SNPS in overweight/obese women could have a positive impact on n-3 LC-PUFA production. Furthermore, among major homozygotes, overweight/obese women also showed nominally higher DHA:EPA index than those in the normal-weight group. Perhaps, regardless of genetic variants in the FADS gene, a high BMI could be linked to increased activity of enzymes involved in n-3 FA synthesis. Nevertheless, there were no differences in amounts of DHA between the weight groups, suggesting that even if enzymatic activity in n-3 FA production is increased in overweight/obese individuals, it is not enough to elicit greater n-3 LC-PUFA levels than in normal-weight subjects.

We analyzed 3 ELOVL2 SNPs (rs2236212, rs3798713 and rs953413) and found that their genetic variants only affected n-3 FA levels in overweight/obese women. Overweight/obese women who were minor allele carriers of rs2236212 [12] and rs3798713 had nominally lower DHA:DPAn3 index compared with major homozygotes. This led to nominally higher amounts of the substrate DPAn3. Lemaitre et al. found decreased DHA levels in the presence of at least one minor allele of rs2236212 [15], which is consistent with the tendency observed in our results. The normal-weight group generally showed the same trends. Moreover, among minor allele carriers of ELOVL2 SNPs, overweight/obese women showed significantly higher DHA:EPA index than normal-weight women (DPA:EPA and DHA:DPA indexes showed the same tendency). This is in accordance with FADS2 results. Therefore, minor alleles of FADS2 and

ELOVL2 SNPs in overweight/obese women, could increase n-3 LC-PUFA production indexes, but without surpassing levels in normal-weight women.

Estrogen facilitates LC-PUFA synthesis [34, 35], probably by regulating delta-6 desaturase. During pregnancy, estrogen levels are higher, leading to increased amounts of DHA and AA until delivery, when the release of prolactin inhibits estrogen activity [35]. Since estrogen is produced in adipocytes, obesity is linked to high estrogen levels which increase proportionally to total body adiposity [36]. This could be an alternative explanation of the increase in n-3 LC-PUFA production observed in overweight/obese individuals. In this case, more studies introducing measurements of estrogen in both populations are needed.

We also studied n3:n6 ratios (EPA:AA and DHA:AA), which are associated with obesity risk. Among minor allele carriers of the *FADS* and *ELOVL* genes, normal-weight women showed nominally higher plasma ratios of EPA:AA and DHA:AA than women who were overweight/obese. The same trend was observed when comparing major homozygotes, but with a weaker (or no) association. This suggests that a high BMI leads to increased levels of AA and/or lower levels of DHA and EPA, more importantly in women who carry at least one copy of the minor allele of *FADS* and *ELOVL* SNPs.

To the best of our knowledge, this is the first report to directly associate *FADS* SNPs with obesity risk and to analyze how weight affects the impact of variations in genes involved in FA metabolism. Our results further justify the need for personalized nutrition by showing that metabolism is affected by nutritional status and genes. Our present study had some limitations: it might be limited by the relatively small sample size; however, we could identify and group participants into different weight and genotype categories. Information on dietary intake was obtained from validated food records and questionnaires and although each participant was guided by a nutritionist, this information could have been affected by recall bias. Likewise, supplementation data (brand, content, doses and frequency) during pregnancy were also obtained from questionnaires answered by the participants; therefore, this information might not be completely accurate.

Conclusions

In conclusion, minor allele carriers of *FADS1* and *FADS2* SNPs have an increased risk of obesity ($p \leq 0.05$). The effects of genotype on FA concentrations differed by maternal pre-pregnancy weight status. Enzymatic activity and FA levels were reduced in normal-weight women who were minor allele carriers of *FADS* SNPs; these reductions were not significant in overweight/obese participants. This suggests that women with a BMI$\geq$25 are less affected by *FADS* genetic variants in this regard. In the presence of *FADS2* and *ELOVL2* SNPs, overweight/obese women showed higher n-3 LC-PUFA production indexes than those women in the normal-weight group, but this was not enough to obtain a higher n-3 LC-PUFA concentration ($p \leq 0.004$). Since genotypes may not have the same effects on all people, it is of interest to continue exploring gene-BMI interactions to pursue personalized health-related recommendations. Alterations in maternal FAs modify the risk of pro-inflammatory diseases and affect FA delivery to the fetus/neonate, which has an impact on child growth and development. Therefore, this study also supports the importance of a healthy pre-pregnancy weight, and identifies groups of women

who could benefit from a high intake of n-3 FAs in order to achieve an improved FA status that fulfills fetal/neonatal requirements.

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Tables and Figures

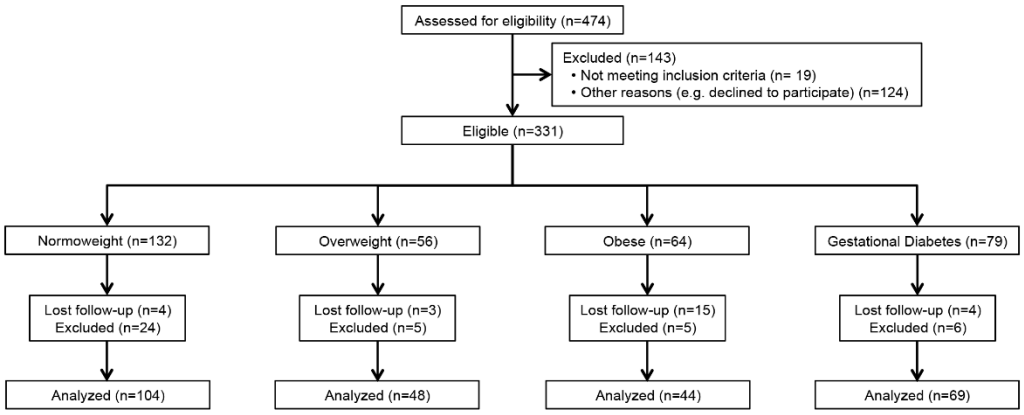


Fig 1. Participants in the PREOBE study and classification following BMI and gestational diabetes criteria.

Table 1. Characteristics of the population.

	NORMAL-WEIGHT (n= 88)	OVERWEIGHT/OBESE (n= 92)	P
	Mean (SD)	Mean (SD)	
Age (years)	30.91 (4.09)	30.64 (4.20)	0.665
Pre-pregnancy BMI (kg/m2)	22.00 (1.64)	30.33 (4.20)	**<0.001***
Weight Gain (kg)	12.64 (5.47)	9.19 (6.29)	**0.001***
Education (%)			
<Highschool	10.23%	15.38%	0.401
Highschool	15.91%	19.78%	
>Highschool	73.86%	64.84%	
Smoking during pregnancy (%)	21.92%	27.27%	0.447
DHA supplementation during pregnancy (%)	25.00%	7.61%	**0.002***
Dietary intakes			
Energy intake (kcal/d)	2177.54 (335.41)	1938.07 (591.28)	**0.017**
EPA intake (g/d)	0.13 (0.13)	0.12 (0.10)	0.713
DHA intake (g/d)	0.25 (0.20)	0.27 (0.18)	0.646
AA intakes (g/d)	0.13 (0.07)	0.13 (0.06)	0.980

P-value derived from global ANOVA and significance level ($p \leq 0.004$) was adjusted for multiple testing by Bonferroni correction. P-values <0.05 are highlighted in bold and significant associations that persisted after Bonferroni correction are additionally denoted by stars (*$p < 0.004$). BMI: body mass index; AA: arachidonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

Table 2. Associations between plasma proportions of PUFAs and *FADS* and *ELOVL* polymorphisms.

Fatty acid	Gene	SNP	*Major/minor allele*	NORMAL-WEIGHT			OVERWEIGHT/OBESE		
				N	β	P	N	β	P
***FADS1* indexes**									
AA:DGLA									
	FADS1	rs174537	*G/T*	22	-0.81	**0.004***	18	-0.48	0.155
	FADS1	rs174545	*C/G*	28	-0.70	**0.003***	17	-0.48	0.174
	FADS1	rs174546	*C/T*	28	-0.70	**0.003***	19	-0.49	0.139
	FADS1	rs174553	*A/G*	28	-0.70	**0.003***	19	-0.49	0.139
	FADS1	rs174547	*T/C*	23	-0.67	**0.013**	18	-0.48	0.155
***FADS2* indexes**									
DGLA:LA									
	FADS2	rs1535	*A/G*	26	0.56	**0.029**	18	0.42	0.226
	FADS2	rs174583	*C/T*	27	0.53	**0.040**	19	0.41	0.226
	FADS2	rs99780	*C/T*	25	0.54	**0.042**	18	0.42	0.226
***ELOVL2* indexes**									
DHA:DPAn3									
	ELOVL2	rs2236212	*G/C*	25	-0.05	0.806	19	-0.58	**0.016**
	ELOVL2	rs3798713	*G/C*	25	0.00	0.995	19	-0.58	**0.016**
Fatty acids involved in *FADS1* indexes									
C20:3n6 (DGLA)									
	FADS1	rs174537	*G/T*	22	0.75	**0.012**	18	0.53	0.119
	FADS1	rs174545	*C/G*	28	0.57	**0.028**	17	0.52	0.137
	FADS1	rs174546	*C/T*	28	0.57	**0.028**	19	0.52	0.118
	FADS1	rs174553	*A/G*	28	0.57	**0.028**	19	0.52	0.118
	FADS1	rs174547	*T/C*	23	0.60	**0.034**	18	0.53	0.119
C20:4n6 (AA)									
	FADS1	rs174545	*C/G*	28	-0.55	**0.042**	17	-0.01	0.977
	FADS1	rs174546	*C/T*	28	-0.55	**0.042**	19	-0.01	0.971
	FADS1	rs174553	*A/G*	28	-0.55	**0.042**	19	-0.01	0.971
C18:3n3 (ALA)									
	FADS1	rs174537	*G/T*	22	0.71	**0.027**	18	-0.09	0.790
	FADS1	rs174547	*T/C*	23	0.66	**0.026**	18	-0.09	0.790
Fatty acids involved in *FADS2* indexes									
C18:3n6 (GLA)									
	FADS2	rs174575	*C/G*	22	0.12	0.672	19	0.64	**0.031**
C20:3n6 (DGLA)									
	FADS2	rs1535	*A/G*	26	0.65	**0.010**	18	0.53	0.119
	FADS2	rs174583	*C/G*	27	0.62	**0.015**	19	0.52	0.118
C18:3n3 (ALA)									
	FADS2	rs174575	*C/G*	23	0.50	**0.043**	19	0.22	0.494
C20:5n3 (EPA)									
	FADS2	rs99780	*C/T*	25	0.52	**0.035**	17	0.11	0.749
C22:5n3 (DPAn3)									
	FADS2	rs99780	*C/T*	25	0.62	**0.011**	18	-0.41	0.173

Associations between SNPs and fatty acids were analyzed using linear regression. SNPs were coded according to minor allele count and analyzed as a numeric variable. "β"= beta per minor allele standardized per the major allele. All associations were adjusted for potential confounders such as age, education, smoking and energy intake. P-values <0.05 are highlighted in bold and significant associations that persisted after Bonferroni correction are additionally denoted by stars (*p≤0.004). LA: Linoleic Acid; GLA: γ-Linolenic Acid; DGLA: Dihomo-γ-Linolenic Acid; AA: Arachidonic Acid; ALA: α-linolenic Acid; EPA: Eicosapentaenoic acid; DPAn3: Docosapentaenoic acid n3; DHA: Docosahexaenoic acid.

Table 3. Associations between FADS and ELOVL genes and maternal obesity.

Gene	SNP *Major/Minor alleles*		N	BMI≥25		BMI≥25c	
				OR	P	ORc	Pc
FADS1							
	rs174537	*G/T*	66	2.12	0.147	2.89	0.069
	rs174545	*C/G*	79	2.34	0.074	3.11	**0.032**
	rs174546	*C/T*	81	2.55	**0.047**	3.28	**0.025**
	rs174548	*C/G*	82	3.11	**0.015**	3.18	**0.021**
	rs174553	*A/G*	82	2.64	**0.039**	3.44	**0.019**
	rs174561	*T/C*	44	0.56	0.351	0.43	0.217
	rs174547	*T/C*	66	1.96	0.191	2.75	0.082
FADS2							
	rs1535	*A/G*	77	2.58	**0.048**	3.42	**0.025**
	rs174575	*C/G*	70	1.57	0.351	1.77	0.273
	rs174583	*C/T*	79	2.62	**0.046**	3.38	**0.024**
	rs99780	*C/T*	71	1.75	0.261	2.37	0.122
	rs174602	*T/C*	46	1.55	0.475	1.18	0.812
ELOVL2							
	rs2236212	*G/C*	76	0.76	0.568	0.88	0.805
	rs3798713	*G/C*	75	0.67	0.430	0.81	0.708
	rs953413	*A/G*	58	1.00	1.000	0.58	0.425
ELOVL5							
	rs2397142	*C/G*	79	0.78	0.573	0.75	0.556
	rs9395855	*T/G*	54	0.50	0.316	0.50	0.345

The association between SNPs and obesity risk was analyzed with logistic regression. SNPs were coded according to minor allele count and analyzed as numeric variable. BMI= Body Mass Index. OR= odds ratio per minor allele with the major allele as reference. ORc and Pc= are corrected values after adjustment for age, education, smoking and energy intake. Nominal associations are highlighted in bold ($p<0.05$).

Table 4. FADS1, FADS2 and ELOVL2 enzymatic indexes according to maternal SNPs and LC-PUFA levels in plasma.

Gene	SNP *Major/minor allele*		NORMAL-WEIGHT					OVERWEIGHT/OBESITY					P (*MM*)	P (*Mm+mm*)
			MM		*Mm+mm*		P	*MM*		*Mm+mm*		P		
			N	Mean (SD)	N	Mean (SD)		N	Mean (SD)	N	Mean (SD)			
***FADS1* indexes**														
AA:LA														
FADS1	rs174537	*G/T*	15	0.45 (0.08)	23	0.39 (0.09)	**0.038**	12	0.46 (0.07)	21	0.43 (0.08)	0.390	0.811	0.085
FADS1	rs174545	*C/G*	22	0.46 (0.09)	27	0.38 (0.08)	**0.003***	13	0.47 (0.07)	22	0.42 (0.08)	0.071	0.877	0.171
FADS1	rs174546	*C/T*	22	0.46 (0.09)	27	0.38 (0.08)	**0.003***	13	0.47 (0.07)	25	0.42 (0.08)	0.098	0.877	0.109
FADS1	rs174548	*C/G*	24	0.45 (0.08)	25	0.39 (0.09)	**0.031**	13	0.45 (0.08)	25	0.43 (0.08)	0.553	0.969	0.115
FADS1	rs174553	*A/G*	22	0.46 (0.09)	27	0.38 (0.08)	**0.003***	13	0.47 (0.07)	25	0.42 (0.08)	0.098	0.877	0.109
FADS1	rs174547	*T/C*	15	0.45 (0.08)	23	0.39 (0.09)	**0.040**	12	0.46 (0.07)	20	0.43 (0.08)	0.427	0.950	0.124
AA:DGLA														
FADS1	rs174537	*G/T*	15	3.14 (0.70)	23	2.20 (0.60)	**<0.001***	12	3.57 (1.73)	21	2.55 (0.80)	**0.032**	0.414	0.107
FADS1	rs174545	*C/G*	22	3.12 (0.65)	27	2.25 (0.57)	**<0.001***	13	3.48 (1.67)	22	2.51 (0.78)	**0.025**	0.362	0.183
FADS1	rs174546	*C/T*	22	3.12 (0.65)	27	2.25 (0.57)	**<0.001***	13	3.48 (1.67)	25	2.54 (0.74)	**0.021**	0.362	0.120
FADS1	rs174548	*C/G*	24	2.97 (0.65)	25	2.33 (0.70)	**0.002***	13	3.42 (1.69)	25	2.57 (0.75)	**0.037**	0.244	0.246
FADS1	rs174553	*A/G*	22	3.12 (0.65)	27	2.25 (0.57)	**<0.001***	13	3.48 (1.67)	25	2.54 (0.74)	**0.021**	0.362	0.120
FADS1	rs174547	*T/C*	15	3.20 (0.67)	23	2.27 (0.55)	**<0.001***	12	3.54 (1.73)	20	2.56 (0.82)	**0.037**	0.291	0.194
***FADS2* indexes**														
DGLA:LA														
FADS2	rs1535	*A/G*	20	0.15 (0.04)	25	0.18 (0.05)	**0.011**	13	0.15 (0.04)	23	0.18 (0.05)	0.115	0.877	0.798
FADS2	rs174575	*C/G*	24	0.15 (0.04)	16	0.19 (0.06)	**0.017**	21	0.17 (0.06)	14	0.17 (0.05)	0.943	0.276	0.315
FADS2	rs174583	*C/T*	19	0.15 (0.04)	27	0.18 (0.05)	**0.036**	13	0.15 (0.04)	25	0.18 (0.05)	0.130	0.967	0.907
FADS2	rs99780	*C/T*	15	0.15 (0.04)	26	0.18 (0.05)	**0.037**	12	0.15 (0.05)	22	0.18 (0.05)	0.076	0.953	0.961
AA:LA														
FADS2	rs1535	*A/G*	20	0.45 (0.08)	25	0.39 (0.09)	**0.025**	13	0.47 (0.07)	23	0.42 (0.08)	0.128	0.504	0.170
FADS2	rs174583	*C/T*	19	0.44 (0.08)	27	0.38 (0.08)	**0.019**	13	0.47 (0.07)	25	0.42 (0.08)	0.098	0.447	0.109
DHA:EPA														
FADS2	rs1535	*A/G*	20	15.02 (5.64)	25	14.66 (5.92)	0.840	13	19.69 (6.63)	23	20.89 (7.87)	0.655	**0.045**	**0.004†**
FADS2	rs174575	*C/G*	24	16.41 (5.99)	16	13.41 (5.21)	0.119	21	20.99 (6.36)	14	20.27 (8.32)	0.778	**0.018**	**0.012**
FADS2	rs174583	*C/T*	19	15.02 (5.64)	27	14.48 (5.71)	0.753	13	19.69 (6.63)	25	20.58 (7.89)	0.737	**0.045**	**0.003†**
FADS2	rs99780	*C/T*	15	16.22 (5.52)	26	13.71 (4.76)	0.138	12	20.50 (6.29)	22	20.19 (8.14)	0.911	0.078	**0.002†**
FADS2	rs174602	*T/C*	19	16.06 (5.17)	13	14.76 (6.12)	0.531	23	19.97 (6.82)	14	18.02 (6.37)	0.393	**0.046**	0.199
DHA:DPAn3														
FADS2	rs1535	*A/G*	20	10.84 (1.96)	25	10.03 (2.40)	0.232	13	11.14 (2.26)	23	11.58 (2.26)	0.577	0.687	**0.027**
FADS2	rs174575	*C/G*	24	10.58 (2.21)	16	10.15 (2.39)	0.574	21	11.23 (2.29)	14	12.20 (2.28)	0.230	0.334	**0.026**
FADS2	rs174583	*C/T*	19	10.76 (1.98)	27	10.13 (2.33)	0.350	13	11.14 (2.26)	25	11.53 (2.50)	0.642	0.613	**0.044**
FADS2	rs99780	*C/T*	15	11.04 (2.07)	26	9.83 (2.17)	0.090	12	11.42 (2.11)	22	11.41 (2.45)	0.990	0.640	**0.024**
***ELOVL2* indexes**														
DPAn3:EPA														
ELOVL2	rs2236212	*G/C*	14	1.51 (0.51)	29	1.48 (0.67)	0.876	13	1.66 (0.71)	22	1.88 (0.71)	0.388	0.530	**0.047**
ELOVL2	rs3798713	*G/C*	11	1.48 (0.52)	31	1.44 (0.67)	0.863	12	1.67 (0.74)	25	1.88 (0.70)	0.410	0.476	**0.021**
DHA:EPA														
ELOVL2	rs2236212	*G/C*	14	16.39 (6.29)	28	14.45 (5.43)	0.307	13	20.58 (8.33)	22	20.05 (7.02)	0.841	0.151	**0.003†**
ELOVL2	rs3798713	*G/C*	11	15.48 (5.10)	30	13.96 (5.33)	0.418	12	20.76 (8.67)	24	20.13 (7.07)	0.819	0.094	**<0.001†**
ELOVL2	rs953413	*A/G*	10	15.33 (5.93)	25	14.78 (5.23)	0.789	7	21.14 (7.60)	20	20.88 (7.57)	0.939	0.096	**0.003†**
DHA:DPAn3														
ELOVL2	rs2236212	*G/C*	14	10.87 (2.20)	29	10.14 (2.32)	0.337	13	12.57 (1.53)	22	10.77 (2.67)	**0.032**	**0.029**	0.375
ELOVL2	rs3798713	*G/C*	11	10.68 (2.24)	31	10.10 (2.20)	0.460	12	12.64 (1.58)	25	10.78 (2.55)	**0.028**	**0.024**	0.291
ELOVL2	rs953413	*A/G*	10	9.83 (2.22)	26	10.38 (1.99)	0.478	7	10.68 (2.80)	20	12.04 (2.09)	0.183	0.493	**0.008**

P-value derived from global ANOVA and significance level ($p \leq 0.004$) was adjusted for multiple testing by Bonferroni correction. Data are means of FAs expressed as percentages of the total phospholipid profile (standard error). P-values <0.05 are highlighted in bold and significant associations that persisted after Bonferroni correction are additionally denoted by stars or daggers ($p \leq 0.004$). *Indicates significant differences within each group of weight and † Indicates significant differences between groups of weight. Major allele: M; minor allele: m; LA: Linoleic Acid; GLA: γ-Linolenic Acid; DGLA: Dihomo-γ-Linolenic Acid; AA: Arachidonic Acid; AdA: Adrenic Acid; DPAn6: Docosapentaenoic acid n6; ALA: α-linolenic Acid; EPA: Eicosapentaenoic acid; DPAn3: Docosapentaenoic acid n3; DHA: Docosahexaenoic acid.

Table 5. Substrates and products of enzymatic indexes according to maternal SNPs and LC-PUFA levels in plasma.

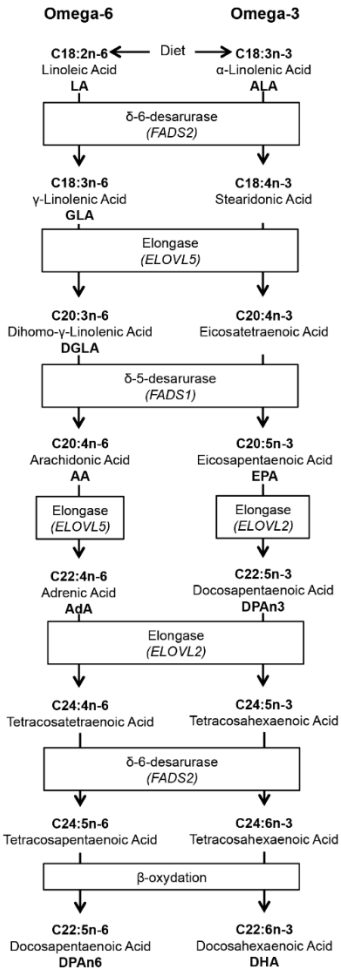
Gene	SNP *Major/minor allele*		NORMAL-WEIGHT					OVERWEIGHT/OBESITY					P (*MM*)	P (*Mm+mm*)
			MM		*Mm+mm*		P	*MM*		*Mm+mm*		P		
			N	Mean (SD)	N	Mean (SD)		N	Mean (SD)	N	Mean (SD)			
Fatty acids involved in *FADS1* indexes														
C20:3n-6 (DGLA)														
FADS1	rs174537	*G/T*	15	3.51 (0.81)	23	4.27 (0.85)	**0.010**	12	3.31 (0.80)	21	4.18 (0.96)	**0.012**	0.529	0.754
FADS1	rs174545	*C/G*	22	3.50 (0.79)	27	4.14 (0.86)	**0.009**	13	3.39 (0.81)	22	4.15 (0.94)	**0.021**	0.691	0.976
FADS1	rs174546	*C/T*	22	3.50 (0.79)	27	4.14 (0.86)	**0.009**	13	3.39 (0.81)	25	4.11 (0.90)	**0.021**	0.691	0.893
FADS1	rs174548	*C/G*	24	3.59 (0.79)	25	4.10 (0.93)	**0.041**	13	3.38 (0.81)	25	4.11 (0.90)	**0.019**	0.431	0.969
FADS1	rs174553	*A/G*	22	3.50 (0.79)	27	4.14 (0.86)	**0.009**	13	3.39 (0.81)	25	4.11 (0.90)	**0.021**	0.691	0.893
FADS1	rs174547	*T/C*	15	3.61 (0.78)	23	4.17 (0.76)	**0.036**	12	3.31 (0.80)	20	4.17 (0.98)	**0.016**	0.335	0.980
C20:4n-6 (AA)														
FADS1	rs174537	*G/T*	15	10.55 (1.33)	23	9.02 (1.54)	**0.003***	12	10.53 (0.89)	21	10.04 (1.36)	0.273	0.973	**0.026**
FADS1	rs174545	*C/G*	22	10.50 (1.39)	27	8.96 (1.49)	**0.001***	13	10.64 (0.93)	22	9.83 (1.31)	0.061	0.759	**0.037**
FADS1	rs174546	*C/T*	22	10.50 (1.39)	27	8.96 (1.49)	**0.001***	13	10.64 (0.93)	25	9.90 (1.31)	0.080	0.759	**0.019**
FADS1	rs174548	*C/G*	24	10.28 (1.39)	25	9.05 (1.64)	**0.007**	13	10.40 (1.02)	25	10.03 (1.33)	0.389	0.790	**0.025**
FADS1	rs174553	*A/G*	22	10.50 (1.39)	27	8.96 (1.49)	**0.001***	13	10.64 (0.93)	25	9.90 (1.31)	0.080	0.759	**0.019**
FADS1	rs174561	*T/C*	15	9.88 (1.32)	14	9.30 (1.12)	0.212	20	10.27 (1.48)	17	10.33 (1.54)	0.910	0.423	**0.046**
FADS1	rs174547	*T/C*	15	10.49 (1.33)	23	9.18 (1.47)	**0.009**	12	10.53 (0.89)	20	10.00 (1.38)	0.243	0.917	0.067
C18:3n-3 (ALA)														
FADS1	rs174537	*G/T*	15	0.12 (0.04)	23	0.16 (0.04)	**0.021**	12	0.11 (0.03)	21	0.11 (0.04)	0.632	0.496	**<0.001†**
FADS1	rs174545	*C/G*	22	0.12 (0.04)	27	0.15 (0.04)	**0.018**	13	0.12 (0.03)	22	0.11 (0.04)	0.532	0.536	**0.001†**
FADS1	rs174546	*C/T*	22	0.12 (0.04)	27	0.15 (0.04)	**0.018**	13	0.12 (0.03)	25	0.11 (0.04)	0.580	0.536	**0.001†**
FADS1	rs174548	*C/G*	24	0.13 (0.04)	22	0.15 (0.04)	0.146	12	0.12 (0.03)	25	0.11 (0.04)	0.454	0.371	**0.001**
FADS1	rs174553	*A/G*	22	0.12 (0.04)	27	0.15 (0.04)	**0.018**	13	0.12 (0.03)	25	0.11 (0.04)	0.580	0.536	**0.001†**
FADS1	rs174561	*T/C*	15	0.13 (0.04)	14	0.13 (0.03)	0.695	20	0.12 (0.05)	17	0.11 (0.03)	0.497	0.397	**0.024**
FADS1	rs174547	*T/C*	15	0.12 (0.04)	23	0.15 (0.04)	**0.027**	12	0.11 (0.03)	20	0.11 (0.04)	0.734	0.444	**<0.001†**
C20:5n3 (EPA)														
FADS1	rs174561	*T/C*	15	0.33 (0.14)	13	0.31 (0.11)	0.683	20	0.26 (0.11)	17	0.23 (0.10)	0.491	0.092	**0.044**
Fatty acids involved in *FADS2* indexes														
C20:3n-6 (DGLA)														
FADS2	rs1535	*A/G*	20	3.41 (0.77)	25	4.23 (0.82)	**0.001***	13	3.39 (0.81)	23	4.15 (0.92)	**0.019**	0.927	0.736
FADS2	rs174575	*C/G*	24	3.57 (0.71)	16	4.28 (0.97)	**0.012**	21	3.82 (1.30)	14	3.90 (0.90)	0.805	0.358	0.283
FADS2	rs174583	*C/T*	19	3.47 (0.75)	27	4.14 (0.86)	**0.009**	13	3.39 (0.81)	25	4.11 (0.90)	**0.021**	0.762	0.893
FADS2	rs99780	*C/T*	15	3.51 (0.78)	26	4.16 (0.89)	**0.025**	12	3.35 (0.84)	22	4.14 (0.94)	**0.022**	0.613	0.941
FADS2	rs174602	*T/C*	19	3.77 (0.82)	13	3.50 (0.85)	0.387	23	3.95 (0.76)	14	4.19 (0.54)	0.297	0.463	**0.018**
C20:4n-6 (AA)														
FADS2	rs1535	*A/G*	20	10.31 (1.30)	25	9.07 (1.50)	**0.005**	13	10.64 (0.93)	23	9.92 (1.35)	0.102	0.439	**0.044**
FADS2	rs174583	*C/T*	19	10.33 (1.34)	27	8.96 (1.49)	**0.003***	13	10.64 (0.93)	25	9.90 (1.31)	0.080	0.482	**0.019**
FADS2	rs99780	*C/T*	15	10.39 (1.48)	26	9.15 (1.51)	**0.015**	12	10.54 (0.91)	22	10.02 (1.40)	0.254	0.757	**0.046**
C18:3n-3 (ALA)														
FADS2	rs1535	*A/G*	20	0.13 (0.04)	25	0.15 (0.04)	**0.025**	13	0.12 (0.03)	23	0.11 (0.04)	0.448	0.500	**<0.001†**
FADS2	rs174575	*C/G*	24	0.13 (0.04)	16	0.16 (0.04)	**0.013**	21	0.11 (0.03)	14	0.11 (0.03)	0.972	**0.035**	**<0.001†**
FADS2	rs174583	*C/T*	19	0.13 (0.04)	27	0.15 (0.04)	**0.033**	13	0.12 (0.03)	25	0.11 (0.04)	0.580	0.498	**0.001†**
FADS2	rs99780	*C/T*	15	0.13 (0.04)	26	0.15 (0.04)	0.060	12	0.12 (0.03)	22	0.11 (0.04)	0.584	0.576	**0.001†**
C20:5n-3 (EPA)														
FADS2	rs174602	*T/C*	19	0.31 (0.14)	12	0.32 (0.10)	0.780	23	0.23 (0.09)	14	0.27 (0.13)	0.347	**0.040**	0.241
C22:5n-3 (DPAn3)														
FADS2	rs1535	*A/G*	20	0.41 (0.10)	25	0.42 (0.06)	0.730	13	0.42 (0.11)	23	0.37 (0.10)	0.190	0.887	**0.034**
FADS2	rs174575	*C/G*	24	0.40 (0.09)	16	0.44 (0.05)	0.056	21	0.39 (0.11)	14	0.37 (0.10)	0.632	0.872	**0.033**
FADS2	rs174583	*C/T*	19	0.40 (0.10)	27	0.42 (0.06)	0.388	13	0.42 (0.11)	25	0.37 (0.10)	0.158	0.674	**0.018**
FADS2	rs99780	*C/T*	15	0.38 (0.07)	26	0.44 (0.08)	**0.017**	12	0.42 (0.12)	22	0.39 (0.10)	0.429	0.297	**0.043**
C22:6n-3 (DHA)														
FADS2	rs1535	*A/G*	20	4.48 (1.35)	25	4.23 (1.04)	0.496	13	4.56 (0.98)	23	4.18 (0.93)	0.255	0.841	0.869
FADS2	rs174575	*C/G*	24	4.19 (1.22)	16	4.50 (1.02)	0.400	21	4.25 (0.92)	14	4.44 (1.01)	0.573	0.856	0.859
FADS2	rs174583	*C/T*	19	4.31 (1.16)	27	4.29 (1.06)	0.955	13	4.56 (0.98)	25	4.11 (0.93)	0.169	0.528	0.511
FADS2	rs99780	*C/T*	15	4.14 (0.95)	26	4.33 (1.19)	0.599	12	4.64 (0.98)	22	4.26 (0.88)	0.260	0.188	0.835
FADS2	rs174602	*T/C*	19	4.32 (1.09)	13	4.52 (1.22)	0.635	23	4.10 (0.91)	14	4.19 (1.02)	0.794	0.480	0.450

Cont. Table 5.

Gene	SNP *Major/minor allele*		NORMAL-WEIGHT					OVERWEIGHT/OBESITY					P (*MM*)	P (*Mm+mm*)
			MM		*Mm+mm*		P	*MM*		*Mm+mm*		P		
			N	Mean (SD)	N	Mean (SD)		N	Mean (SD)	N	Mean (SD)			
Fatty acids involved in *ELOVL2* indexes														
C20:5n-3 (EPA)														
ELOVL2	rs2236212	*G/C*	14	0.31 (0.12)	29	0.33 (0.15)	0.776	13	0.27 (0.23)	22	0.24 (0.11)	0.612	0.571	**0.032**
ELOVL2	rs3798713	*G/C*	11	0.32 (0.13)	31	0.34 (0.15)	0.765	12	0.28 (0.24)	25	0.24 (0.11)	0.490	0.626	**0.011**
C22:5n-3 (DPAn3)														
ELOVL2	rs2236212	*G/C*	14	0.43 (0.10)	29	0.41 (0.07)	0.424	13	0.34 (0.06)	22	0.41 (0.12)	**0.047**	**0.011**	0.787
ELOVL2	rs3798713	*G/C*	11	0.43 (0.11)	31	0.41 (0.07)	0.465	12	0.35 (0.06)	25	0.41 (0.12)	0.099	**0.033**	0.992
C22:6n-3 (DHA)														
ELOVL2	rs2236212	*G/C*	14	4.64 (1.32)	29	4.10 (0.98)	0.139	13	4.30 (0.99)	22	4.28 (0.97)	0.942	0.466	0.514
ELOVL2	rs3798713	*G/C*	11	4.60 (1.49)	31	4.10 (0.91)	0.200	12	4.41 (0.94)	25	4.24 (0.96)	0.617	0.728	0.565
ELOVL2	rs953413	*A/G*	10	3.82 (0.83)	26	4.31 (1.22)	0.250	7	3.85 (0.73)	20	4.61 (0.92)	0.059	0.929	0.357
Fatty acids involved in *ELOVL5* indexes														
C18:3n-3 (ALA)														
ELOVL5	rs2397142	*C/G*	23	0.13 (0.04)	20	0.15 (0.04)	0.168	19	0.11 (0.03)	16	0.11 (0.04)	0.605	**0.022**	**0.016**
ELOVL5	rs9395855	*T/G*	6	0.16 (0.05)	25	0.14 (0.04)	0.405	8	0.10 (0.02)	20	0.11 (0.04)	0.519	**0.010**	**0.047**
C20:5n-3 (EPA)														
ELOVL5	rs2397142	*C/G*	24	0.33 (0.14)	22	0.34 (0.14)	0.855	18	0.21 (0.09)	17	0.29 (0.22)	0.168	**0.004†**	0.411

P-value derived from global ANOVA and significance level ($p \leq 0.004$) was adjusted for multiple testing by Bonferroni correction. Data are means of FAs expressed as percentages of the total phospholipid profile (standard error). P-values <0.05 are highlighted in bold and significant associations that persisted after Bonferroni correction are additionally denoted by stars or daggers ($p \leq 0.004$). *Indicates significant differences within each group of weight and † Indicates significant differences between groups of weight. Major allele: M; minor allele: m; LA: Linoleic Acid; GLA: γ-Linolenic Acid; DGLA: Dihomo-γ-Linolenic Acid; AA: Arachidonic Acid; AdA: Adrenic Acid; DPAn6: Docosapentaenoic acid n6; ALA: α-linolenic Acid; EPA: Eicosapentaenoic acid; DPAn3: Docosapentaenoic acid n3; DHA: Docosahexaenoic acid.

Supporting Information



S1 Figure. Metabolism pathways of omega-6 and omega-3 PUFAs.

S1 Table. DHA in plasma according to DHA supplementation.

	DHA supplementation		No DHA supplementation		P
	N	Mean % (SD)	N	Mean % (SD)	
Total population (n= 110)	17	4.42 (0.88)	93	4.28 (1.10)	0.636
Normal-weight (n= 59)	11	4.21 (0.96)	48	4.48 (1.21)	0.485
Overweight/Obese (n= 51)	6	4.82 (0.57)	45	4.09 (0.95)	0.071

* Indicates significant differences ($p<0.05$) after ANOVA test between groups of weight
Abbreviations: DHA, docosahexaenoic acid.

S2 Table a). Characteristics of the studied SNPs within the *FADS* genes.

Gene SNP	*Major/minor allele*	NORMAL-WEIGHT (n= 88)				OVERWEIGHT/OBESE (n= 92)			
		N	%	HWE*	Missing Analysis (%)	N	%	HWE*	Missing Analysis (%)
FADS1									
rs174537	***G/T***								
	MM	22	46.80			18	36.00		
	Mm	18	38.30	0.313	46.59	27	54.00	0.263	45.65
	mm	7	14.90			5	10.00		
rs174545	***C/G***								
	MM	29	48.30			20	33.90		
	Mm	24	40.00	0.557	31.82	34	57.60	0.075	35.87
	mm	7	11.70			5	8.50		
rs174546	***C/T***								
	MM	30	49.20			20	32.80		
	Mm	24	39.30	0.518	30.68	36	59.00	0.045	33.70
	mm	7	11.50			5	8.20		
rs174548	***C/G***								
	MM	33	54.10			21	33.90		
	Mm	21	34.40	0.215	30.68	37	59.70	0.022	32.61
	mm	7	11.50			4	6.50		
rs174553	***A/G***								
	MM	30	49.20			20	32.30		
	Mm	24	39.30	0.518	30.68	37	59.70	0.350	32.61
	mm	7	11.50			5	8.10		
rs174561	***T/C***								
	MM	27	50.9%			34	52.3%		
	Mm	21	39.6%	0.757	39.77	26	40.0%	0.992	29.35
	mm	5	9.4%			5	7.7%		
rs174547	***T/C***								
	MM	22	44.90			17	37.00		
	Mm	21	42.90	0.777	44.32	25	54.30	0.219	50.00
	mm	6	12.20			4	8.70		
FADS2									
rs1535	***A/G***								
	MM	28	49.10			20	35.10		
	Mm	22	38.60	0.420	35.23	32	56.10	0.119	38.04
	mm	7	12.30			5	8.80		
rs174575	***C/G***								
	MM	32	61.50			30	56.60		
	Mm	14	26.90	0.042	40.91	19	35.80	0.685	42.39
	mm	6	11.50			4	7.50		
rs174583	***C/T***								
	MM	26	45.60			19	31.70		
	Mm	23	40.40	0.434	35.23	35	58.30	0.083	34.78
	mm	8	14.00			6	10.00		
rs99780	***C/T***								
	MM	23	43.40			19	35.20		
	Mm	20	37.70	0.152	39.77	29	53.70	0.303	41.30
	mm	10	18.90			6	11.10		
rs174602	***T/C***								
	MM	40	70.2%			39	60.0%		
				0.254	35.23			0.707	29.35
	Mm	14	24.6%			22	33.8%		
	mm	3	5.3%			4	6.2%		

PREOBE cohort.
Major allele: M; minor allele: m
Data are expressed as percentages of total women in each weight group.
*P-values of deviation from HWE among all subjects were tested by chi-square tests.

S2 Table b). Characteristics of the studied SNPs within the *ELOVL* genes.

Gene SNP	Major/minor allele	NORMAL-WEIGHT (n= 88) N	%	HWE*	Missing Analysis (%)	OVERWEIGHT/OBESE (n= 92) N	%	HWE*	Missing Analysis (%)
ELOVL2									
rs2236212	***G/C***								
	MM	16	29.10			22	36.70		
	Mm	27	49.10	0.923	37.50	24	40.00	0.151	34.78
	mm	12	21.80			14	23.30		
rs3798713	***G/C***								
	MM	13	24.50			19	32.20		
	Mm	24	45.30	0.506	39.77	25	42.40	0.254	35.87
	mm	16	30.20			15	25.40		
rs953413	***A/G***								
	MM	12	27.90			11	26.20		
	Mm	18	41.90	0.957	51.14	17	40.50	0.227	54.35
	mm	13	30.20			14	33.30		
ELOVL5									
rs2397142	***C/G***								
	MM	28	48.30			29	50.00		
	Mm	23	39.70	0.506	34.09	21	36.20	0.205	36.96
	mm	7	12.10			8	13.80		
rs9395855	***T/G***								
	MM	9	23.10			11	29.70		
	Mm	18	46.20	0.656	55.68	19	51.40	0.812	59.78
	mm	12	30.80			7	18.90		

PREOBE cohort. Major allele: M; minor allele: m. Data are expressed as percentages of total women in each weight group.
*P-values of deviation from HWE among all subjects were tested by chi-square tests.

S3 Table a). Associations between fatty acid indexes and *FADS1* SNPs.

Fatty acid	Gene	SNP	Major/minor allele	NORMAL-WEIGHT N	β	P	OVERWEIGHT/OBESE N	β	P
***FADS1* indexes**									
AA:LA									
	FADS1	rs174537	*G/T*	22	-0.23	0.498	18	-0.03	0.935
	FADS1	rs174545	*C/G*	28	-0.39	0.171	17	-0.05	0.865
	FADS1	rs174546	*C/T*	28	-0.39	0.171	19	-0.04	0.890
	FADS1	rs174548	*C/G*	28	-0.21	0.361	19	-0.04	0.890
	FADS1	rs174553	*A/G*	28	-0.39	0.171	19	-0.04	0.890
	FADS1	rs174561	*T/C*	9	-0.20	0.703	11	0.35	0.397
	FADS1	rs174547	*T/C*	23	-0.34	0.289	18	-0.03	0.935
AA:DGLA									
	FADS1	rs174537	*G/T*	22	-0.81	**0.004***	18	-0.48	0.155
	FADS1	rs174545	*C/G*	28	-0.70	**0.003***	17	-0.48	0.174
	FADS1	rs174546	*C/T*	28	-0.70	**0.003***	19	-0.49	0.139
	FADS1	rs174548	*C/G*	28	-0.36	0.071	19	-0.49	0.139
	FADS1	rs174553	*A/G*	28	-0.70	**0.003***	19	-0.49	0.139
	FADS1	rs174561	*T/C*	9	-0.21	0.655	11	-0.22	0.692
	FADS1	rs174547	*T/C*	23	-0.67	**0.013**	18	-0.48	0.155
EPA:ALA									
	FADS1	rs174537	*G/T*	22	-0.30	0.343	17	0.12	0.721
	FADS1	rs174545	*C/G*	28	-0.35	0.183	16	0.12	0.733
	FADS1	rs174546	*C/T*	28	-0.35	0.183	18	0.10	0.761
	FADS1	rs174548	*C/G*	28	0.06	0.778	18	0.10	0.761
	FADS1	rs174553	*A/G*	28	-0.35	0.183	18	0.10	0.761
	FADS1	rs174561	*T/C*	9	-0.45	0.318	11	0.25	0.597
	FADS1	rs174547	*T/C*	23	-0.35	0.236	17	0.12	0.721

Associations between SNPs and fatty acids were analyzed using linear regression. SNPs were coded according to minor allele count and analyzed as a numeric variable. "β"= beta per minor allele standardized per the major allele. All associations were adjusted for potential confounders such as age, education, smoking and energy intake. P-values <0.05 are highlighted in bold and significant associations that persisted after Bonferroni correction are additionally denoted by stars (*p≤0.004). LA: Linoleic Acid; GLA: γ-Linolenic Acid; DGLA: Dihomo-γ-Linolenic Acid; AA: Arachidonic Acid; AdA: Adrenic Acid; DPAn6: Docosapentaenoic acid n6; ALA: α-linolenic Acid; EPA: Eicosapentaenoic acid; DPAn3: Docosapentaenoic acid n3; DHA: Docosahexaenoic acid.

S3 Table b). Associations between fatty acid indexes and *FADS2* SNPs.

Fatty acid	Gene	SNP	*Major/minor allele*	NORMAL-WEIGHT			OVERWEIGHT/OBESE		
				N	β	P	N	β	P
***FADS2* indexes**									
GLA:LA									
	FADS2	rs1535	*A/G*	25	0.01	0.984	18	-0.10	0.761
	FADS2	rs174575	*C/G*	22	0.20	0.451	19	0.59	0.054
	FADS2	rs174583	*C/T*	26	-0.04	0.891	19	-0.13	0.699
	FADS2	rs99780	*C/T*	24	-0.08	0.786	18	-0.10	0.761
	FADS2	rs174602	*T/C*	11	0.23	0.630	11	-0.50	0.372
DGLA:LA									
	FADS2	rs1535	*A/G*	26	0.56	**0.029**	18	0.42	0.226
	FADS2	rs174575	*C/G*	23	0.51	0.062	19	-0.05	0.889
	FADS2	rs174583	*C/T*	27	0.53	**0.040**	19	0.41	0.226
	FADS2	rs99780	*C/T*	25	0.54	**0.042**	18	0.42	0.226
	FADS2	rs174602	*T/C*	11	0.38	0.181	11	0.44	0.489
AA:LA									
	FADS2	rs1535	*A/G*	26	-0.32	0.271	18	-0.03	0.935
	FADS2	rs174575	*C/G*	23	-0.03	0.909	19	0.11	0.720
	FADS2	rs174583	*C/T*	27	-0.35	0.218	19	-0.04	0.890
	FADS2	rs99780	*C/T*	25	-0.06	0.849	18	-0.03	0.935
	FADS2	rs174602	*T/C*	11	0.15	0.772	11	-0.51	0.335
DPAn6:AA									
	FADS2	rs1535	*A/G*	26	0.27	0.271	18	0.18	0.484
	FADS2	rs174575	*C/G*	23	-0.05	0.836	19	-0.07	0.772
	FADS2	rs174583	*C/T*	27	0.29	0.236	19	0.18	0.466
	FADS2	rs99780	*C/T*	25	0.01	0.981	18	0.18	0.484
	FADS2	rs174602	*T/C*	11	0.46	0.124	11	0.02	0.958
DPAn6:AdA									
	FADS2	rs1535	*A/G*	26	0.04	0.873	17	0.27	0.336
	FADS2	rs174575	*C/G*	23	-0.15	0.579	18	-0.26	0.333
	FADS2	rs174583	*C/T*	27	0.03	0.916	18	0.28	0.304
	FADS2	rs99780	*C/T*	25	-0.42	0.094	17	0.27	0.336
	FADS2	rs174602	*T/C*	11	-0.10	0.792	10	0.52	0.318
EPA:ALA									
	FADS2	rs1535	*A/G*	26	-0.33	0.228	17	0.12	0.721
	FADS2	rs174575	*C/G*	23	-0.19	0.484	18	0.35	0.254
	FADS2	rs174583	*C/T*	27	-0.33	0.216	18	0.10	0.761
	FADS2	rs99780	*C/T*	25	0.26	0.338	17	0.12	0.721
	FADS2	rs174602	*T/C*	11	-0.13	0.739	11	0.34	0.566
DHA:EPA									
	FADS2	rs1535	*A/G*	26	0.20	0.486	17	-0.10	0.703
	FADS2	rs174575	*C/G*	23	-0.13	0.665	18	-0.23	0.375
	FADS2	rs174583	*C/T*	27	0.19	0.497	18	-0.08	0.762
	FADS2	rs99780	*C/T*	25	-0.29	0.276	17	-0.10	0.703
	FADS2	rs174602	*T/C*	11	0.23	0.556	11	-0.47	0.388
DHA:DPAn3									
	FADS2	rs1535	*A/G*	26	0.12	0.658	18	0.34	0.273
	FADS2	rs174575	*C/G*	23	0.00	0.995	19	0.12	0.695
	FADS2	rs174583	*C/T*	27	0.14	0.617	19	0.35	0.251
	FADS2	rs99780	*C/T*	25	-0.13	0.641	18	0.34	0.273
	FADS2	rs174602	*T/C*	11	-0.52	0.164	11	-0.52	0.312

Associations between SNPs and fatty acids were analyzed using linear regression. SNPs were coded according to minor allele count and analyzed as a numeric variable. "β"= beta per minor allele standardized per the major allele. All associations were adjusted for potential confounders such as age, education, smoking and energy intake. P-values <0.05 are highlighted in bold and significant associations that persisted after Bonferroni correction are additionally denoted by stars (*p≤0.004). LA: Linoleic Acid; GLA: γ-Linolenic Acid; DGLA: Dihomo-γ-Linolenic Acid; AA: Arachidonic Acid; AdA: Adrenic Acid; DPAn6: Docosapentaenoic acid n6; ALA: α-linolenic Acid; EPA: Eicosapentaenoic acid; DPAn3: Docosapentaenoic acid n3; DHA: Docosahexaenoic acid.

S3 Table c). Associations between fatty acid indexes and *ELOVL* SNPs.

Fatty acid	Gene	SNP	*Major/minor allele*	NORMAL-WEIGHT			OVERWEIGHT/OBESE		
				N	β	P	N	β	P
***ELOVL2* indexes**									
DPAn6:AdA									
	ELOVL2	rs2236212	*G/C*	25	0.07	0.744	18	-0.19	0.427
	ELOVL2	rs3798713	*G/C*	25	0.12	0.579	18	-0.19	0.427
	ELOVL2	rs953413	*A/G*	21	-0.01	0.978	16	-0.04	0.887
DPAn3:EPA									
	ELOVL2	rs2236212	*G/C*	25	0.05	0.822	18	0.00	0.987
	ELOVL2	rs3798713	*G/C*	25	0.09	0.679	18	0.00	0.987
	ELOVL2	rs953413	*A/G*	21	0.14	0.554	16	0.30	0.115
DHA:EPA									
	ELOVL2	rs2236212	*G/C*	25	-0.07	0.750	18	-0.12	0.642
	ELOVL2	rs3798713	*G/C*	25	0.01	0.953	18	-0.12	0.642
	ELOVL2	rs953413	*A/G*	21	0.04	0.871	16	0.24	0.271
DHA:DPAn3									
	ELOVL2	rs2236212	*G/C*	25	-0.05	0.806	19	-0.58	**0.016**
	ELOVL2	rs3798713	*G/C*	25	0.00	0.995	19	-0.58	**0.016**
	ELOVL2	rs953413	*A/G*	21	-0.21	0.402	17	-0.06	0.834
***ELOVL5* indexes**									
DGLA:LA									
	ELOVL5	rs2397142	*C/G*	27	-0.33	0.153	19.00	-0.03	0.923
	ELOVL5	rs9395855	*T/G*	19	0.00	0.988	17.00	0.20	0.507
DGLA:GLA									
	ELOVL5	rs2397142	*C/G*	26	-0.12	0.637	19.00	-0.01	0.977
	ELOVL5	rs9395855	*T/G*	18	-0.18	0.490	17.00	-0.10	0.745
AA:LA									
	ELOVL5	rs2397142	*C/G*	27	-0.11	0.644	19.00	-0.24	0.330
	ELOVL5	rs9395855	*T/G*	19	0.30	0.289	17.00	0.35	0.155
AdA:AA									
	ELOVL5	rs2397142	*C/G*	27	-0.03	0.903	18.00	0.08	0.726
	ELOVL5	rs9395855	*T/G*	19	-0.20	0.422	16.00	-0.06	0.800
EPA:ALA									
	ELOVL5	rs2397142	*C/G*	27	0.11	0.634	18	0.35	0.182
	ELOVL5	rs9395855	*T/G*	19	0.09	0.757	16	-0.39	0.124

Associations between SNPs and fatty acids were analyzed using linear regression. SNPs were coded according to minor allele count and analyzed as a numeric variable. "β"= beta per minor allele standardized per the major allele. All associations were adjusted for potential confounders such as age, education, smoking and energy intake. P-values <0.05 are highlighted in bold and significant associations that persisted after Bonferroni correction are additionally denoted by stars (*p≤0.004). LA: Linoleic Acid; GLA: γ-Linolenic Acid; DGLA: Dihomo-γ-Linolenic Acid; AA: Arachidonic Acid; AdA: Adrenic Acid; DPAn6: Docosapentaenoic acid n6; ALA: α-linolenic Acid; EPA: Eicosapentaenoic acid; DPAn3: Docosapentaenoic acid n3; DHA: Docosahexaenoic acid.

S3 Table d). Associations between fatty acids and *FADS1* SNPs.

Fatty acid	Gene	SNP	*Major/minor allele*	NORMAL-WEIGHT			OVERWEIGHT/OBESE		
				N	β	P	N	β	P
Fatty acids involved in *FADS1* indexes									
C18:2n-6 (LA)									
	FADS1	rs174537	*G/T*	22	-0.37	0.263	18	0.01	0.978
	FADS1	rs174545	*C/G*	28	-0.04	0.888	17	0.02	0.948
	FADS1	rs174546	*C/T*	28	-0.04	0.888	19	0.01	0.965
	FADS1	rs174548	*C/G*	28	-0.13	0.573	19	0.01	0.965
	FADS1	rs174553	*A/G*	28	-0.04	0.888	19	0.01	0.965
	FADS1	rs174561	*T/C*	9	0.14	0.763	11	-0.31	0.507
	FADS1	rs174547	*T/C*	23	-0.20	0.370	18	0.01	0.978
C20:3n-6 (DGLA)									
	FADS1	rs174537	*G/T*	22	0.75	**0.012**	18	0.53	0.119
	FADS1	rs174545	*C/G*	28	0.57	**0.028**	17	0.52	0.137
	FADS1	rs174546	*C/T*	28	0.57	**0.028**	19	0.52	0.118
	FADS1	rs174548	*C/G*	28	0.34	0.109	19	0.52	0.118
	FADS1	rs174553	*A/G*	28	0.57	**0.028**	19	0.52	0.118
	FADS1	rs174561	*T/C*	9	0.11	0.495	11	0.36	0.503
	FADS1	rs174547	*T/C*	23	0.60	**0.034**	18	0.53	0.119
C20:4n-6 (AA)									
	FADS1	rs174537	*G/T*	22	-0.57	0.092	18	0.01	0.984
	FADS1	rs174545	*C/G*	28	-0.55	**0.042**	17	-0.01	0.977
	FADS1	rs174546	*C/T*	28	-0.55	**0.042**	19	-0.01	0.971
	FADS1	rs174548	*C/G*	28	-0.36	0.102	19	-0.01	0.971
	FADS1	rs174553	*A/G*	28	-0.55	**0.042**	19	-0.01	0.971
	FADS1	rs174561	*T/C*	9	-0.16	0.793	11	0.18	0.666
	FADS1	rs174547	*T/C*	23	-0.53	0.087	18	0.01	0.984
C18:3n-3 (ALA)									
	FADS1	rs174537	*G/T*	22	0.71	**0.027**	18	-0.09	0.790
	FADS1	rs174545	*C/G*	28	0.47	0.084	17	-0.08	0.814
	FADS1	rs174546	*C/T*	28	0.47	0.084	19	-0.09	0.774
	FADS1	rs174548	*C/G*	28	0.17	0.436	19	-0.09	0.774
	FADS1	rs174553	*A/G*	28	0.47	0.084	19	-0.09	0.774
	FADS1	rs174561	*T/C*	9	0.25	0.557	11	-0.57	0.081
	FADS1	rs174547	*T/C*	23	0.66	**0.026**	18	-0.09	0.790
C20:5n3 (EPA)									
	FADS1	rs174537	*G/T*	22	0.21	0.478	17	0.11	0.749
	FADS1	rs174545	*C/G*	28	-0.05	0.856	16	0.11	0.751
	FADS1	rs174546	*C/T*	28	-0.05	0.856	18	0.10	0.780
	FADS1	rs174548	*C/G*	28	0.22	0.285	18	0.10	0.780
	FADS1	rs174553	*A/G*	28	-0.05	0.856	18	0.10	0.780
	FADS1	rs174561	*T/C*	9	-0.26	0.559	11	-0.03	0.943
	FADS1	rs174547	*T/C*	23	0.05	0.866	17	0.11	0.749

Associations between SNPs and fatty acids were analyzed using linear regression. SNPs were coded according to minor allele count and analyzed as a numeric variable. "β"= beta per minor allele standardized per the major allele. All associations were adjusted for potential confounders such as age, education, smoking and energy intake. P-values <0.05 are highlighted in bold and significant associations that persisted after Bonferroni correction are additionally denoted by stars (*p≤0.004). LA: Linoleic Acid; GLA: γ-Linolenic Acid; DGLA: Dihomo-γ-Linolenic Acid; AA: Arachidonic Acid; AdA: Adrenic Acid; DPAn6: Docosapentaenoic acid n6; ALA: α-linolenic Acid; EPA: Eicosapentaenoic acid; DPAn3: Docosapentaenoic acid n3; DHA: Docosahexaenoic acid.

S3 Table e). Associations between fatty acids and *FADS2* SNPs.

Fatty acid	Gene	SNP	*Major/minor allele*	NORMAL-WEIGHT			OVERWEIGHT/OBESE		
				N	β	P	N	β	P
Fatty acids involved in *FADS2* indexes									
C18:2n-6 (LA)									
	FADS2	rs1535	*A/G*	26	-0.10	0.728	18	0.01	0.978
	FADS2	rs174575	*C/G*	23	-0.24	0.424	19	-0.14	0.669
	FADS2	rs174583	*C/T*	27	-0.09	0.763	19	0.01	0.965
	FADS2	rs99780	*C/T*	25	-0.48	0.085	18	0.01	0.978
	FADS2	rs174602	*T/C*	11	-0.39	0.446	11	0.21	0.734
C18:3n6 (GLA)									
	FADS2	rs1535	*A/G*	25	-0.07	0.811	18	-0.08	0.806
	FADS2	rs174575	*C/G*	22	0.12	0.672	19	0.64	**0.031**
	FADS2	rs174583	*C/T*	26	-0.12	0.695	19	-0.11	0.740
	FADS2	rs99780	*C/T*	24	-0.24	0.429	18	-0.08	0.806
	FADS2	rs174602	*T/C*	11	0.19	0.690	11	-0.48	0.327
C20:3n-6 (DGLA)									
	FADS2	rs1535	*A/G*	26	0.65	**0.010**	18	0.53	0.119
	FADS2	rs174575	*C/T*	23	0.51	0.054	19	-0.11	0.750
	FADS2	rs174583	*C/G*	27	0.62	**0.015**	19	0.52	0.118
	FADS2	rs99780	*C/T*	25	0.43	0.109	18	0.53	0.119
	FADS2	rs174602	*T/C*	11	0.33	0.065	11	0.77	0.230
C20:4n-6 (AA)									
	FADS2	rs1535	*A/G*	26	-0.50	0.073	18	0.01	0.984
	FADS2	rs174575	*C/T*	23	-0.24	0.396	19	0.06	0.838
	FADS2	rs174583	*C/G*	27	-0.52	0.054	19	-0.01	0.971
	FADS2	rs99780	*C/T*	25	-0.41	0.155	18	0.01	0.984
	FADS2	rs174602	*T/C*	11	-0.03	0.957	11	-0.51	0.319
C22:4n-6 (AdA)									
	FADS2	rs1535	*A/G*	26	-0.04	0.882	17	-0.01	0.965
	FADS2	rs174575	*C/G*	23	-0.07	0.819	18	-0.13	0.616
	FADS2	rs174583	*C/T*	27	-0.04	0.873	18	-0.03	0.906
	FADS2	rs99780	*C/T*	25	0.12	0.678	17	-0.01	0.965
	FADS2	rs174602	*T/C*	11	0.61	0.138	10	0.02	0.964
C22:5n-6 (DPAn6)									
	FADS2	rs1535	*A/G*	26	0.01	0.973	18	0.21	0.341
	FADS2	rs174575	*C/G*	23	-0.15	0.578	19	-0.07	0.732
	FADS2	rs174583	*C/T*	27	0.00	0.999	19	0.20	0.339
	FADS2	rs99780	*C/T*	25	-0.17	0.501	18	0.21	0.341
	FADS2	rs174602	*T/C*	11	0.42	0.095	11	-0.11	0.768
C18:3n-3 (ALA)									
	FADS2	rs1535	*A/G*	26	0.45	0.105	18	-0.09	0.790
	FADS2	rs174575	*C/G*	23	0.50	**0.043**	19	0.22	0.494
	FADS2	rs174583	*C/T*	27	0.44	0.106	19	-0.09	0.774
	FADS2	rs99780	*C/T*	25	0.32	0.270	18	-0.09	0.790
	FADS2	rs174602	*T/C*	11	-0.38	0.397	11	-0.15	0.753
C20:5n3 (EPA)									
	FADS2	rs1535	*A/G*	26	-0.04	0.887	17	0.11	0.749
	FADS2	rs174575	*C/G*	23	0.12	0.671	18	0.37	0.252
	FADS2	rs174583	*C/T*	27	-0.05	0.856	18	0.10	0.780
	FADS2	rs99780	*C/T*	25	0.52	**0.035**	17	0.11	0.749
	FADS2	rs174602	*T/C*	11	-0.17	0.622	11	0.39	0.494
C22:5n-3 (DPAn3)									
	FADS2	rs1535	*A/G*	26	-0.03	0.916	18	-0.41	0.173
	FADS2	rs174575	*C/G*	23	0.01	0.969	19	0.05	0.867
	FADS2	rs174583	*C/T*	27	-0.06	0.825	19	-0.42	0.153
	FADS2	rs99780	*C/T*	25	0.62	**0.011**	18	-0.41	0.173
	FADS2	rs174602	*T/C*	11	0.22	0.517	11	0.33	0.467
C22:6n-3 (DHA)									
	FADS2	rs1535	*A/G*	26	0.08	0.761	18	-0.16	0.563
	FADS2	rs174575	*C/G*	23	0.01	0.974	19	0.24	0.365
	FADS2	rs174583	*C/T*	27	0.07	0.795	19	-0.17	0.522
	FADS2	rs99780	*C/T*	25	0.42	0.106	18	-0.16	0.563
	FADS2	rs174602	*T/C*	11	-0.32	0.403	11	-0.24	0.620

Associations between SNPs and fatty acids were analyzed using linear regression. SNPs were coded according to minor allele count and analyzed as a numeric variable. "β"= beta per minor allele standardized per the major allele. All associations were adjusted for potential confounders such as age, education, smoking and energy intake. P-values <0.05 are highlighted in bold and significant associations that persisted after Bonferroni correction are additionally denoted by stars (*p≤0.004). LA: Linoleic Acid; GLA: γ-Linolenic Acid; DGLA: Dihomo-γ-Linolenic Acid; AA: Arachidonic Acid; AdA: Adrenic Acid; DPAn6: Docosapentaenoic acid n6; ALA: α-linolenic Acid; EPA: Eicosapentaenoic acid; DPAn3: Docosapentaenoic acid n3; DHA: Docosahexaenoic acid.

S3 Table e). Associations between fatty acids and *ELOVL* SNPs.

Fatty acid	Gene	SNP	*Major/minor allele*	NORMAL-WEIGHT			OVERWEIGHT/OBESE		
				N	β	P	N	β	P
Fatty acids involved in *ELOVL2* indexes									
C22:4n-6 (AdA)									
	ELOVL2	rs2236212	*G/C*	25	-0.06	0.793	18	0.02	0.922
	ELOVL2	rs3798713	*G/C*	25	-0.06	0.793	18	0.02	0.922
	ELOVL2	rs953413	*A/G*	21	-0.15	0.543	16	0.10	0.613
C22:5n-6 (DPAn6)									
	ELOVL2	rs2236212	*G/C*	25	0.03	0.903	19	0.02	0.928
	ELOVL2	rs3798713	*G/C*	25	0.10	0.637	19	0.02	0.928
	ELOVL2	rs953413	*A/G*	21	-0.09	0.679	17	-0.09	0.645
C20:5n3 (EPA)									
	ELOVL2	rs2236212	*G/C*	25	-0.15	0.479	18	-0.16	0.598
	ELOVL2	rs3798713	*G/C*	25	-0.17	0.427	18	-0.16	0.598
	ELOVL2	rs953413	*A/G*	21	-0.12	0.602	16	-0.10	0.743
C22:5n-3 (DPAn3)									
	ELOVL2	rs2236212	*G/C*	25	-0.32	0.114	19	0.44	0.072
	ELOVL2	rs3798713	*G/C*	25	-0.34	0.104	19	0.44	0.072
	ELOVL2	rs953413	*A/G*	21	0.21	0.375	17	-0.09	0.758
C22:6n-3 (DHA)									
	ELOVL2	rs2236212	*G/C*	25	-0.32	0.109	19	-0.02	0.943
	ELOVL2	rs3798713	*G/C*	25	-0.29	0.176	19	-0.02	0.943
	ELOVL2	rs953413	*A/G*	21	0.04	0.869	17	-0.06	0.768
Fatty acids involved in *ELOVL5* indexes									
C18:2n6 (LA)									
	ELOVL5	rs2397142	*C/G*	27	0.01	0.965	19	0.04	0.898
	ELOVL5	rs9395855	*T/G*	19	0.05	0.858	17	-0.31	0.232
C18:3n6 (GLA)									
	ELOVL5	rs2397142	*C/G*	26	-0.04	0.867	19	-0.18	0.518
	ELOVL5	rs9395855	*T/G*	18	0.02	0.949	17	0.41	0.136
C20:3n-6 (DGLA)									
	ELOVL5	rs2397142	*C/G*	27	-0.39	0.081	19	0.03	0.931
	ELOVL5	rs9395855	*T/G*	19	-0.05	0.847	17	0.09	0.766
C20:4n-6 (AA)									
	ELOVL5	rs2397142	*C/G*	27	-0.11	0.641	19	-0.30	0.241
	ELOVL5	rs9395855	*T/G*	19	0.44	0.101	17	0.24	0.366
C22:4n-6 (AdA)									
	ELOVL5	rs2397142	*C/G*	27	-0.13	0.583	18	-0.14	0.523
	ELOVL5	rs9395855	*T/G*	19	0.28	0.294	16	0.04	0.845
C18:3n-3 (ALA)									
	ELOVL5	rs2397142	*C/G*	27	0.15	0.546	19	-0.07	0.815
	ELOVL5	rs9395855	*T/G*	19	-0.18	0.515	17	0.17	0.569
C20:5n3 (EPA)									
	ELOVL5	rs2397142	*C/G*	27	0.15	0.486	18	0.29	0.298
	ELOVL5	rs9395855	*T/G*	19	-0.05	0.841	16	-0.36	0.187

Associations between SNPs and fatty acids were analyzed using linear regression. SNPs were coded according to minor allele count and analyzed as a numeric variable. "β"= beta per minor allele standardized per the major allele. All associations were adjusted for potential confounders such as age, education, smoking and energy intake. P-values <0.05 are highlighted in bold and significant associations that persisted after Bonferroni correction are additionally denoted by stars (*p≤0.004). LA: Linoleic Acid; GLA: γ-Linolenic Acid; DGLA: Dihomo-γ-Linolenic Acid; AA: Arachidonic Acid; AdA: Adrenic Acid; DPAn6: Docosapentaenoic acid n6; ALA: α-linolenic Acid; EPA: Eicosapentaenoic acid; DPAn3: Docosapentaenoic acid n3; DHA: Docosahexaenoic acid.

S4 Table a). Fatty acid indexes according to *FADS1* SNPs.

Gene	SNP *Major/minor allele*		NORMAL-WEIGHT *MM* N	*MM* Mean (SD)	*Mm+mm* N	*Mm+mm* Mean (SD)	P	OVERWEIGHT/OBESITY *MM* N	*MM* Mean (SD)	*Mm+mm* N	*Mm+mm* Mean (SD)	P	P (*MM*)	P (*Mm+mm*)
***FADS1* indexes**														
AA:LA														
FADS1	rs174537	*G/T*	15	0.45 (0.08)	23	0.39 (0.09)	**0.038**	12	0.46 (0.07)	21	0.43 (0.08)	0.390	0.811	0.085
FADS1	rs174545	*C/G*	22	0.46 (0.09)	27	0.38 (0.08)	**0.003***	13	0.47 (0.07)	22	0.42 (0.08)	0.071	0.877	0.171
FADS1	rs174546	*C/T*	22	0.46 (0.09)	27	0.38 (0.08)	**0.003***	13	0.47 (0.07)	25	0.42 (0.08)	0.098	0.877	0.109
FADS1	rs174548	*C/G*	24	0.45 (0.08)	25	0.39 (0.09)	**0.031**	13	0.45 (0.08)	25	0.43 (0.08)	0.553	0.969	0.115
FADS1	rs174553	*A/G*	22	0.46 (0.09)	27	0.38 (0.08)	**0.003***	13	0.47 (0.07)	25	0.42 (0.08)	0.098	0.877	0.109
FADS1	rs174561	*T/C*	15	0.42 (0.07)	14	0.41 (0.09)	0.832	20	0.45 (0.11)	17	0.44 (0.11)	0.939	0.332	0.336
FADS1	rs174547	*T/C*	15	0.45 (0.08)	23	0.39 (0.09)	**0.040**	12	0.46 (0.07)	20	0.43 (0.08)	0.427	0.950	0.124
AA:DGLA														
FADS1	rs174537	*G/T*	15	3.14 (0.70)	23	2.20 (0.60)	**<0.001***	12	3.57 (1.73)	21	2.55 (0.80)	**0.032**	0.414	0.107
FADS1	rs174545	*C/G*	22	3.12 (0.65)	27	2.25 (0.57)	**<0.001***	13	3.48 (1.67)	22	2.51 (0.78)	**0.025**	0.362	0.183
FADS1	rs174546	*C/T*	22	3.12 (0.65)	27	2.25 (0.57)	**<0.001***	13	3.48 (1.67)	25	2.54 (0.74)	**0.021**	0.362	0.120
FADS1	rs174548	*C/G*	24	2.97 (0.65)	25	2.33 (0.70)	**0.002***	13	3.42 (1.69)	25	2.57 (0.75)	**0.037**	0.244	0.246
FADS1	rs174553	*A/G*	22	3.12 (0.65)	27	2.25 (0.57)	**<0.001***	13	3.48 (1.67)	25	2.54 (0.74)	**0.021**	0.362	0.120
FADS1	rs174561	*T/C*	15	2.78 (0.59)	14	2.71 (0.78)	0.771	20	2.65 (0.67)	17	2.61 (0.60)	0.843	0.550	0.691
FADS1	rs174547	*T/C*	15	3.20 (0.67)	23	2.27 (0.55)	**<0.001***	12	3.54 (1.73)	20	2.56 (0.82)	**0.037**	0.291	0.194
EPA:ALA														
FADS1	rs174537	*G/T*	15	2.63 (1.29)	20	2.26 (1.03)	0.351	10	2.33 (1.17)	21	2.40 (1.43)	0.904	0.568	0.721
FADS1	rs174545	*C/G*	21	2.74 (1.19)	23	2.26 (0.96)	0.147	11	2.31 (1.11)	22	2.33 (1.41)	0.971	0.338	0.834
FADS1	rs174546	*C/T*	21	2.74 (1.19)	23	2.26 (0.96)	0.147	11	2.31 (1.11)	25	2.30 (1.34)	0.967	0.338	0.910
FADS1	rs174548	*C/G*	23	2.58 (1.06)	21	2.39 (1.14)	0.571	11	2.27 (1.09)	25	2.31 (1.35)	0.927	0.442	0.848
FADS1	rs174553	*A/G*	21	2.74 (1.19)	23	2.26 (0.96)	0.147	11	2.31 (1.11)	25	2.30 (1.34)	0.967	0.338	0.910
FADS1	rs174561	*T/C*	14	2.77 (1.19)	12	2.24 (0.67)	0.185	19	2.36 (0.97)	17	2.30 (1.25)	0.879	0.285	0.873
FADS1	rs174547	*T/C*	15	2.74 (1.32)	21	2.26 (1.00)	0.220	10	2.33 (1.17)	20	2.40 (1.47)	0.898	0.437	0.713

P-value derived from global ANOVA and significance level ($p \leq 0.004$) was adjusted for multiple testing by Bonferroni correction. Data are means of FAs expressed as percentages of the total phospholipid profile (standard error). P-values <0.05 are highlighted in bold and significant associations that persisted after Bonferroni correction are additionally denoted by stars or daggers ($p \leq 0.004$). *Indicates significant differences within each group of weight and † Indicates significant differences between groups of weight. Major allele: M; minor allele: m; LA: Linoleic Acid; DGLA: Dihomo-γ-Linolenic Acid; AA: Arachidonic Acid; ALA: α-linolenic Acid; EPA: Eicosapentaenoic acid.

S4 Table b). Fatty acid indexes according to *FADS2* SNPs.

Gene	SNP *Major/minor allele*		NORMAL-WEIGHT *MM* N	Mean (SD)	*Mm+mm* N	Mean (SD)	P	OVERWEIGHT/OBESITY *MM* N	Mean (SD)	*Mm+mm* N	Mean (SD)	P	P (*MM*)	P (*Mm+mm*)
***FADS2* indexes**														
GLA:LA														
FADS2	rs1535	*A/G*	19	0.002 (0.001)	23	0.003 (0.001)	0.307	12	0.002 (0.001)	23	0.002 (0.001)	0.492	0.416	0.359
FADS2	rs174575	*C/G*	23	0.002 (0.001)	14	0.003 (0.001)	0.266	20	0.002 (0.001)	14	0.003 (0.001)	0.260	0.870	0.746
FADS2	rs174583	*C/T*	18	0.002 (0.001)	25	0.002 (0.001)	0.562	12	0.002 (0.001)	25	0.002 (0.001)	0.541	0.496	0.604
FADS2	rs99780	*C/T*	15	0.002 (0.001)	24	0.002 (0.001)	0.625	11	0.002 (0.001)	22	0.002 (0.001)	0.973	0.982	0.601
FADS2	rs174602	*T/C*	18	0.002 (0.001)	13	0.003 (0.001)	0.879	23	0.002 (0.001)	13	0.003 (0.001)	0.465	0.590	0.932
DGLA:LA														
FADS2	rs1535	*A/G*	20	0.15 (0.04)	25	0.18 (0.05)	**0.011**	13	0.15 (0.04)	23	0.18 (0.05)	0.115	0.877	0.798
FADS2	rs174575	*C/G*	24	0.15 (0.04)	16	0.19 (0.06)	**0.017**	21	0.17 (0.06)	14	0.17 (0.05)	0.943	0.276	0.315
FADS2	rs174583	*C/T*	19	0.15 (0.04)	27	0.18 (0.05)	**0.036**	13	0.15 (0.04)	25	0.18 (0.05)	0.130	0.967	0.907
FADS2	rs99780	*C/T*	15	0.15 (0.04)	26	0.18 (0.05)	**0.037**	12	0.15 (0.05)	22	0.18 (0.05)	0.076	0.953	0.961
FADS2	rs174602	*T/C*	19	0.16 (0.04)	13	0.15 (0.05)	0.678	23	0.17 (0.05)	14	0.18 (0.03)	0.811	0.323	0.124
AA:LA														
FADS2	rs1535	*A/G*	20	0.45 (0.08)	25	0.39 (0.09)	**0.025**	13	0.47 (0.07)	23	0.42 (0.08)	0.128	0.504	0.170
FADS2	rs174575	*C/G*	24	0.43 (0.09)	16	0.40 (0.08)	0.301	21	0.45 (0.07)	14	0.43 (0.10)	0.521	0.368	0.320
FADS2	rs174583	*C/T*	19	0.44 (0.08)	27	0.38 (0.08)	**0.019**	13	0.47 (0.07)	25	0.42 (0.08)	0.098	0.447	0.109
FADS2	rs99780	*C/T*	15	0.44 (0.09)	26	0.40 (0.09)	0.152	12	0.46 (0.07)	22	0.43 (0.09)	0.435	0.549	0.144
FADS2	rs174602	*T/C*	19	0.41 (0.07)	13	0.42 (0.09)	0.730	23	0.46 (0.11)	14	0.42 (0.11)	0.351	0.111	0.928
DPAn6:AA														
FADS2	rs1535	*A/G*	20	0.05 (0.01)	25	0.05 (0.01)	0.178	13	0.05 (0.02)	23	0.05 (0.02)	0.428	0.943	0.765
FADS2	rs174575	*C/G*	24	0.05 (0.01)	16	0.05 (0.02)	0.999	21	0.05 (0.01)	14	0.05 (0.02)	0.627	0.690	0.863
FADS2	rs174583	*C/T*	19	0.05 (0.01)	27	0.05 (0.01)	0.371	13	0.05 (0.02)	25	0.05 (0.02)	0.470	0.787	0.813
FADS2	rs99780	*C/T*	15	0.05 (0.01)	26	0.05 (0.01)	0.364	12	0.05 (0.02)	22	0.05 (0.02)	0.359	0.762	0.987
FADS2	rs174602	*T/C*	19	0.05 (0.01)	13	0.05 (0.02)	0.979	23	0.05 (0.01)	14	0.05 (0.01)	0.731	0.621	0.533
DPAn6:AdA														
FADS2	rs1535	*A/G*	20	1.27 (0.17)	24	1.25 (0.29)	0.839	13	1.22 (0.24)	22	1.25 (0.22)	0.721	0.560	0.980
FADS2	rs174575	*C/G*	24	1.28 (0.24)	15	1.21 (0.27)	0.354	21	1.25 (0.22)	13	1.26 (0.23)	0.880	0.276	0.775
FADS2	rs174583	*C/T*	19	1.28 (0.17)	26	1.24 (0.28)	0.566	13	1.22 (0.24)	24	1.25 (0.23)	0.766	0.455	0.868
FADS2	rs99780	*C/T*	15	1.29 (0.16)	25	1.23 (0.27)	0.441	12	1.22 (0.25)	21	1.25 (0.22)	0.759	0.371	0.847
FADS2	rs174602	*T/C*	19	1.28 (0.22)	12	1.27 (0.27)	0.939	22	1.20 (0.23)	14	0.20 (0.18)	0.959	0.285	0.475
EPA:ALA														
FADS2	rs1535	*A/G*	19	2.74 (1.24)	22	2.24 (0.98)	0.162	11	2.31 (1.11)	23	2.36 (1.38)	0.923	0.361	0.738
FADS2	rs174575	*C/G*	23	2.43 (1.19)	13	2.18 (0.78)	0.509	19	2.12 (0.91)	14	2.65 (1.69)	0.256	0.365	0.369
FADS2	rs174583	*C/T*	19	2.74 (1.24)	23	2.26 (0.96)	0.167	11	2.31 (1.11)	25	2.30 (1.34)	0.967	0.361	0.910
FADS2	rs99780	*C/T*	15	2.40 (1.07)	23	2.49 (1.21)	0.808	10	2.24 (1.14)	22	2.44 (1.40)	0.688	0.726	0.903
FADS2	rs174602	*T/C*	18	2.39 (1.07)	11	2.52 (0.87)	0.729	23	2.29 (1.03)	13	2.42 (1.24)	0.738	0.754	0.811
DHA:EPA														
FADS2	rs1535	*A/G*	20	15.02 (5.64)	25	14.66 (5.92)	0.840	13	19.69 (6.63)	23	20.89 (7.87)	0.655	**0.045**	**0.004†**
FADS2	rs174575	*C/G*	24	16.41 (5.99)	16	13.41 (5.21)	0.119	21	20.99 (6.36)	14	20.27 (8.32)	0.778	**0.018**	**0.012**
FADS2	rs174583	*C/T*	19	15.02 (5.64)	27	14.48 (5.71)	0.753	13	19.69 (6.63)	25	20.58 (7.89)	0.737	**0.045**	**0.003†**
FADS2	rs99780	*C/T*	15	16.22 (5.52)	26	13.71 (4.76)	0.138	12	20.50 (6.29)	22	20.19 (8.14)	0.911	0.078	**0.002†**
FADS2	rs174602	*T/C*	19	16.06 (5.17)	13	14.76 (6.12)	0.531	23	19.97 (6.82)	14	18.02 (6.37)	0.393	**0.046**	0.199
DHA:DPAn3														
FADS2	rs1535	*A/G*	20	10.84 (1.96)	25	10.03 (2.40)	0.232	13	11.14 (2.26)	23	11.58 (2.26)	0.577	0.687	**0.027**
FADS2	rs174575	*C/G*	24	10.58 (2.21)	16	10.15 (2.39)	0.574	21	11.23 (2.29)	14	12.20 (2.28)	0.230	0.334	**0.026**
FADS2	rs174583	*C/T*	19	10.76 (1.98)	27	10.13 (2.33)	0.350	13	11.14 (2.26)	25	11.53 (2.50)	0.642	0.613	**0.044**
FADS2	rs99780	*C/T*	15	11.04 (2.07)	26	9.83 (2.17)	0.090	12	11.42 (2.11)	22	11.41 (2.45)	0.990	0.640	**0.024**
FADS2	rs174602	*T/C*	19	11.04 (2.08)	13	10.67 (2.43)	0.641	23	11.14 (2.75)	14	11.40 (2.50)	0.768	0.902	0.443

P-value derived from global ANOVA and significance level ($p \leq 0.004$) was adjusted for multiple testing by Bonferroni correction. Data are means of FAs expressed as percentages of the total phospholipid profile (standard error). P-values <0.05 are highlighted in bold and significant associations that persisted after Bonferroni correction are additionally denoted by stars or daggers ($p \leq 0.004$). *Indicates significant differences within each group of weight and † Indicates significant differences between groups of weight. Major allele: M; minor allele: m; LA: Linoleic Acid; GLA: γ-Linolenic Acid; DGLA: Dihomo-γ-Linolenic Acid; AA: Arachidonic Acid; AdA: Adrenic Acid; DPAn6: Docosapentaenoic acid n6; ALA: α-linolenic Acid; EPA: Eicosapentaenoic acid; DPAn3: Docosapentaenoic acid n3; DHA: Docosahexaenoic acid.

S4 Table c). Fatty acid indexes according to *ELOVL* SNPs.

Gene	SNP *Major/minor allele*		NORMAL-WEIGHT					OVERWEIGHT/OBESITY					P (*MM*)	P (*Mm+mm*)
			MM		*Mm+mm*		P	*MM*		*Mm+mm*		P		
			N	Mean (SD)	N	Mean (SD)		N	Mean (SD)	N	Mean (SD)			
***ELOVL2* indexes**														
DPAn6:AdA														
ELOVL2	rs2236212	*G/C*	14	1.30 (0.29)	29	1.27 (0.25)	0.732	13	1.26 (0.18)	22	1.23 (0.26)	0.726	0.654	0.562
ELOVL2	rs3798713	*G/C*	11	1.30 (0.28)	31	1.24 (0.21)	0.438	12	1.26 (0.19)	25	1.23 (0.25)	0.724	0.659	0.889
ELOVL2	rs953413	*A/G*	10	1.19 (0.28)	26	1.29 (0.23)	0.261	7	1.30 (0.28)	20	1.23 (0.21)	0.527	0.442	0.386
DPAn3:EPA														
ELOVL2	rs2236212	*G/C*	14	1.51 (0.51)	29	1.48 (0.67)	0.876	13	1.66 (0.71)	22	1.88 (0.71)	0.388	0.530	**0.047**
ELOVL2	rs3798713	*G/C*	11	1.48 (0.52)	31	1.44 (0.67)	0.863	12	1.67 (0.74)	25	1.88 (0.70)	0.410	0.476	**0.021**
ELOVL2	rs953413	*A/G*	10	1.64 (0.73)	26	1.46 (0.62)	0.479	7	2.11 (1.01)	20	1.73 (0.63)	0.244	0.278	0.166
DHA:EPA														
ELOVL2	rs2236212	*G/C*	14	16.39 (6.29)	28	14.45 (5.43)	0.307	13	20.58 (8.33)	22	20.05 (7.02)	0.841	0.151	**0.003†**
ELOVL2	rs3798713	*G/C*	11	15.48 (5.10)	30	13.96 (5.33)	0.418	12	20.76 (8.67)	24	20.13 (7.07)	0.819	0.094	**<0.001†**
ELOVL2	rs953413	*A/G*	10	15.33 (5.93)	25	14.78 (5.23)	0.789	7	21.14 (7.60)	20	20.88 (7.57)	0.939	0.096	**0.003†**
DHA:DPAn3														
ELOVL2	rs2236212	*G/C*	14	10.87 (2.20)	29	10.14 (2.32)	0.337	13	12.57 (1.53)	22	10.77 (2.67)	**0.032**	**0.029**	0.375
ELOVL2	rs3798713	*G/C*	11	10.68 (2.24)	31	10.10 (2.20)	0.460	12	12.64 (1.58)	25	10.78 (2.55)	**0.028**	**0.024**	0.291
ELOVL2	rs953413	*A/G*	10	9.83 (2.22)	26	10.38 (1.99)	0.478	7	10.68 (2.80)	20	12.04 (2.09)	0.183	0.493	**0.008**
***ELOVL5* indexes**														
DGLA:LA														
ELOVL5	rs2397142	*C/G*	24	0.17 (0.05)	21	0.16 (0.04)	0.380	19	0.17 (0.06)	17	0.17 (0.04)	0.786	0.730	0.424
ELOVL5	rs9395855	*T/G*	6	0.19 (0.04)	25	0.16 (0.04)	0.246	8	0.17 (0.04)	21	0.17 (0.06)	0.930	0.579	0.593
DGLA:GLA														
ELOVL5	rs2397142	*C/G*	22	86.68 (33.02)	21	75.75 (33.15)	0.436	19	76.97 (35.87)	15	75.73 (18.30)	0.904	0.537	0.999
ELOVL5	rs9395855	*T/G*	6	87.14 (52.95)	23	73.81 (30.40)	0.422	7	75.33 (21.41)	20	77.97 (34.17)	0.850	0.597	0.675
AA:LA														
ELOVL5	rs2397142	*C/G*	24	0.42 (0.08)	21	0.39 (0.09)	0.207	19	0.45 (0.08)	17	0.42 (0.08)	0.285	0.252	0.235
ELOVL5	rs9395855	*T/G*	6	0.39 (0.14)	25	0.42 (0.09)	0.504	8	0.41 (0.07)	21	0.46 (0.08)	0.174	0.760	0.188
AdA:AA														
ELOVL5	rs2397142	*C/G*	24	0.04 (0.01)	21	0.04 (0.01)	0.934	18	0.04 (0.01)	17	0.04 (0.01)	0.469	0.367	0.937
ELOVL5	rs9395855	*T/G*	6	0.04 (0.01)	25	0.04 (0.01)	0.653	8	0.04 (0.01)	20	0.04 (0.01)	0.197	0.917	0.252
EPA:ALA														
ELOVL5	rs2397142	*C/G*	23	2.50 (1.13)	19	2.44 (1.12)	0.858	18	2.04 (0.74)	16	2.61 (1.69)	0.202	0.142	0.719
ELOVL5	rs9395855	*T/G*	5	2.16 (0.50)	25	2.41 (1.27)	0.673	8	2.97 (2.13)	19	2.28 (0.91)	0.241	0.425	0.717

P-value derived from global ANOVA and significance level ($p \leq 0.004$) was adjusted for multiple testing by Bonferroni correction. Data are means of FAs expressed as percentages of the total phospholipid profile (standard error). P-values <0.05 are highlighted in bold and significant associations that persisted after Bonferroni correction are additionally denoted by stars or daggers ($p \leq 0.004$). *Indicates significant differences within each group of weight and † Indicates significant differences between groups of weight. Major allele: M; minor allele: m; LA: Linoleic Acid; GLA: γ-Linolenic Acid; DGLA: Dihomo-γ-Linolenic Acid; AA: Arachidonic Acid; AdA: Adrenic Acid; DPAn6: Docosapentaenoic acid n6; ALA: α-linolenic Acid; EPA: Eicosapentaenoic acid; DPAn3: Docosapentaenoic acid n3; DHA: Docosahexaenoic acid.

S4 Table d). Fatty acids involved in *FADS1* indexes.

Gene	SNP Major/minor allele		NORMAL-WEIGHT MM N	MM Mean (SD)	Mm+mm N	Mm+mm Mean (SD)	P	OVERWEIGHT/OBESITY MM N	MM Mean (SD)	Mm+mm N	Mm+mm Mean (SD)	P	P (MM)	P (Mm+mm)
Fatty acids involved in *FADS1* indexes														
C18:2n-6 (LA)														
FADS1	rs174537	*G/T*	15	23.77 (2.02)	23	23.56 (2.39)	0.782	12	23.35 (2.12)	21	23.50 (2.42)	0.855	0.603	0.937
FADS1	rs174545	*C/G*	22	23.11 (2.19)	27	23.58 (2.21)	0.460	13	23.13 (2.17)	22	23.93 (2.50)	0.345	0.975	0.602
FADS1	rs174546	*C/T*	22	23.11 (2.19)	27	23.58 (2.21)	0.460	13	23.13 (2.17)	25	23.84 (2.41)	0.380	0.975	0.684
FADS1	rs174548	*C/G*	24	23.26 (2.17)	25	23.47 (2.25)	0.736	13	23.60 (2.30)	25	23.60 (2.39)	0.998	0.657	0.848
FADS1	rs174553	*A/G*	22	23.11 (2.19)	27	23.58 (2.21)	0.460	13	23.13 (2.17)	25	23.84 (2.41)	0.380	0.975	0.684
FADS1	rs174561	*T/C*	15	23.92 (1.79)	14	23.11 (2.47)	0.321	20	23.43 (2.52)	17	23.80 (2.97)	0.683	0.524	0.497
FADS1	rs174547	*T/C*	15	23.39 (2.22)	23	23.66 (2.32)	0.730	12	23.35 (2.12)	20	23.33 (2.35)	0.989	0.955	0.652
C20:3n-6 (DGLA)														
FADS1	rs174537	*G/T*	15	3.51 (0.81)	23	4.27 (0.85)	**0.010**	12	3.31 (0.80)	21	4.18 (0.96)	**0.012**	0.529	0.754
FADS1	rs174545	*C/G*	22	3.50 (0.79)	27	4.14 (0.86)	**0.009**	13	3.39 (0.81)	22	4.15 (0.94)	**0.021**	0.691	0.976
FADS1	rs174546	*C/T*	22	3.50 (0.79)	27	4.14 (0.86)	**0.009**	13	3.39 (0.81)	25	4.11 (0.90)	**0.021**	0.691	0.893
FADS1	rs174548	*C/G*	24	3.59 (0.79)	25	4.10 (0.93)	**0.041**	13	3.38 (0.81)	25	4.11 (0.90)	**0.019**	0.431	0.969
FADS1	rs174553	*A/G*	22	3.50 (0.79)	27	4.14 (0.86)	**0.009**	13	3.39 (0.81)	25	4.11 (0.90)	**0.021**	0.691	0.893
FADS1	rs174561	*T/C*	15	3.70 (0.90)	14	3.63 (0.83)	0.828	20	3.99 (0.64)	17	4.09 (0.76)	0.662	0.270	0.115
FADS1	rs174547	*T/C*	15	3.61 (0.78)	23	4.17 (0.76)	**0.036**	12	3.31 (0.80)	20	4.17 (0.98)	**0.016**	0.335	0.980
C20:4n-6 (AA)														
FADS1	rs174537	*G/T*	15	10.55 (1.33)	23	9.02 (1.54)	**0.003***	12	10.53 (0.89)	21	10.04 (1.36)	0.273	0.973	**0.026**
FADS1	rs174545	*C/G*	22	10.50 (1.39)	27	8.96 (1.49)	**0.001***	13	10.64 (0.93)	22	9.83 (1.31)	0.061	0.759	**0.037**
FADS1	rs174546	*C/T*	22	10.50 (1.39)	27	8.96 (1.49)	**0.001***	13	10.64 (0.93)	25	9.90 (1.31)	0.080	0.759	**0.019**
FADS1	rs174548	*C/G*	24	10.28 (1.39)	25	9.05 (1.64)	**0.007**	13	10.40 (1.02)	25	10.03 (1.33)	0.389	0.790	**0.025**
FADS1	rs174553	*A/G*	22	10.50 (1.39)	27	8.96 (1.49)	**0.001***	13	10.64 (0.93)	25	9.90 (1.31)	0.080	0.759	**0.019**
FADS1	rs174561	*T/C*	15	9.88 (1.32)	14	9.30 (1.12)	0.212	20	10.27 (1.48)	17	10.33 (1.54)	0.910	0.423	**0.046**
FADS1	rs174547	*T/C*	15	10.49 (1.33)	23	9.18 (1.47)	**0.009**	12	10.53 (0.89)	20	10.00 (1.38)	0.243	0.917	0.067
C18:3n-3 (ALA)														
FADS1	rs174537	*G/T*	15	0.12 (0.04)	23	0.16 (0.04)	**0.021**	12	0.11 (0.03)	21	0.11 (0.04)	0.632	0.496	**<0.001†**
FADS1	rs174545	*C/G*	22	0.12 (0.04)	27	0.15 (0.04)	**0.018**	13	0.12 (0.03)	22	0.11 (0.04)	0.532	0.536	**0.001†**
FADS1	rs174546	*C/T*	22	0.12 (0.04)	27	0.15 (0.04)	**0.018**	13	0.12 (0.03)	25	0.11 (0.04)	0.580	0.536	**0.001†**
FADS1	rs174548	*C/G*	24	0.13 (0.04)	22	0.15 (0.04)	0.146	12	0.12 (0.03)	25	0.11 (0.04)	0.454	0.371	**0.001**
FADS1	rs174553	*A/G*	22	0.12 (0.04)	27	0.15 (0.04)	**0.018**	13	0.12 (0.03)	25	0.11 (0.04)	0.580	0.536	**0.001†**
FADS1	rs174561	*T/C*	15	0.13 (0.04)	14	0.13 (0.03)	0.695	20	0.12 (0.05)	17	0.11 (0.03)	0.497	0.397	**0.024**
FADS1	rs174547	*T/C*	15	0.12 (0.04)	23	0.15 (0.04)	**0.027**	12	0.11 (0.03)	20	0.11 (0.04)	0.734	0.444	**<0.001†**
C20:5n3 (EPA)														
FADS1	rs174537	*G/T*	15	0.31 (0.16)	23	0.33 (0.14)	0.683	11	0.25 (0.11)	21	0.26 (0.20)	0.919	0.254	0.140
FADS1	rs174545	*C/G*	21	0.33 (0.14)	27	0.33 (0.13)	0.922	12	0.26 (0.10)	22	0.26 (0.20)	0.999	0.126	0.112
FADS1	rs174546	*C/T*	21	0.33 (0.14)	27	0.33 (0.13)	0.922	12	0.26 (0.10)	25	0.25 (0.19)	0.937	0.126	0.074
FADS1	rs174548	*C/G*	23	0.32 (0.13)	25	0.34 (0.14)	0.646	12	0.26 (0.10)	25	0.25 (0.19)	0.937	0.145	0.063
FADS1	rs174553	*A/G*	21	0.33 (0.14)	27	0.33 (0.13)	0.922	12	0.26 (0.10)	25	0.25 (0.19)	0.937	0.126	0.074
FADS1	rs174561	*T/C*	15	0.33 (0.14)	13	0.31 (0.11)	0.683	20	0.26 (0.11)	17	0.23 (0.10)	0.491	0.092	**0.044**
FADS1	rs174547	*T/C*	15	0.33 (0.16)	23	0.32 (0.12)	0.815	11	0.25 (0.11)	20	0.26 (0.20)	0.863	0.160	0.250

P-value derived from global ANOVA and significance level ($p \leq 0.004$) was adjusted for multiple testing by Bonferroni correction. Data are means of FAs expressed as percentages of the total phospholipid profile (standard error). P-values <0.05 are highlighted in bold and significant associations that persisted after Bonferroni correction are additionally denoted by stars or daggers ($p \leq 0.004$). *Indicates significant differences within each group of weight and † Indicates significant differences between groups of weight. Major allele: M; minor allele: m; LA: Linoleic Acid; DGLA: Dihomo-γ-Linolenic Acid; AA: Arachidonic Acid; ALA: α-linolenic Acid; EPA: Eicosapentaenoic acid.

S4 Table e). Fatty acids involved in *FADS2* indexes.

Gene	SNP Major/minor allele		NORMAL-WEIGHT					OVERWEIGHT/OBESITY					P (*MM*)	P (*Mm+mm*)
			MM		*Mm+mm*		P	*MM*		*Mm+mm*		P		
			N	Mean (SD)	N	Mean (SD)		N	Mean (SD)	N	Mean (SD)			
Fatty acids involved in *FADS2* indexes														
C18:2n-6 (LA)														
FADS2	rs1535	*A/G*	20	23.35 (2.15)	25	23.56 (2.30)	0.751	13	23.13 (2.17)	23	23.81 (2.52)	0.425	0.781	0.728
FADS2	rs174575	*C/G*	24	23.82 (2.06)	16	23.06 (2.41)	0.291	21	23.39 (2.37)	14	23.61 (2.39)	0.795	0.519	0.538
FADS2	rs174583	*C/T*	19	23.54 (2.02)	27	23.58 (2.21)	0.955	13	23.13 (2.17)	25	23.84 (2.41)	0.380	0.588	0.684
FADS2	rs99780	*C/T*	15	23.98 (1.98)	26	23.36 (2.35)	0.396	12	23.32 (2.15)	22	23.39 (2.43)	0.932	0.412	0.970
FADS2	rs174602	*T/C*	19	23.89 (2.07)	13	23.27 (2.39)	0.443	23	23.38 (3.02)	14	23.95 (2.15)	0.545	0.541	0.444
C18:3n-6 (GLA)														
FADS2	rs1535	*A/G*	19	0.05 (0.02)	24	0.06 (0.02)	0.349	12	0.06 (0.01)	23	0.05 (0.02)	0.685	0.525	0.460
FADS2	rs174575	*C/G*	23	0.05 (0.02)	15	0.06 (0.03)	0.493	20	0.05 (0.02)	14	0.06 (0.02)	0.164	0.645	0.942
FADS2	rs174583	*C/T*	18	0.05 (0.02)	26	0.06 (0.02)	0.660	12	0.06 (0.01)	25	0.05 (0.02)	0.765	0.648	0.767
FADS2	rs99780	*C/T*	15	0.06 (0.02)	25	0.06 (0.02)	0.840	11	0.05 (0.01)	22	0.05 (0.02)	0.939	0.810	0.653
FADS2	rs174602	*T/C*	18	0.06 (0.02)	13	0.06 (0.02)	0.883	23	0.05 (0.02)	13	0.06 (0.02)	0.229	0.307	0.723
C20:3n-6 (DGLA)														
FADS2	rs1535	*A/G*	20	3.41 (0.77)	25	4.23 (0.82)	**0.001***	13	3.39 (0.81)	23	4.15 (0.92)	**0.019**	0.927	0.736
FADS2	rs174575	*C/G*	24	3.57 (0.71)	16	4.28 (0.97)	**0.012**	21	3.82 (1.30)	14	3.90 (0.90)	0.805	0.358	0.283
FADS2	rs174583	*C/T*	19	3.47 (0.75)	27	4.14 (0.86)	**0.009**	13	3.39 (0.81)	25	4.11 (0.90)	**0.021**	0.762	0.893
FADS2	rs99780	*C/T*	15	3.51 (0.78)	26	4.16 (0.89)	**0.025**	12	3.35 (0.84)	22	4.14 (0.94)	**0.022**	0.613	0.941
FADS2	rs174602	*T/C*	19	3.77 (0.82)	13	3.50 (0.85)	0.387	23	3.95 (0.76)	14	4.19 (0.54)	0.297	0.463	**0.018**
C20:4n-6 (AA)														
FADS2	rs1535	*A/G*	20	10.31 (1.30)	25	9.07 (1.50)	**0.005**	13	10.64 (0.93)	23	9.92 (1.35)	0.102	0.439	**0.044**
FADS2	rs174575	*C/G*	24	10.02 (1.55)	15	9.12 (1.30)	0.063	21	10.37 (0.97)	14	10.01 (1.64)	0.413	0.374	0.109
FADS2	rs174583	*C/T*	19	10.33 (1.34)	27	8.96 (1.49)	**0.003***	13	10.64 (0.93)	25	9.90 (1.31)	0.080	0.482	**0.019**
FADS2	rs99780	*C/T*	15	10.39 (1.48)	26	9.15 (1.51)	**0.015**	12	10.54 (0.91)	22	10.02 (1.40)	0.254	0.757	**0.046**
FADS2	rs174602	*T/C*	19	9.73 (1.34)	13	9.66 (1.23)	0.874	23	10.48 (1.39)	14	10.00 (1.66)	0.347	0.085	0.553
C22:4n-6 (AdA)														
FADS2	rs1535	*A/G*	20	0.39 (0.07)	25	0.40 (0.10)	0.742	13	0.41 (0.10)	22	0.40 (0.09)	0.781	0.430	0.817
FADS2	rs174575	*C/G*	24	0.40 (0.07)	16	0.40 (0.12)	0.946	21	0.42 (0.10)	13	0.39 (0.09)	0.459	0.575	0.543
FADS2	rs174583	*C/T*	19	0.39 (0.07)	27	0.39 (0.10)	0.865	13	0.41 (0.10)	24	0.40 (0.09)	0.753	0.553	0.644
FADS2	rs99780	*C/T*	15	0.39 (0.07)	26	0.40 (0.10)	0.685	12	0.40 (0.10)	21	0.41 (0.09)	0.784	0.649	0.651
FADS2	rs174602	*T/C*	19	0.39 (0.13)	12	0.39 (0.09)	0.962	22	0.42 (0.09)	14	0.39 (0.08)	0.318	0.467	0.926
C22:5n-6 (DPAn6)														
FADS2	rs1535	*A/G*	20	0.50 (0.14)	25	0.50 (0.14)	0.966	13	0.51 (0.17)	23	0.52 (0.15)	0.922	0.782	0.594
FADS2	rs174575	*C/G*	24	0.51 (0.14)	16	0.48 (0.15)	0.435	21	0.52 (0.14)	14	0.52 (0.19)	0.986	0.935	0.507
FADS2	rs174583	*C/T*	19	0.51 (0.13)	27	0.48 (0.14)	0.534	13	0.51 (0.17)	25	0.51 (0.15)	0.990	0.943	0.471
FADS2	rs99780	*C/T*	15	0.51 (0.14)	26	0.49 (0.14)	0.758	12	0.50 (0.18)	22	0.53 (0.16)	0.642	0.921	0.408
FADS2	rs174602	*T/C*	19	0.49 (0.14)	13	0.49 (0.16)	0.942	23	0.51 (0.15)	14	0.47 (0.12)	0.361	0.692	0.686
C18:3n-3 (ALA)														
FADS2	rs1535	*A/G*	20	0.13 (0.04)	25	0.15 (0.04)	**0.025**	13	0.12 (0.03)	23	0.11 (0.04)	0.448	0.500	**<0.001†**
FADS2	rs174575	*C/G*	24	0.13 (0.04)	16	0.16 (0.04)	**0.013**	21	0.11 (0.03)	14	0.11 (0.03)	0.972	**0.035**	**<0.001†**
FADS2	rs174583	*C/T*	19	0.13 (0.04)	27	0.15 (0.04)	**0.033**	13	0.12 (0.03)	25	0.11 (0.04)	0.580	0.498	**0.001†**
FADS2	rs99780	*C/T*	15	0.13 (0.04)	26	0.15 (0.04)	0.060	12	0.12 (0.03)	22	0.11 (0.04)	0.584	0.576	**0.001†**
FADS2	rs174602	*T/C*	18	0.13 (0.04)	12	0.14 (0.034)	0.539	23	0.11 (0.04)	13	0.11 (0.04)	0.684	0.133	0.142
C20:5n-3 (EPA)														
FADS2	rs1535	*A/G*	19	0.33 (0.15)	25	0.33 (0.14)	0.912	12	0.26 (0.10)	23	0.25 (0.19)	0.975	0.132	0.136
FADS2	rs174575	*C/G*	24	0.30 (0.14)	16	0.37 (0.13)	0.129	20	0.22 (0.10)	14	0.28 (0.22)	0.293	0.061	0.220
FADS2	rs174583	*C/T*	19	0.33 (0.15)	27	0.33 (0.13)	0.974	12	0.26 (0.10)	25	0.25 (0.19)	0.937	0.132	0.074
FADS2	rs99780	*C/T*	15	0.29 (0.14)	26	0.35 (0.14)	0.212	11	0.25 (0.10)	22	0.27 (0.19)	0.748	0.388	0.108
FADS2	rs174602	*T/C*	19	0.31 (0.14)	12	0.32 (0.10)	0.780	23	0.23 (0.09)	14	0.27 (0.13)	0.347	**0.040**	0.241
C22:5n-3 (DPAn3)														
FADS2	rs1535	*A/G*	20	0.41 (0.10)	25	0.42 (0.06)	0.730	13	0.42 (0.11)	23	0.37 (0.10)	0.190	0.887	**0.034**
FADS2	rs174575	*C/G*	24	0.40 (0.09)	16	0.44 (0.05)	0.056	21	0.39 (0.11)	14	0.37 (0.10)	0.632	0.872	**0.033**
FADS2	rs174583	*C/T*	19	0.40 (0.10)	27	0.42 (0.06)	0.388	13	0.42 (0.11)	25	0.37 (0.10)	0.158	0.674	**0.018**
FADS2	rs99780	*C/T*	15	0.38 (0.07)	26	0.44 (0.08)	**0.017**	12	0.42 (0.12)	22	0.39 (0.10)	0.429	0.297	**0.043**
FADS2	rs174602	*T/C*	19	0.40 (0.10)	13	0.43 (0.08)	0.386	23	0.38 (0.10)	14	0.38 (0.10)	0.805	0.641	0.158
C22:6n-3 (DHA)														
FADS2	rs1535	*A/G*	20	4.48 (1.35)	25	4.23 (1.04)	0.496	13	4.56 (0.98)	23	4.18 (0.93)	0.255	0.841	0.869
FADS2	rs174575	*C/G*	24	4.19 (1.22)	16	4.50 (1.02)	0.400	21	4.25 (0.92)	14	4.44 (1.01)	0.573	0.856	0.859
FADS2	rs174583	*C/T*	19	4.31 (1.16)	27	4.29 (1.06)	0.955	13	4.56 (0.98)	25	4.11 (0.93)	0.169	0.528	0.511
FADS2	rs99780	*C/T*	15	4.14 (0.95)	26	4.33 (1.19)	0.599	12	4.64 (0.98)	22	4.26 (0.88)	0.260	0.188	0.835
FADS2	rs174602	*T/C*	19	4.32 (1.09)	13	4.52 (1.22)	0.635	23	4.10 (0.91)	14	4.19 (1.02)	0.794	0.480	0.450

P-value derived from global ANOVA and significance level ($p \leq 0.004$) was adjusted for multiple testing by Bonferroni correction. Data are means of FAs expressed as percentages of the total phospholipid profile (standard error). P-values <0.05 are highlighted in bold and significant associations that persisted after Bonferroni correction are additionally denoted by stars or daggers ($p \leq 0.004$). *Indicates significant differences within each group of weight and † Indicates significant differences between groups of weight. Major allele: M; minor allele: m; LA: Linoleic Acid; GLA: γ-Linolenic Acid; DGLA: Dihomo-γ-Linolenic Acid; AA: Arachidonic Acid; AdA: Adrenic Acid; DPAn6: Docosapentaenoic acid n6; ALA: α-linolenic Acid; EPA: Eicosapentaenoic acid; DPAn3: Docosapentaenoic acid n3; DHA: Docosahexaenoic acid.

S4 Table f). Fatty acids involved in *ELOVL* indexes.

Gene	SNP *Major/minor allele*		NORMAL-WEIGHT *MM* N	Mean (SD)	*Mm+mm* N	Mean (SD)	P	OVERWEIGHT/OBESITY *MM* N	Mean (SD)	*Mm+mm* N	Mean (SD)	P	P (*MM*)	P (*Mm+mm*)
Fatty acids involved in *ELOVL2* indexes														
C22:4n-6 (AdA)														
ELOVL2	rs2236212	*G/C*	14	0.38 (0.06)	29	0.41 (0.10)	0.396	13	0.38 (0.11)	22	0.42 (0.09)	0.253	0.941	0.540
ELOVL2	rs3798713	*G/C*	11	0.37 (0.07)	31	0.40 (0.10)	0.363	12	0.39 (0.11)	25	0.42 (0.08)	0.377	0.739	0.576
ELOVL2	rs953413	*A/G*	10	0.43 (0.14)	26	0.39 (0.06)	0.225	7	0.46 (0.11)	20	0.39 (0.09)	0.139	0.714	0.957
C22:5n-6 (DPAn6)														
ELOVL2	rs2236212	*G/C*	14	0.49 (0.12)	29	0.52 (0.16)	0.538	13	0.49 (0.16)	22	0.53 (0.16)	0.456	0.957	0.832
ELOVL2	rs3798713	*G/C*	11	0.48 (0.11)	31	0.50 (0.14)	0.652	12	0.49 (0.17)	25	0.52 (0.15)	0.595	0.851	0.618
ELOVL2	rs953413	*A/G*	10	0.50 (0.16)	26	0.51 (0.13)	0.898	7	0.60 (0.13)	20	0.49 (0.16)	0.113	0.207	0.635
C20:5n-3 (EPA)														
ELOVL2	rs2236212	*G/C*	14	0.31 (0.12)	29	0.33 (0.15)	0.776	13	0.27 (0.23)	22	0.24 (0.11)	0.612	0.571	**0.032**
ELOVL2	rs3798713	*G/C*	11	0.32 (0.13)	31	0.34 (0.15)	0.765	12	0.28 (0.24)	25	0.24 (0.11)	0.490	0.626	**0.011**
ELOVL2	rs953413	*A/G*	10	0.30 (0.16)	26	0.32 (0.13)	0.611	7	0.22 (0.11)	20	0.27 (0.19)	0.513	0.258	0.243
C22:5n-3 (DPAn3)														
ELOVL2	rs2236212	*G/C*	14	0.43 (0.10)	29	0.41 (0.07)	0.424	13	0.34 (0.06)	22	0.41 (0.12)	**0.047**	**0.011**	0.787
ELOVL2	rs3798713	*G/C*	11	0.43 (0.11)	31	0.41 (0.07)	0.465	12	0.35 (0.06)	25	0.41 (0.12)	0.099	**0.033**	0.992
ELOVL2	rs953413	*A/G*	10	0.39 (0.06)	26	0.41 (0.09)	0.475	7	0.38 (0.10)	20	0.39 (0.11)	0.758	0.711	0.466
C22:6n-3 (DHA)														
ELOVL2	rs2236212	*G/C*	14	4.64 (1.32)	29	4.10 (0.98)	0.139	13	4.30 (0.99)	22	4.28 (0.97)	0.942	0.466	0.514
ELOVL2	rs3798713	*G/C*	11	4.60 (1.49)	31	4.10 (0.91)	0.200	12	4.41 (0.94)	25	4.24 (0.96)	0.617	0.728	0.565
ELOVL2	rs953413	*A/G*	10	3.82 (0.83)	26	4.31 (1.22)	0.250	7	3.85 (0.73)	20	4.61 (0.92)	0.059	0.929	0.357
Fatty acids involved in *ELOVL5* indexes														
C18:2n6 (LA)														
ELOVL5	rs2397142	*C/G*	24	23.49 (2.14)	22	23.65 (2.13)	0.806	19	23.41 (2.47)	17	23.57 (2.20)	0.839	0.906	0.911
ELOVL5	rs9395855	*T/G*	7	23.31 (2.70)	25	23.76 (2.36)	0.668	8	23.54 (2.04)	21	23.02 (2.38)	0.587	0.854	0.294
C18:3n6 (GLA)														
ELOVL5	rs2397142	*C/G*	22	0.05 (0.02)	22	0.06 (0.02)	0.684	19	0.05 (0.02)	16	0.05 (0.02)	0.787	0.787	0.571
ELOVL5	rs9395855	*T/G*	7	0.06 (0.03)	23	0.06 (0.02)	0.990	8	0.05 (0.02)	20	0.05 (0.02)	0.653	0.557	0.496
C20:3n-6 (DGLA)														
ELOVL5	rs2397142	*C/G*	24	3.96 (0.86)	22	3.76 (0.89)	0.450	19	3.78 (1.17)	17	3.95 (0.66)	0.554	0.554	0.469
ELOVL5	rs9395855	*T/G*	7	4.38 (0.87)	25	3.82 (0.77)	0.107	8	4.05 (0.88)	21	3.86 (1.08)	0.663	0.479	0.881
C20:4n-6 (AA)														
ELOVL5	rs2397142	*C/G*	24	9.87 (1.58)	22	9.16 (1.51)	0.128	19	10.46 (1.16)	17	9.90 (1.29)	0.176	0.176	0.112
ELOVL5	rs9395855	*T/G*	7	9.10 (1.86)	25	9.93 (1.52)	0.232	8	9.63 (1.26)	21	10.39 (1.21)	0.145	0.528	0.273
C22:4n-6 (AdA)														
ELOVL5	rs2397142	*C/G*	24	0.40 (0.07)	22	0.38 (0.11)	0.402	18	0.40 (0.08)	17	0.41 (0.10)	0.977	0.977	0.420
ELOVL5	rs9395855	*T/G*	7	0.39 (0.07)	25	0.41 (0.10)	0.608	8	0.43 (0.13)	20	0.40 (0.08)	0.452	0.524	0.566
C18:3n-3 (ALA)														
ELOVL5	rs2397142	*C/G*	23	0.13 (0.04)	20	0.15 (0.04)	0.168	19	0.11 (0.03)	16	0.11 (0.04)	0.605	**0.022**	**0.016**
ELOVL5	rs9395855	*T/G*	6	0.16 (0.05)	25	0.14 (0.04)	0.405	8	0.10 (0.02)	20	0.11 (0.04)	0.519	**0.010**	**0.047**
C20:5n-3 (EPA)														
ELOVL5	rs2397142	*C/G*	24	0.33 (0.14)	22	0.34 (0.14)	0.855	18	0.21 (0.09)	17	0.29 (0.22)	0.168	**0.004†**	0.411
ELOVL5	rs9395855	*T/G*	7	0.30 (0.15)	25	0.31 (0.13)	0.916	8	0.33 (0.29)	20	0.25 (0.11)	0.280	0.844	0.089

P-value derived from global ANOVA and significance level ($p \leq 0.004$) was adjusted for multiple testing by Bonferroni correction. Data are means of FAs expressed as percentages of the total phospholipid profile (standard error). P-values <0.05 are highlighted in bold and significant associations that persisted after Bonferroni correction are additionally denoted by stars or daggers ($p \leq 0.004$). *Indicates significant differences within each group of weight and † Indicates significant differences between groups of weight. Major allele: M; minor allele: m; LA: Linoleic Acid; GLA: γ-Linolenic Acid; DGLA: Dihomo-γ-Linolenic Acid; AA: Arachidonic Acid; AdA: Adrenic Acid; DPAn6: Docosapentaenoic acid n6; ALA: α-linolenic Acid; EPA: Eicosapentaenoic acid; DPAn3: Docosapentaenoic acid n3; DHA: Docosahexaenoic acid.

S5 Table. Maternal EPA:AA and DHA:AA ratios in plasma according to their genotypes.

Gene	SNP *Major/minor allele*		NORMAL-WEIGHT					OVERWEIGHT/OBESITY					P (*MM*)	P (*Mm+mm*)
			MM		*Mm+mm*		P	*MM*		*Mm+mm*		P		
			N	Mean (SD)	N	Mean (SD)		N	Mean (SD)	N	Mean (SD)			
Indexes														
EPA:AA														
FADS1	rs174537	*G/T*	15	0.03 (0.02)	23	0.04 (0.02)	0.218	12	0.02 (0.01)	21	0.03 (0.02)	0.690	0.231	**0.040**
FADS1	rs174545	*C/G*	22	0.03 (0.02)	27	0.04 (0.02)	0.221	13	0.02 (0.01)	22	0.03 (0.02)	0.663	0.108	**0.031**
FADS1	rs174546	*C/T*	22	0.03 (0.02)	27	0.04 (0.02)	0.221	13	0.02 (0.01)	25	0.03 (0.02)	0.728	0.108	**0.016**
FADS1	rs174548	*C/G*	24	0.03 (0.02)	25	0.04 (0.02)	0.173	13	0.02 (0.01)	25	0.03 (0.02)	0.899	0.135	**0.013**
FADS1	rs174553	*A/G*	22	0.03 (0.02)	27	0.04 (0.02)	0.221	13	0.02 (0.01)	25	0.03 (0.02)	0.728	0.108	**0.016**
FADS1	rs174561	*T/C*	15	0.03 (0.02)	14	0.03 (0.01)	0.991	20	0.03 (0.01)	17	0.02 (0.01)	0.375	0.100	**0.010**
FADS1	rs174547	*T/C*	15	0.03 (0.02)	23	0.04 (0.01)	0.561	20	0.02 (0.01)	17	0.03 (0.02)	0.634	0.143	0.097
FADS2	rs1535	*A/G*	20	0.03 (0.02)	25	0.04 (0.02)	0.434	13	0.02 (0.01)	23	0.03 (0.02)	0.703	0.092	**0.045**
FADS2	rs174575	*C/G*	24	0.03 (0.02)	16	0.04 (0.02)	**0.029**	21	0.02 (0.01)	14	0.03 (0.02)	0.165	**0.029**	0.088
FADS2	rs174583	*C/T*	19	0.03 (0.02)	27	0.04 (0.02)	0.308	13	0.02 (0.01)	25	0.03 (0.02)	0.728	0.092	**0.016**
FADS2	rs99780	*C/T*	15	0.03 (0.02)	26	0.04 (0.02)	0.087	12	0.02 (0.01)	22	0.03 (0.02)	0.529	0.300	**0.033**
FADS2	rs174602	*T/C*	19	0.03 (0.02)	13	0.03 (0.01)	0.793	23	0.02 (0.01)	14	0.03 (0.01)	0.206	**0.014**	0.242
ELOVL2	rs2236212	*G/C*	14	0.03 (0.01)	29	0.04 (0.02)	0.605	13	0.03 (0.02)	22	0.02 (0.01)	0.427	0.517	**0.011**
ELOVL2	rs3798713	*G/C*	11	0.03 (0.01)	31	0.04 (0.02)	0.638	12	0.03 (0.02)	25	0.02 (0.01)	0.348	0.554	**0.004†**
ELOVL2	rs953413	*A/G*	10	0.03 (0.01)	26	0.03 (0.02)	0.483	7	0.02 (0.01)	20	0.03 (0.02)	0.455	0.129	0.137
ELOVL5	rs2397142	*C/G*	24	0.04 (0.02)	22	0.04 (0.02)	0.564	19	0.02 (0.01)	17	0.03 (0.02)	0.102	**0.005**	0.161
ELOVL5	rs9395855	*T/G*	7	0.04 (0.02)	25	0.03 (0.01)	0.372	8	0.03 (0.03)	21	0.02 (0.01)	0.203	0.768	**0.047**
DHA:AA														
FADS1	rs174537	*G/T*	15	0.41 (0.16)	23	0.47 (0.12)	0.241	12	0.43 (0.10)	21	0.43 (0.10)	0.995	0.839	0.197
FADS1	rs174545	*C/G*	22	0.45 (0.16)	27	0.49 (0.13)	0.287	13	0.43 (0.09)	22	0.43 (0.10)	0.988	0.772	0.091
FADS1	rs174546	*C/T*	22	0.45 (0.16)	27	0.49 (0.13)	0.287	13	0.43 (0.09)	25	0.42 (0.10)	0.753	0.772	**0.039**
FADS1	rs174548	*C/G*	24	0.43 (0.13)	25	0.51 (0.14)	**0.032**	13	0.45 (0.09)	25	0.41 (0.10)	0.276	0.586	**0.005**
FADS1	rs174553	*A/G*	22	0.45 (0.16)	27	0.49 (0.13)	0.287	13	0.43 (0.09)	25	0.42 (0.10)	0.753	0.772	**0.039**
FADS1	rs174561	*T/C*	15	0.44 (0.14)	14	0.51 (0.14)	0.242	20	0.40 (0.12)	17	0.41 (0.08)	0.809	0.350	**0.024**
FADS1	rs174547	*T/C*	15	0.42 (0.16)	23	0.47 (0.12)	0.293	12	0.43 (0.10)	20	0.43 (0.10)	0.835	0.871	0.330
FADS2	rs1535	*A/G*	20	0.45 (0.16)	25	0.47 (0.12)	0.505	13	0.43 (0.09)	23	0.43 (0.10)	0.916	0.764	0.148
FADS2	rs174575	*C/G*	24	0.43 (0.14)	16	0.51 (0.14)	0.091	21	0.41 (0.09)	14	0.45 (0.11)	0.229	0.636	0.239
FADS2	rs174583	*C/T*	19	0.43 (0.15)	27	0.49 (0.13)	0.157	13	0.43 (0.09)	25	0.42 (0.10)	0.753	0.971	**0.039**
FADS2	rs99780	*C/T*	15	0.41 (0.14)	26	0.48 (0.13)	0.118	12	0.44 (0.09)	22	0.43 (0.10)	0.796	0.521	0.163
FADS2	rs174602	*T/C*	19	0.46 (0.16)	13	0.47 (0.13)	0.760	23	0.40 (0.09)	14	0.43 (0.12)	0.387	0.117	0.322
ELOVL2	rs2236212	*G/C*	14	0.48 (0.13)	29	0.44 (0.14)	0.305	13	0.44 (0.12)	22	0.41 (0.09)	0.333	0.415	0.424
ELOVL2	rs3798713	*G/C*	11	0.48 (0.14)	31	0.44 (0.13)	0.387	12	0.46 (0.11)	25	0.41 (0.09)	0.184	0.643	0.384
ELOVL2	rs953413	*A/G*	10	0.40 (0.08)	26	0.46 (0.16)	0.308	7	0.38 (0.06)	20	0.46 (0.09)	0.055	0.588	0.921
ELOVL5	rs2397142	*C/G*	24	0.44 (0.13)	22	0.49 (0.15)	0.287	19	0.42 (0.09)	17	0.44 (0.10)	0.546	0.449	0.215
ELOVL5	rs9395855	*T/G*	7	0.49 (0.20)	25	0.43 (0.14)	0.402	8	0.44 (0.12)	21	0.44 (0.09)	0.864	0.599	0.898

P-value derived from global ANOVA and significance level was adjusted for multiple testing by Bonferroni correction. Data are means of FAs expressed as percentages of the total phospholipid profile (standard error). P-values <0.05 are highlighted in bold and significant associations that persisted after Bonferroni correction are additionally denoted by stars or daggers ($p \leq 0.004$). *Indicates significant differences within each group of weight and † Indicates significant differences between groups of weight. Major allele: M; minor allele: m; AA: Arachidonic acid; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid.

5.2. OBESITY AND FADS POLYMORPHISMS AFFECT PUFAS IN BREAST MILK – THE PREOBE FOLLOW-UP

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ABSTRACT

Few studies have explored the relationship of breast milk polyunsaturated fatty acids (PUFAs) with maternal weight and polymorphisms in genes associated with PUFA metabolism. The aim of this study was to analyze variation in breast milk PUFA composition in relation to maternal weight and fatty acid desaturase (*FADS*) genotype. Participants (n=61) from the PREOBE cohort were grouped according to their pre-pregnancy body mass index (BMI): normal-weight (n=26) and overweight/obese (n=35) women. Colostrum samples (collected 2-4 days postpartum) were used to analyze PUFAs and tag polymorphisms were genotyped (3 in *FADS1* and 3 in *FADS2*). A high BMI was associated with high n6 FA levels and low DHA:AA ratio in colostrum. Among the major allele carriers of *FADS* polymorphisms, normal-weight women had higher n3 and lower n6 FA levels than overweight/obese women. These differences, however, were not observed in the minor allele carriers since this genotype was linked to decreased *FADS* enzyme activity and, consequently, lower PUFA levels in the normal-weight group. Thus, colostrum PUFA levels in minor allele carriers were similar for normal-weight and overweight/obese women. Dietary intake of DHA in late pregnancy affected the colostrum DHA level. In conclusion, a high BMI was associated with an altered breast milk FA profile; which could be modulated by *FADS* genotypes. Unlike in normal-weight individuals, *FADS* genetic variation in overweight and obese women seems to have a beneficial effect on their FA status. Moreover, high dietary DHA intake might prevent the reduction in colostrum DHA caused by overweight or *FADS* minor alleles.

Keywords: Fatty acids, colostrum, DHA, AA, omega, SNPs.

INTRODUCTION

Maternal nutritional status could be determinant of the metabolic health of future generations (106, 107). The incidence of obesity is higher in women than men, with pregnancy being a possibly key factor influencing this prevalence (108). Obesity during pregnancy compromises the woman's nutritional status and, consequently, the nutrient supply to the fetus or neonate. It is therefore important to follow up the nutritional and health status of both mother and child to prevent further cases of obesity and obesity-related diseases (106, 109).

Adequate nutrition is crucial for the newborn since it affects growth and neurodevelopment, and it could prevent later diseases during adulthood (110, 111). Breast milk is usually the only source of nutrients for the newborn, its composition thus greatly affects the child's nutritional and health status (76, 112, 113). Some of the most studied nutrients in breast milk are fatty acids (FAs), especially long-chain (LC) polyunsaturated fatty acids (PUFAs) that play an important role in many physiological conditions (114). Several studies suggest that LC-PUFAs in breast milk are linked to obesity in the offspring (110, 111, 115); (110, 111, 115). Furthermore, it has been reported that n3 LC-PUFAs play a protective role in the development of obesity, while n6 LC-PUFAs have an adverse effect (111, 116, 117).

PUFA levels in breast milk vary and reflect the mother's diet (109, 118). However, little is known on the effect of maternal obesity since only a few studies have analyzed differences in breast milk PUFA composition between women with different weights. Overweight women have been reported to present higher levels of the n6 FA linoleic acid (LA, C18:2n6) and lower amounts of n3 FAs, particularly n3 LC-PUFAs, than normal-weight mothers (96, 119, 120).

Apart from diet, LC-PUFAs can be obtained through endogenous synthesis from their essential n6 and n3 PUFA precursors linoleic acid (LA, C18:2n6) and α-linolenic acid (ALA, C18:3n3), respectively (121) (Supplemental Figure 1). The genes involved in PUFA metabolism are *FADS1* and *FADS2* (fatty acid desaturases), which encode the desaturase enzymes, and *ELOVL2* and *ELOVL5* (elongation of very long chain fatty acids), which encode the elongase enzymes (121-123). Therefore, genetic variation resulting from single nucleotide polymorphisms (SNPs) in these genes influence LC-PUFA production and consequently alter FA levels, as observed in previous studies analyzing tissues such as maternal and infant blood and breast milk (37, 121, 122, 124-128). For instance, SNPs (minor allele carriers) in *FADS* (121, 122, 125) and *ELOVL* (122, 129, 130) have been linked to slower enzyme conversion rates and decrease formation of those enzymatic reaction products. Hence, people with *FADS* and *ELOVL* minor alleles are known to present a lower LC-PUFA production.

Several studies have demonstrated separately that obesity and *FADS* and *ELOVL* SNPs affect PUFA levels in breast milk. However, the results are not yet conclusive. Since breast milk LC-PUFA composition is influenced by both maternal obesity and SNPs, it is of interest to determine whether PUFA levels vary according to both variables. Nutritional studies involving pregnant women are of great importance since LC-PUFA synthesis is induced in pregnancy and, therefore, SNPs in the genes involved in LC-PUFA metabolism might have a different effect in these individuals than in the general population. Therefore, the aim of the present study was to analyze the differences in breast milk PUFA composition according to maternal pre-gestational BMI and *FADS* genotype.

METHODS

Ethics statement

The study was carried out following the ethical standards recognized by the Declaration of Helsinki (2004), the Good Clinical Practice recommendations of the EEC (document 111/3976/88 July 1990) and the current Spanish legislation governing clinical research in humans (Royal Decree 561/1993 on clinical trials). Moreover, the study was approved by the Ethics Committee of San Cecilio University Hospital and the Faculty of Medicine at the University of Granada.

Study population and design

Pregnant women (n=61) were selected from the 331 individuals participating in the observational PREOBE cohort study (Role of Nutrition and Maternal Genetics on the Programming of Development of Fetal Adipose Tissue). The information regarding the PREOBE study has been published elsewhere (131) and it was registered at www.ClinicalTrials.gov (NCT01634464).

Briefly, participants were recruited at San Cecilio University Hospital and Mother-Infant Hospital in the city of Granada, Spain, where samples and information were also collected. Study design and information about PREOBE participants are exhibited in Supplemental Figure 2. The inclusion criteria were singleton pregnancy, gestation between 12 and 20 weeks at enrollment, and intention to deliver at the centers involved in the study. Women were excluded if they were participating in other research studies, receiving drug treatment or supplements of docosahexaenoic acid (DHA) or folate for more than the first 3 months of pregnancy, suffering from disorders such as hypertension, pre-eclampsia, fetal intrauterine growth retardation, infections, hypo- or hyperthyroidism and hepatic renal diseases or those following an extravagant or vegan diet. Using questionnaires and medical records, some baseline and background characteristics were recorded such as maternal age, pre-pregnancy BMI, parity, smoking status, diet, alcohol drinking habits, socio-demographic information, educational level, and weight gain during pregnancy.

For the present study, 61 lactating women were divided into two groups according to their pre-pregnancy BMI, normal-weight (BMI=18.5-24.9, n=26) and overweight/obese (BMI$\geq$25, n=35) women.

Breast milk sample collection

After the mother was trained, colostrum was collected 2–4 days postpartum by an experienced nurse at the hospital. Samples were collected during 24 hours, beginning in the morning and before and after of each feeding, from the two breasts, and along the whole day. Milk samples were gathered in sterile polypropylene tubes by the mechanical expression of each breast using a breast pump. Milk samples were transported to the laboratory in ice boxes every time, which lasted a maximum of 2 hours after collection, and the samples were stored at –80°C until analysis. Before the analysis, all samples from each woman were mixed and aliquoted.

Breast milk fatty acid analysis

The PUFA composition of breast milk was determined according to the method described by Chisaguano *et al.* (132). FA methyl esters (FAMEs) were obtained by the reaction of FAs with sodium methylate in methanol (0.5 M) and boron trifluoride methanol solution (14% v/v), before being separated and quantified by fast gas chromatography (GC) with flame ionization detection (FID). The relative amount of each PUFA was quantified and expressed as the percentage of the total amount of FAs.

DNA analysis

Maternal material for DNA analysis was collected by scraping the inside of the cheek of the pregnant participant with a buccal swab. SNPs from genes involved in lipid metabolism (*FADS1* and *FADS2*) were genotyped from 5 µl of maternal DNA mixed with 5 µl of 2X TaqMan® OpenArray® Genotyping Master Mix. Analysis was then performed with 3 µl of the mixture in a microplate using the TaqMan® OpenArray® genotyping technology. Analyses were carried out at the *Autonomous University of Barcelona* (UAB) using the OpenArray® instrument, which consists of a Dual Flat Block GeneAmp® PCR System 9700, a Bio-Rad® thermal cycler with Slide Chambers Dual-Block Alpha units, a Thermo Electron PX2 thermal cycler, and the corresponding OpenArray® SNP Genotyping Analysis software. Genotyping required two phases: the thermal cycle (PCR amplification) and detection of the final fluorescence signal. The reagents used were supplied by Applied Biosystems (Foster City, USA).

Statistical analysis

Statistical analyses were performed using the SPSS statistical software package for Windows (SPSS Inc., Chicago, IL). The Kolmogorov-Smirnov test was used to study the normal distribution of the data, with non-normally distributed data log transformed. Agreement between genotype frequencies and Hardy-Weinberg equilibrium expectations was tested by the chi-square test. Due to the limited sample size, heterozygotes and minor allele homozygotes were analyzed as one group. Even so, the sample size is small, but remains consistent with the recommendations in clinical pilot studies (133). SNPs were coded according to the minor allele count (0 for major homozygotes and 1 for the carriers of at least one minor allele) and studied as a numeric variable. Although this coding implies an additive and dominant model, we tested the analyses with the three allele groups and the trends remained the same. The associations between SNPs and PUFAs were determined using linear regression. All associations were corrected for potential confounders such as gestational weight gain, maternal age, education, smoking status, parity, energy intake and the dietary n6:n3 ratio. Breast milk PUFA percentages were expressed as mean ± standard deviation and compared by univariate ANOVA. We analyzed all the available PUFAs involved in enzymatic reactions encoded by the *FADS* genes. Total FA levels were derived by adding the single FAs to n6 PUFAs, n3 PUFAs, n6 LC-PUFAs, n3 LC-PUFAs, n6:n3 PUFAs and n6:n3 LC-PUFAs. Furthermore, we studied n3:n6 ratios (eicosapentaenoic acid (EPA):arachidonic acid (AA) and DHA:AA) that are associated with obesity risk (134-137). Pearson's correlation was used to assess the relationship between DHA dietary intake and DHA level in the colostrum. DHA supplementation was largely absent within the whole population and most of the supplemented women were of normal weight, which could have affected comparisons between the two BMI groups. Therefore, women with DHA supplementation

during pregnancy were excluded from the analyses. The confidence level was established at 95% for all the tests. Thus, results obtaining a *P* value of below 0.05 were considered statistically significant.

RESULTS

Population characteristics

The distribution of baseline characteristics of the women participating in the PREOBE cohort study is given in Table 1. Normal-weight women showed greater gestational weight gain and lower AA intake. DHA levels in the colostrum positively correlated with the dietary DHA intake during late pregnancy regardless of maternal weight (Figure 1). Supplemental Table 1 presents details of the studied SNPs.

Fatty acid profile according to maternal weight and genotype

A high pre-gestational BMI was positively associated with colostrum dihomo-γ-linolenic acid (DGLA), AA, adrenic acid (AdA), docosapentaenoic acid n6 (DPAn6) and n6 LC-PUFA levels and negatively associated with colostrum DHA:AA ratio (Table 2). Maternal weight also altered PUFA concentrations when the women were divided according to their genotype, with differences mainly found in the major allele homozygotes. Among the major allele homozygotes for *FADS1* and/or *FADS2* SNPs, normal-weight women showed higher levels of EPA, DHA, EPA:AA and DHA:AA and a lower n6:n3 LC-PUFA ratio than overweight/obese women. Among the minor allele carriers of the SNP rs1535 (*FADS2*), overweight/obese women showed higher EPA:ALA index than those with normal weight. Genotype was also linked to alterations in PUFA concentrations. Normal-weight women carrying minor alleles of *FADS1* and/or *FADS2* SNPs showed lower DHA levels and AA:DGLA index and higher DGLA levels and n6:n3 LC-PUFA ratio than major allele homozygotes in colostrum milk. In overweight/obese women, minor allele carriers of *FADS2* SNPs showed higher levels of EPA, EPA:AA and EPA:ALA than major allele homozygotes (Table 3). Supplemental Table 2 presents all the results from the analysis of colostrum PUFA concentrations.

The impact of *FADS* SNPs on colostrum PUFA profile was also assessed by linear regression and the statistically significant associations are shown in Table 4. For normal-weight women, the presence of minor alleles of *FADS* SNPs was associated with a low DHA:AA ratio and a high DGLA level and n6:n3 LC-PUFA ratio. In addition, for overweight/obese women, the presence of minor alleles of *FADS* SNPs was associated with low DHA:EPA index and high levels of EPA, EPA:ALA and EPA:AA in colostrum milk. Supplemental Table 3 shows all the results from this analysis.

DISCUSSION

This study describes the relationship between colostrum PUFA content and maternal weight and *FADS* genotype. There are few studies analyzing breast milk PUFA content according to maternal weight (96, 119, 120, 138). Although some authors have studied the influence of *FADS* SNPs on breast milk PUFA composition (37, 126-128), our study is the first to demonstrate the effect of both maternal weight and *FADS* genotype on colostrum PUFA profiles.

To the best of our knowledge, only four studies have evaluated the FA composition of breast milk according to maternal BMI. Marin *et al.* compared the FA composition of mature breast milk (collected 1-3 months postpartum) from normal, overweight and obese mothers. Regarding PUFAs, they reported a significant increase in LA and total n6 PUFAs at a high BMI; while patterns of n3 PUFAs, including DHA, did not differ significantly between the three groups (119). A recent study found that overweight/obese women had a higher n6:n3 ratio and lower amounts of EPA, DHA and total n3 PUFAs in mature milk (collected at 3 months postpartum) than normal-weight women (96). Another study collected breast milk between 4 and 10 weeks postpartum and compared its FA composition between normal-weight and obese mothers. They showed that obese women presented lower levels of PUFAn3, ALA, EPA, DPAn3, and DHA and a higher n6:n3 ratio than normal-weight women (138). By contrast, Storck Lindholm *et al.* comparing the colostrum (collected at 3 days postpartum) from normal-weight and obese mothers, revealed that the obese group showed lower LA, ALA, EPA, DHA, total n6 and n3 PUFA levels and higher DGLA and AA levels (120). Thus, existing results in the literature are not entirely consistent and the studies differ on the groups of weight tested and the timing of sample collection. Our colostrum samples were collected 2-4 days postpartum, similar to Storck Lindholm *et al.* (2013), but our results are not entirely alike. In accordance with the other study, we found that a high BMI was associated with high DGLA and AA levels and a low DHA:AA ratio; however, we also observed associations between a high BMI and high levels of AdA, DPAn6 and total n6 LC-PUFAs (see Table 2) (120).

We also compared PUFA levels between normal-weight and overweight/obese women taking into account their genotype. Among the major homozygotes of *FADS* SNPs, normal-weight women had higher levels of EPA, DHA, EPA:AA and DHA:AA and a lower n6:n3 LC-PUFA ratio than overweight/obese women, which is consistent with the results of previous studies (96, 120, 138). However, these differences were not observed among the minor allele carriers (see Table 3).

Based on our results, the effects of the *FADS* genetic variants on PUFA levels differed in their extent depending on maternal weight. Previous studies have reported that minor alleles of the *FADS* SNPs are associated with lower enzymatic activity and, therefore, lower amounts of FA products and higher levels of substrates (121, 124, 126). Within our normal-weight population, *FADS* minor allele carriers had higher DGLA levels and lower DHA concentrations and AA:DGLA index compared to major allele carriers, which is consistent with the studies that have additionally reported lower EPA concentrations (37, 126-128). We also observed that normal-weight minor allele carriers presented higher n6:n3 LC-PUFA ratio. The overweight/obese group did not show the same behavior; minor allele carriers presented higher levels of EPA, EPA:ALA and EPA:AA than major allele carriers (but not higher than normal-weight women). This could explain why normal-weight women did not have higher EPA levels than overweight/obese women amongst the minor allele carriers (see Table 3).

We also tried to confirm the impact of *FADS* genetic variation by testing the associations between PUFAs and minor alleles of the *FADS* SNPs (Table 4). We used linear regression analysis that was adjusted for several potential confounders. Although we found few significant associations, all the results followed the same trend. The significant results that persisted in the normal-weight population were that the minor alleles were positively associated with the DGLA concentration and n6:n3 LC-PUFA ratio and negatively associated with the DHA:AA ratio. In overweight women, the minor alleles of *FADS* SNPs

were positively associated with EPA levels, EPA:ALA index and EPA:AA ratio and negatively associated with the DHA:EPA index. Perhaps, this last negative association (*FADS* minor alleles with DHA:EPA) is a direct cause of the positive association with EPA, which is the substrate of the enzymatic reaction. When the enzymatic reactions are affected by genotype, the substrates can be accumulated in comparison with the products of the reactions. Thus, overweight/obese women with *FADS* minor alleles would accumulate EPA.

Our results suggest that *FADS* polymorphisms might have a positive effect in overweight and obese lactating women by regulating the PUFA composition of breast milk. However, estrogen could also influence these results. Estrogen is a hormone that facilitates LC-PUFA synthesis (139, 140) (probably by regulating delta-6 desaturase) and increases during a regular pregnancy (140) and also in obese individuals (141). Thus, this could be an alternative explanation for the increased LC-PUFA production observed in the overweight/obese group. In this case, more studies are needed to assess the effect of estrogen in both populations.

Our analyses also confirmed that the DHA content of breast milk was affected by maternal diet during the third trimester of pregnancy (142). As mentioned before, we found reduced DHA concentrations in two different situations: (1) normal-weight women carrying *FADS* minor alleles showed lower DHA levels than those with two major alleles; and (2) overweight/obese women had lower DHA amounts than normal-weight participants among the *FADS* major allele carriers. It would be interesting to follow this line of research as it could develop into a clinical recommendation for these subgroups of women (*FADS* minor allele carriers and obese women) to optimize the colostrum DHA concentration and improve the supply of this crucial FA to the neonate.

To our knowledge, this is the first study to analyze breast milk PUFA composition according to maternal weight and *FADS* genotype. Our study supports the personalization of nutrition given that breast milk PUFA profiles are affected by maternal nutritional status and genes. We acknowledge some limitations in our study such as the small sample size; however, it is important to note that this subsample is representative of the PREOBE study with regard to PUFA analysis. Furthermore, validated food records and questionnaires were used to obtain information on dietary intake and although each participant was guided to answer them, this information might have been affected by recall bias.

CONCLUSION

We observed that a high maternal pre-pregnancy BMI was associated with high n6 FA levels and a low DHA:AA ratio in breast milk, however *FADS* genotypes could modulate these results. When carrying two major alleles, normal-weight women had higher n3 and lower n6 FA levels in their colostrum than overweight/obese women. These differences were not observed in minor allele carriers because this genotype resulted in decreased enzymatic activity and PUFA levels only in normal-weight individuals, making their FA levels similar to those of overweight/obese women. Therefore, *FADS* genetic variation in overweight/obese women has a different impact, possibly improving their FA status as a physiological compensatory mechanism. Dietary intake of DHA in late pregnancy affected the colostrum DHA level; a high intake of this FA may prevent the reduction of colostrum DHA caused by overweight or *FADS* minor alleles. Since genotypes could affect people differently, it is of interest to continue exploring gene-

BMI interactions to pursue personalized health-related recommendations. Alterations in breast milk FAs affect the nutritional supply to the neonate, which in turn impacts on the child's growth and development. Therefore, this study also highlights the importance of a healthy pre-pregnancy weight and identifies the groups of women who could benefit from a high intake of n3 FAs to optimize their breast milk FA status according to neonatal requirements.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

The authors' responsibilities were as follows: CC, MCL-S and AIC designed the project; MCL-S, AIC, ADLGP, AMCT and RMG data analysis and manuscript design; RMG, ADLGP and AMCT sample processing; ADLGP and AMCT data management and drafting of the paper. LMGV, FJTE and MTSM recruited the participants, follow-up them and collected and prepared the samples for later analysis. They

also collected all the information regarding characteristics of the population and the dietary intake reported in the present paper. All authors performed a critical review of the final manuscript.

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TABLES AND FIGURES

Table 1. Characteristics of the population.

	NORMAL-WEIGHT (n= 26)	OVERWEIGHT/OBESE (n= 35)	
	Mean (SD)	Mean (SD)	*P*
Age (years)	30.69 (4.24)	31.91 (3.94)	0.251
Pre-pregnancy BMI (kg/m2)	22.23 (1.60)	29.30 (3.10)	**<0.001***
Weight Gain (kg)	13.03 (3.07)	9.98 (6.33)	**0.038***
Parity (%)			0.161
0	68.00	51.43	
1	32.00	37.14	
2+	0	11.43	
Education (%)			0.830
>High School	73.10	68.60	
High School	11.54	17.14	
<High School	15.38	14.29	
Smoking during pregnancy (%)			0.916
No, never	75.00	74.07	
Quit	10.00	7.41	
Yes	15.00	18.52	
Dietary intakes			
Energy intake (kcal/d)	2048.96 (266.66)	1986.07 (412.58)	0.528
AA intake (g/d)	0.12 (0.06)	0.16 (0.07)	**0.014***
EPA intake (g/d)	0.13 (0.11)	0.12 (0.12)	0.972
DHA intake (g/d)	0.25 (0.18)	0.27 (0.22)	0.744
n3 from fish intake (g/d)	0.36 (0.32)	0.37 (0.35)	0.829
n6:n3 intake (g/d)	12.92 (2.87)	15.30 (6.60)	0.126

Indicates significant differences ($p<0.05$) between groups of weight are marked by stars.

BMI: body mass index; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; AA: arachidonic acid.

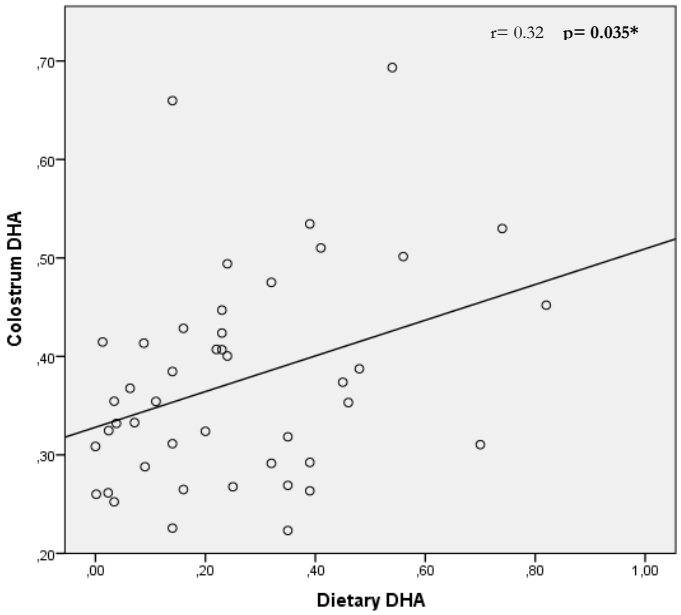


Figure 1. Dietary DHA intake referred to DHA level in colostrum. Correlation coefficient derived from Pearson's (*p<0.05). DHA: Docosahexaenoic acid.

Table 2. Associations between colostrum proportions of PUFAs and maternal pre-pregnancy BMI.

Fatty acid	BMI	
	β	*P*
C18:2n6 (LA)	0.14	0.569
C18:3n6 (GLA)	0.20	0.356
C20:3n6 (DGLA)	0.69	**0.003***
C20:4n6 (AA)	0.52	**0.044***
C22:4n6 (AdA)	0.57	**0.020***
C22:5n6 (DPAn6)	0.59	**0.012***
C18:3n3 (ALA)	-0.24	0.335
C20:5n3 (EPA)	0.02	0.924
C22:5n3 (DPAn3)	0.32	0.197
C22:6n3 (DHA)	-0.15	0.547
n6 PUFA	0.31	0.204
n3 PUFA	-0.07	0.774
n6 LC-PUFA	0.70	**0.003***
n3 LC-PUFA	0.06	0.801
n6:n3 PUFA	0.24	0.331
n6:n3 LC-PUFA	0.49	0.051
EPA:AA	-0.29	0.191
DHA:AA	-0.55	**0.016***

Associations were analyzed using linear regression and adjusted for potential confounders such as gestational weight gain, age, education, smoking, parity, dietary n6:n3 ratio and energy intake. Significant associations are marked by stars (p<0.05). n= 31 participants.

Table 3. PUFA levels in colostrum according to maternal *FADS* SNPs.

Fatty acids and Gene	SNP	M/m	NORMAL-WEIGHT							OVERWEIGHT/OBESITY								
			MM			Mm+mm				MM			Mm+mm					
			N	Mean	SD	N	Mean	SD	P^a	N	Mean	SD	N	Mean	SD	P^a	P^b (MM)	P^b (Mm+mm)
DGLA (C20:3n6)																		
FADS1	rs174545	C/G	12	0.53	0.10	9	0.66	0.16	**0.036***	8	0.61	0.19	20	0.66	0.26	0.776	0.291	0.693
FADS1	rs174546	C/T	12	0.53	0.10	9	0.66	0.16	**0.036***	8	0.61	0.19	20	0.67	0.26	0.734	0.291	0.745
FADS1	rs174553	A/G	12	0.53	0.10	9	0.66	0.16	**0.036***	8	0.61	0.19	21	0.67	0.26	0.692	0.291	0.780
FADS2	rs1535	A/G	10	0.51	0.09	8	0.69	0.15	**0.009***	8	0.61	0.19	19	0.68	0.27	0.650	0.188	0.652
FADS2	rs174583	C/T	10	0.51	0.09	9	0.66	0.16	**0.020***	8	0.61	0.19	19	0.68	0.27	0.650	0.188	0.846
EPA (C20:5n3)																		
FADS2	rs1535	A/G	10	0.05	0.02	8	0.04	0.02	0.109	8	0.03	0.02	19	0.05	0.02	**0.044***	**0.033†**	0.162
FADS2	rs174583	C/T	10	0.05	0.02	9	0.04	0.02	0.186	8	0.03	0.02	19	0.05	0.02	**0.044***	**0.033†**	0.310
FADS2	rs99780	C/T	6	0.06	0.01	11	0.05	0.02	0.173	8	0.03	0.02	17	0.05	0.02	0.077	**0.009†**	0.742
DHA (C22:6n3)																		
FADS1	rs174545	C/G	12	0.46	0.16	9	0.33	0.10	**0.034***	8	0.33	0.09	20	0.36	0.10	0.552	**0.049†**	0.469
FADS1	rs174546	C/T	12	0.46	0.16	9	0.33	0.10	**0.034***	8	0.33	0.09	20	0.36	0.09	0.589	**0.049†**	0.503
FADS1	rs174553	A/G	12	0.46	0.16	9	0.33	0.10	**0.034***	8	0.33	0.09	21	0.36	0.09	0.502	**0.049†**	0.420
FADS2	rs1535	A/G	10	0.44	0.14	8	0.32	0.10	**0.035***	8	0.33	0.09	19	0.36	0.09	0.533	0.063	0.332
FADS2	rs174583	C/T	10	0.44	0.14	9	0.33	0.10	**0.046***	8	0.33	0.09	19	0.36	0.09	0.533	0.063	0.451
n6:n3 LC-PUFA																		
FADS1	rs174545	C/G	12	3.29	0.81	9	4.62	0.99	**0.007***	8	4.83	0.93	20	4.18	1.05	0.171	**0.003†**	0.320
FADS1	rs174546	C/T	12	3.29	0.81	9	4.62	0.99	**0.007***	8	4.83	0.93	20	4.23	1.06	0.207	**0.003†**	0.376
FADS1	rs174553	A/G	12	3.29	0.81	9	4.62	0.99	**0.007***	8	4.83	0.93	21	4.22	1.04	0.195	**0.003†**	0.363
FADS2	rs1535	A/G	10	3.19	0.83	8	4.75	0.96	**0.004***	8	4.83	0.93	19	4.15	1.03	0.158	**0.003†**	0.210
FADS2	rs174583	C/T	10	3.19	0.83	9	4.62	0.99	**0.006***	8	4.83	0.93	19	4.15	1.03	0.158	**0.003†**	0.297
FADS2	rs99780	C/T	6	3.33	0.97	11	4.35	1.07	0.073	8	4.83	0.93	17	4.21	1.04	0.206	**0.020†**	0.728
EPA:AA																		
FADS1	rs174545	C/G	12	0.08	0.03	9	0.08	0.04	0.490	8	0.05	0.02	20	0.07	0.04	0.098	**0.025†**	0.843
FADS1	rs174546	C/T	12	0.08	0.03	9	0.08	0.04	0.490	8	0.05	0.02	20	0.08	0.03	0.066	**0.025†**	0.727
FADS1	rs174553	A/G	12	0.08	0.03	9	0.08	0.04	0.490	8	0.05	0.02	21	0.07	0.03	0.084	**0.025†**	0.815
FADS2	rs1535	A/G	10	0.09	0.02	8	0.07	0.05	0.152	8	0.05	0.02	19	0.08	0.03	**0.031***	**0.003†**	0.395
FADS2	rs174583	C/T	10	0.09	0.02	9	0.08	0.04	0.225	8	0.05	0.02	19	0.08	0.03	**0.031***	**0.003†**	0.559
FADS2	rs99780	C/T	6	0.09	0.02	11	0.08	0.04	0.226	8	0.05	0.02	17	0.08	0.03	**0.042***	**0.006†**	0.782
DHA:AA																		
FADS1	rs174545	C/G	12	0.73	0.27	9	0.57	0.14	0.098	8	0.50	0.09	20	0.60	0.21	0.250	**0.017†**	0.734
FADS1	rs174546	C/T	12	0.73	0.27	9	0.57	0.14	0.098	8	0.50	0.09	20	0.59	0.21	0.372	**0.017†**	0.914
FADS1	rs174553	A/G	12	0.73	0.27	9	0.57	0.14	0.098	8	0.50	0.09	21	0.59	0.21	0.332	**0.017†**	0.865
FADS2	rs1535	A/G	10	0.74	0.28	8	0.56	0.15	0.102	8	0.50	0.09	19	0.59	0.22	0.347	**0.020†**	0.782
FADS2	rs174583	C/T	10	0.74	0.28	9	0.57	0.14	0.103	8	0.50	0.09	19	0.59	0.22	0.347	**0.020†**	0.863
EPA:ALA																		
FADS2	rs1535	A/G	10	0.10	0.04	8	0.07	0.04	0.075	8	0.07	0.05	19	0.11	0.05	**0.022***	0.060	**0.032†**
FADS2	rs174583	C/T	10	0.10	0.04	9	0.07	0.04	0.141	8	0.07	0.05	19	0.11	0.05	**0.022***	0.060	0.064
FADS2	rs99780	C/T	6	0.10	0.02	11	0.08	0.05	0.274	8	0.07	0.05	17	0.10	0.05	**0.039***	0.070	0.213
AA:DGLA																		
FADS1	rs174545	C/G	12	1.24	0.27	9	0.97	0.38	**0.037***	8	1.15	0.32	20	1.02	0.25	0.279	0.408	0.425
FADS1	rs174546	C/T	12	1.24	0.27	9	0.97	0.38	**0.037***	8	1.15	0.32	20	1.03	0.26	0.337	0.408	0.379
FADS1	rs174553	A/G	12	1.24	0.27	9	0.97	0.38	**0.037***	8	1.15	0.32	21	1.03	0.25	0.326	0.408	0.365

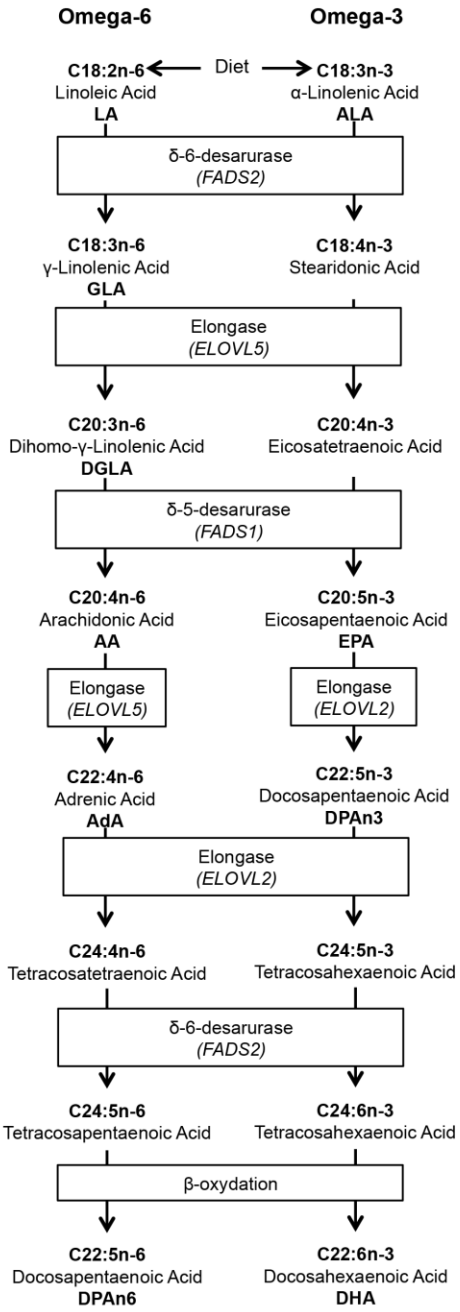
Data are means of PUFAs expressed as percentages of the total phospholipid profile (standard error).
[a]P-values derived from ANOVA comparing PUFAs between allele groups within each group of weight. Significant differences are marked by stars ($p<0.05$).
[b]P-values derived from ANOVA comparing PUFAs between groups of weight. Significant differences are marked by daggers ($p<0.05$).
Major allele: M; minor allele: m; DGLA: Dihomo-γ-Linolenic Acid; AA: Arachidonic Acid; ALA: α-linolenic Acid; EPA: Eicosapentaenoic acid, DHA: Docosahexaenoic acid.

Table 4. Associations between colostrum proportions of PUFAs and *FADS* polymorphisms.

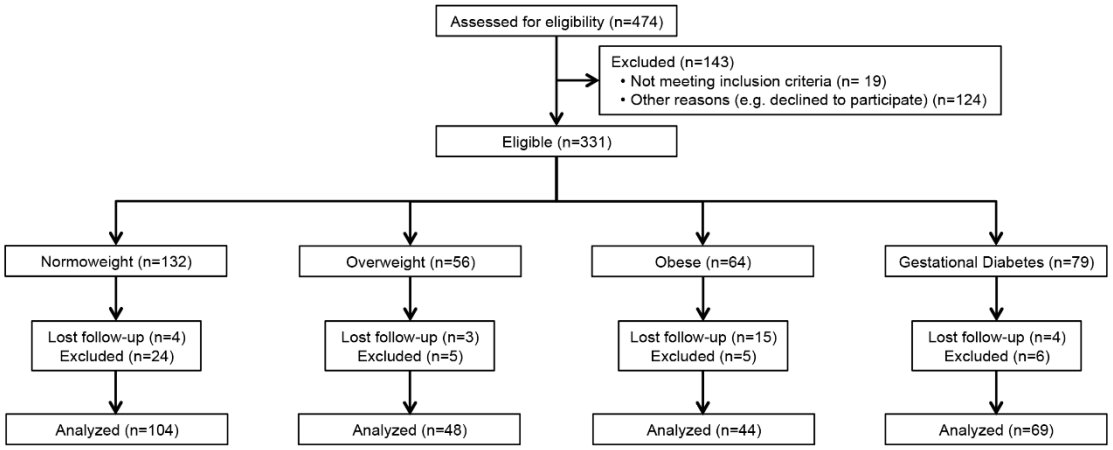
Fatty acids and Genes	SNP	*M/m*	NORMAL-WEIGHT			OVERWEIGHT/OBESE		
			N	β	P	N	β	P
DGLA (C20:3n6)								
FADS1	rs174545	*C/G*	13	1.02	**0.018***	16	0.03	0.936
FADS1	rs174546	*C/T*	13	1.02	**0.018***	17	0.22	0.492
FADS1	rs174553	*A/G*	13	1.02	**0.018***	17	0.22	0.492
FADS2	rs1535	*A/G*	11	1.29	**0.040***	17	0.22	0.492
FADS2	rs99780	*C/T*	11	0.94	**0.024***	17	0.22	0.492
EPA (C20:5n3)								
FADS1	rs174545	*C/G*	13	-0.23	0.428	16	0.82	**0.026***
FADS1	rs174546	*C/T*	13	-0.23	0.428	17	0.73	**0.011***
FADS1	rs174553	*A/G*	13	-0.23	0.428	17	0.73	**0.011***
FADS2	rs1535	*A/G*	11	0.17	0.367	17	0.73	**0.011***
FADS2	rs174583	*C/T*	12	-0.04	0.869	17	0.73	**0.011***
FADS2	rs99780	*C/T*	11	0.15	0.217	17	0.73	**0.011***
EPA:ALA								
FADS1	rs174546	*C/T*	13	-0.23	0.625	17	0.54	**0.041***
FADS1	rs174553	*A/G*	13	-0.23	0.625	17	0.54	**0.041***
FADS2	rs1535	*A/G*	11	-0.70	0.349	17	0.54	**0.041***
FADS2	rs174583	*C/T*	12	-0.37	0.500	17	0.54	**0.041***
FADS2	rs99780	*C/T*	11	-0.18	0.766	17	0.54	**0.041***
DHA:EPA								
FADS2	rs1535	*A/G*	11	-0.46	0.611	17	-0.64	**0.017***
FADS2	rs174583	*C/T*	12	-0.37	0.550	17	-0.64	**0.017***
FADS2	rs99780	*C/T*	11	-0.50	0.416	17	-0.64	**0.017***
EPA:AA								
FADS1	rs174545	*C/G*	13	-0.46	0.405	16	0.91	**0.005***
FADS1	rs174546	*C/T*	13	-0.46	0.405	17	0.72	**0.023***
FADS1	rs174553	*A/G*	13	-0.46	0.405	17	0.72	**0.023***
FADS2	rs1535	*A/G*	11	-0.03	0.977	17	0.72	**0.023***
FADS2	rs174583	*C/T*	12	-0.34	0.626	17	0.72	**0.023***
FADS2	rs99780	*C/T*	11	0.27	0.674	17	0.72	**0.023***
DHA:AA								
FADS1	rs174545	*C/G*	13	-1.04	**0.014***	16	0.46	0.201
FADS1	rs174546	*C/T*	13	-1.04	**0.014***	17	0.20	0.574
FADS1	rs174553	*A/G*	13	-1.04	**0.014***	17	0.20	0.574
FADS2	rs174583	*C/T*	12	-0.96	**0.049***	17	0.20	0.574
n6:n3 LC-PUFA								
FADS1	rs174545	C/G	13	0.94	**0.022***	16	-0.59	0.241
FADS1	rs174546	C/T	13	0.94	**0.022***	17	-0.36	0.457
FADS1	rs174553	A/G	13	0.94	**0.022***	17	-0.36	0.457

Associations between SNPs and fatty acids were analyzed using linear regression. SNPs were coded according to minor allele count and analyzed as a numeric variable. "β"= beta per minor allele standardized per the major allele. Significant associations after adjustment for potential confounders such as gestational weight gain, age, education, smoking, parity, dietary n6:n3 ratio and energy intake are denoted by stars ($p<0.05$). DGLA: Dihomo-γ-Linolenic Acid; AA: Arachidonic Acid; ALA: α-linolenic Acid; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid.

SUPPLEMENTARY APPENDIX



Supplemental Figure 1. Metabolism pathways of omega-6 and omega-3 PUFAs.



Supplemental Figure 2. Participants in PREOBE study and classification following BMI and gestational diabetes criteria.

Supplemental Table 1. Genetic variants studied within the *FADS* gene.

				NORMAL-WEIGHT (n= 26)			OVERWEIGHT/OBESE (n= 35)			
Gene	SNP	*Alleles*	N	MAF (%)	HWE[a]	Missingness (%)	MAF (%)	HWE[a]	Missingness (%)	*P*[b]
FADS1	rs174545	*C/G*	60	42.30	0.079	0	73.50	0.023	2.86	0.014
FADS1	rs174546	*C/T*	60	42.30	0.079	0	73.50	0.023	2.86	0.014
FADS1	rs174553	*A/G*	61	42.30	0.079	0	74.30	0.016	0	0.011
FADS2	rs1535	*A/G*	56	43.50	0.066	11.54	72.70	0.069	5.71	0.027
FADS2	rs174583	*C/T*	56	47.80	0.140	11.54	72.70	0.167	5.71	0.058
FADS2	rs99780	*C/T*	52	61.90	0.134	19.23	71.00	0.128	11.43	0.494

MAF: Minor allele frequency (%)
[a]P-values of deviation from HWE among all subjects were tested by chi-square tests.
[b]P-values comparing MAF% between groups of weight.

Supplemental Table 2. PUFA levels in colostrum according to maternal *FADS* SNPs.

			NORMAL-WEIGHT							OVERWEIGHT/OBESITY								
			MM			Mm+mm				MM			Mm+mm					
Fatty acids and Gene	SNP	M/m	N	Mean	SD	N	Mean	SD	P[a]	N	Mean	SD	N	Mean	SD	P[a]	P[b] (MM)	P[b] (Mm+mm)
LA (C18:2n6)																		
FADS1	rs174545	C/G	12	12.62	2.43	9	12.86	2.14	0.770	8	13.79	3.33	20	13.06	3.33	0.570	0.405	0.956
FADS1	rs174546	C/T	12	12.62	2.43	9	12.86	2.14	0.770	8	13.79	3.33	20	13.09	3.33	0.585	0.405	0.976
FADS1	rs174553	A/G	12	12.62	2.43	9	12.86	2.14	0.770	8	13.79	3.33	21	13.06	3.25	0.570	0.405	0.968
FADS2	rs1535	A/G	10	12.59	2.53	8	12.91	2.29	0.747	8	13.79	3.33	19	12.96	3.37	0.525	0.417	0.878
FADS2	rs174583	C/T	10	12.59	2.53	9	12.86	2.14	0.751	8	13.79	3.33	19	12.96	3.37	0.525	0.417	0.888
FADS2	rs99780	C/T	6	12.12	1.82	11	12.61	2.24	0.687	8	13.79	3.33	17	12.90	3.48	0.504	0.320	0.988
GLA (C18:3n6)																		
FADS1	rs174545	C/G	12	0.11	0.04	9	0.09	0.03	0.248	8	0.11	0.06	20	0.13	0.09	0.618	0.656	0.289
FADS1	rs174546	C/T	12	0.11	0.04	9	0.09	0.03	0.248	8	0.11	0.06	20	0.14	0.09	0.399	0.656	0.149
FADS1	rs174553	A/G	12	0.11	0.04	9	0.09	0.03	0.248	8	0.11	0.06	21	0.13	0.09	0.543	0.656	0.238
FADS2	rs1535	A/G	10	0.12	0.05	8	0.09	0.04	0.242	8	0.11	0.06	19	0.14	0.09	0.315	0.516	0.148
FADS2	rs174583	C/T	10	0.12	0.05	9	0.09	0.03	0.184	8	0.11	0.06	19	0.14	0.09	0.315	0.516	0.108
FADS2	rs99780	C/T	6	0.10	0.03	11	0.10	0.04	0.957	8	0.11	0.06	17	0.14	0.09	0.398	0.960	0.280
DGLA (C20:3n6)																		
FADS1	rs174545	C/G	12	0.53	0.10	9	0.66	0.16	**0.036***	8	0.61	0.19	20	0.66	0.26	0.776	0.291	0.693
FADS1	rs174546	C/T	12	0.53	0.10	9	0.66	0.16	**0.036***	8	0.61	0.19	20	0.67	0.26	0.734	0.291	0.745
FADS1	rs174553	A/G	12	0.53	0.10	9	0.66	0.16	**0.036***	8	0.61	0.19	21	0.67	0.26	0.692	0.291	0.780
FADS2	rs1535	A/G	10	0.51	0.09	8	0.69	0.15	**0.009***	8	0.61	0.19	19	0.68	0.27	0.650	0.188	0.652
FADS2	rs174583	C/T	10	0.51	0.09	9	0.66	0.16	**0.020***	8	0.61	0.19	19	0.68	0.27	0.650	0.188	0.846
FADS2	rs99780	C/T	6	0.52	0.09	11	0.65	0.14	0.064	8	0.61	0.19	17	0.64	0.26	0.885	0.366	0.669
AA (C20:4n6)																		
FADS1	rs174545	C/G	12	0.65	0.17	9	0.60	0.13	0.522	8	0.66	0.08	20	0.63	0.19	0.500	0.714	0.705
FADS1	rs174546	C/T	12	0.65	0.17	9	0.60	0.13	0.522	8	0.66	0.08	20	0.65	0.21	0.662	0.714	0.599
FADS1	rs174553	A/G	12	0.65	0.17	9	0.60	0.13	0.522	8	0.66	0.08	21	0.65	0.21	0.707	0.714	0.545
FADS2	rs1535	A/G	10	0.62	0.16	8	0.59	0.14	0.683	8	0.66	0.08	19	0.65	0.22	0.684	0.477	0.540
FADS2	rs174583	C/T	10	0.62	0.16	9	0.60	0.13	0.756	8	0.66	0.08	19	0.65	0.22	0.684	0.477	0.596
FADS2	rs99780	C/T	6	0.62	0.12	11	0.62	0.15	0.889	8	0.66	0.08	17	0.64	0.22	0.578	0.505	0.963
AdA (C22:4n6)																		
FADS1	rs174545	C/G	12	0.23	0.13	9	0.21	0.08	0.842	8	0.32	0.15	20	0.25	0.13	0.179	0.153	0.458
FADS1	rs174546	C/T	12	0.23	0.13	9	0.21	0.08	0.842	8	0.32	0.15	20	0.26	0.15	0.225	0.153	0.435
FADS1	rs174553	A/G	12	0.23	0.13	9	0.21	0.08	0.842	8	0.32	0.15	21	0.27	0.16	0.319	0.153	0.336
FADS2	rs1535	A/G	10	0.21	0.11	8	0.20	0.08	0.918	8	0.32	0.15	19	0.26	0.15	0.249	0.091	0.309
FADS2	rs174583	C/T	10	0.21	0.11	9	0.21	0.08	0.893	8	0.32	0.15	19	0.26	0.15	0.249	0.091	0.426
FADS2	rs99780	C/T	6	0.19	0.04	11	0.23	0.11	0.651	8	0.32	0.15	17	0.24	0.13	0.151	0.068	0.756
DPAn6 (C22:5n6)																		
FADS1	rs174545	C/G	12	0.10	0.04	9	0.13	0.05	0.258	8	0.12	0.05	20	0.12	0.06	0.728	0.298	0.667
FADS1	rs174546	C/T	12	0.10	0.04	9	0.13	0.05	0.258	8	0.12	0.05	20	0.12	0.07	0.812	0.298	0.754
FADS1	rs174553	A/G	12	0.10	0.04	9	0.13	0.05	0.258	8	0.12	0.05	21	0.12	0.06	0.869	0.298	0.811
FADS2	rs1535	A/G	10	0.10	0.04	8	0.12	0.05	0.290	8	0.12	0.05	19	0.12	0.07	0.769	0.197	0.991
FADS2	rs174583	C/T	10	0.10	0.04	9	0.13	0.05	0.168	8	0.12	0.05	19	0.12	0.07	0.769	0.197	0.711
FADS2	rs99780	C/T	6	0.09	0.02	11	0.12	0.04	0.101	8	0.12	0.05	17	0.12	0.07	0.683	0.140	0.693
ALA (C18:3n3)																		
FADS1	rs174545	C/G	12	0.56	0.12	9	0.61	0.24	0.743	8	0.56	0.15	20	0.49	0.12	0.205	0.983	0.127
FADS1	rs174546	C/T	12	0.56	0.12	9	0.61	0.24	0.743	8	0.56	0.15	20	0.49	0.12	0.179	0.983	0.111
FADS1	rs174553	A/G	12	0.56	0.12	9	0.61	0.24	0.743	8	0.56	0.15	21	0.49	0.12	0.171	0.983	0.104
FADS2	rs1535	A/G	10	0.57	0.12	8	0.62	0.26	0.812	8	0.56	0.15	19	0.48	0.12	0.145	0.907	0.106
FADS2	rs174583	C/T	10	0.57	0.12	9	0.61	0.24	0.822	8	0.56	0.15	19	0.48	0.12	0.145	0.907	0.092
FADS2	rs99780	C/T	6	0.60	0.11	11	0.60	0.22	0.799	8	0.56	0.15	17	0.49	0.13	0.183	0.544	0.102
EPA (C20:5n3)																		
FADS1	rs174545	C/G	12	0.05	0.02	9	0.04	0.02	0.297	8	0.03	0.02	20	0.04	0.02	0.148	0.059	0.645
FADS1	rs174546	C/T	12	0.05	0.02	9	0.04	0.02	0.297	8	0.03	0.02	20	0.05	0.02	0.098	0.059	0.474
FADS1	rs174553	A/G	12	0.05	0.02	9	0.04	0.02	0.297	8	0.03	0.02	21	0.05	0.02	0.111	0.059	0.521
FADS2	rs1535	A/G	10	0.05	0.02	8	0.04	0.02	0.109	8	0.03	0.02	19	0.05	0.02	**0.044***	**0.033†**	0.162
FADS2	rs174583	C/T	10	0.05	0.02	9	0.04	0.02	0.186	8	0.03	0.02	19	0.05	0.02	**0.044***	**0.033†**	0.310
FADS2	rs99780	C/T	6	0.06	0.01	11	0.05	0.02	0.173	8	0.03	0.02	17	0.05	0.02	0.077	**0.009†**	0.742

Supplemental Table 2. Continuation…

Fatty acids and Gene	SNP	M/m	NORMAL-WEIGHT							OVERWEIGHT/OBESITY								
			MM			Mm+mm				MM			Mm+mm					
			N	Mean	SD	N	Mean	SD	P[a]	N	Mean	SD	N	Mean	SD	P[a]	P[b] (MM)	P[b] (Mm+mm)
DPAn3 (C22:5n3)																		
FADS1	rs174545	C/G	12	0.17	0.09	9	0.14	0.04	0.512	8	0.16	0.06	20	0.16	0.08	0.942	0.979	0.499
FADS1	rs174546	C/T	12	0.17	0.09	9	0.14	0.04	0.512	8	0.16	0.06	20	0.16	0.08	0.926	0.979	0.508
FADS1	rs174553	A/G	12	0.17	0.09	9	0.14	0.04	0.512	8	0.16	0.06	21	0.16	0.08	0.944	0.979	0.408
FADS2	rs1535	A/G	10	0.17	0.09	8	0.13	0.04	0.317	8	0.16	0.06	19	0.16	0.08	0.936	0.875	0.246
FADS2	rs174583	C/T	10	0.17	0.09	9	0.14	0.04	0.440	8	0.16	0.06	19	0.16	0.08	0.936	0.875	0.389
FADS2	rs99780	C/T	6	0.18	0.10	11	0.14	0.06	0.378	8	0.16	0.06	17	0.15	0.08	0.810	0.669	0.782
DHA (C22:6n3)																		
FADS1	rs174545	C/G	12	0.46	0.16	9	0.33	0.10	**0.034***	8	0.33	0.09	20	0.36	0.10	0.552	**0.049†**	0.469
FADS1	rs174546	C/T	12	0.46	0.16	9	0.33	0.10	**0.034***	8	0.33	0.09	20	0.36	0.09	0.589	**0.049†**	0.503
FADS1	rs174553	A/G	12	0.46	0.16	9	0.33	0.10	**0.034***	8	0.33	0.09	21	0.36	0.09	0.502	**0.049†**	0.420
FADS2	rs1535	A/G	10	0.44	0.14	8	0.32	0.10	**0.035***	8	0.33	0.09	19	0.36	0.09	0.533	0.063	0.332
FADS2	rs174583	C/T	10	0.44	0.14	9	0.33	0.10	**0.046***	8	0.33	0.09	19	0.36	0.09	0.533	0.063	0.451
FADS2	rs99780	C/T	6	0.42	0.15	11	0.37	0.13	0.373	8	0.33	0.09	17	0.34	0.08	0.793	0.188	0.726
n6 PUFA																		
FADS1	rs174545	C/G	12	14.84	2.34	9	15.21	2.12	0.680	8	16.36	3.00	20	15.47	3.63	0.479	0.224	0.977
FADS1	rs174546	C/T	12	14.84	2.34	9	15.21	2.12	0.680	8	16.36	3.00	20	15.54	3.65	0.508	0.224	0.985
FADS1	rs174553	A/G	12	14.84	2.34	9	15.21	2.12	0.680	8	16.36	3.00	21	15.54	3.56	0.508	0.224	0.969
FADS2	rs1535	A/G	10	14.73	2.27	8	15.24	2.26	0.624	8	16.36	3.00	19	15.42	3.71	0.462	0.209	0.933
FADS2	rs174583	C/T	10	14.73	2.27	9	15.21	2.12	0.613	8	16.36	3.00	19	15.42	3.71	0.462	0.209	0.939
FADS2	rs99780	C/T	6	14.24	1.87	11	14.97	2.14	0.502	8	16.36	3.00	17	15.27	3.87	0.415	0.156	0.978
n3 PUFA																		
FADS1	rs174545	C/G	12	1.23	0.31	9	1.12	0.29	0.391	8	1.09	0.18	20	1.06	0.18	0.634	0.313	0.627
FADS1	rs174546	C/T	12	1.23	0.31	9	1.12	0.29	0.391	8	1.09	0.18	20	1.05	0.17	0.581	0.313	0.581
FADS1	rs174553	A/G	12	1.23	0.31	9	1.12	0.29	0.391	8	1.09	0.18	21	1.06	0.17	0.671	0.313	0.657
FADS2	rs1535	A/G	10	1.23	0.30	8	1.10	0.30	0.342	8	1.09	0.18	19	1.05	0.18	0.612	0.291	0.759
FADS2	rs174583	C/T	10	1.23	0.30	9	1.12	0.29	0.384	8	1.09	0.18	19	1.05	0.18	0.612	0.291	0.610
FADS2	rs99780	C/T	6	1.27	0.34	11	1.16	0.28	0.506	8	1.09	0.18	17	1.03	0.17	0.414	0.278	0.222
n6 LC-PUFA																		
FADS1	rs174545	C/G	12	2.11	0.56	9	2.25	0.34	0.394	8	2.46	0.59	20	2.28	0.72	0.414	0.184	0.837
FADS1	rs174546	C/T	12	2.11	0.56	9	2.25	0.34	0.394	8	2.46	0.59	20	2.31	0.77	0.475	0.184	0.908
FADS1	rs174553	A/G	12	2.11	0.56	9	2.25	0.34	0.394	8	2.46	0.59	21	2.35	0.78	0.565	0.184	0.968
FADS2	rs1535	A/G	10	2.02	0.50	8	2.23	0.36	0.286	8	2.46	0.59	19	2.32	0.80	0.491	0.111	0.977
FADS2	rs174583	C/T	10	2.02	0.50	9	2.25	0.34	0.227	8	2.46	0.59	19	2.32	0.80	0.491	0.111	0.916
FADS2	rs99780	C/T	6	2.03	0.33	11	2.26	0.37	0.208	8	2.46	0.59	17	2.24	0.76	0.339	0.126	0.648
n3 LC-PUFA																		
FADS1	rs174545	C/G	12	0.67	0.24	9	0.51	0.13	0.072	8	0.53	0.16	20	0.56	0.17	0.606	0.133	0.436
FADS1	rs174546	C/T	12	0.67	0.24	9	0.51	0.13	0.072	8	0.53	0.16	20	0.56	0.16	0.616	0.133	0.444
FADS1	rs174553	A/G	12	0.67	0.24	9	0.51	0.13	0.072	8	0.53	0.16	21	0.57	0.17	0.523	0.133	0.362
FADS2	rs1535	A/G	10	0.67	0.23	8	0.49	0.13	0.050	8	0.53	0.16	19	0.57	0.16	0.513	0.144	0.221
FADS2	rs174583	C/T	10	0.67	0.23	9	0.51	0.13	0.079	8	0.53	0.16	19	0.57	0.16	0.513	0.144	0.352
FADS2	rs99780	C/T	6	0.67	0.25	11	0.55	0.19	0.308	8	0.53	0.16	17	0.54	0.14	0.775	0.233	0.941
n6:n3 PUFA																		
FADS1	rs174545	C/G	12	12.84	4.22	9	14.30	3.79	0.328	8	15.56	5.00	20	15.03	4.33	0.776	0.170	0.731
FADS1	rs174546	C/T	12	12.84	4.22	9	14.30	3.79	0.328	8	15.56	5.00	20	15.15	4.29	0.834	0.170	0.668
FADS1	rs174553	A/G	12	12.84	4.22	9	14.30	3.79	0.328	8	15.56	5.00	21	15.02	4.22	0.781	0.170	0.713
FADS2	rs1535	A/G	10	12.80	4.59	8	14.57	3.95	0.302	8	15.56	5.00	19	15.01	4.35	0.768	0.185	0.870
FADS2	rs174583	C/T	10	12.80	4.59	9	14.30	3.79	0.331	8	15.56	5.00	19	15.01	4.35	0.768	0.185	0.745
FADS2	rs99780	C/T	6	12.00	3.78	11	13.68	3.95	0.362	8	15.56	5.00	17	15.21	4.52	0.845	0.133	0.384
n6:n3 LC-PUFA																		
FADS1	rs174545	C/G	12	3.29	0.81	9	4.62	0.99	**0.007***	8	4.83	0.93	20	4.18	1.05	0.171	**0.003†**	0.320
FADS1	rs174546	C/T	12	3.29	0.81	9	4.62	0.99	**0.007***	8	4.83	0.93	20	4.23	1.06	0.207	**0.003†**	0.376
FADS1	rs174553	A/G	12	3.29	0.81	9	4.62	0.99	**0.007***	8	4.83	0.93	21	4.22	1.04	0.195	**0.003†**	0.363
FADS2	rs1535	A/G	10	3.19	0.83	8	4.75	0.96	**0.004***	8	4.83	0.93	19	4.15	1.03	0.158	**0.003†**	0.210
FADS2	rs174583	C/T	10	3.19	0.83	9	4.62	0.99	**0.006***	8	4.83	0.93	19	4.15	1.03	0.158	**0.003†**	0.297
FADS2	rs99780	C/T	6	3.33	0.97	11	4.35	1.07	0.073	8	4.83	0.93	17	4.21	1.04	0.206	**0.020†**	0.728

Supplemental Table 2. Continuation…

Fatty acids and Gene	SNP	M/m	NORMAL-WEIGHT							OVERWEIGHT/OBESITY								
			MM			Mm+mm				MM			Mm+mm					
			N	Mean	SD	N	Mean	SD	P^a	N	Mean	SD	N	Mean	SD	P^a	P^b (MM)	P^b (Mm+mm)
EPA:AA																		
FADS1	rs174545	C/G	12	0.08	0.03	9	0.08	0.04	0.490	8	0.05	0.02	20	0.07	0.04	0.098	**0.025†**	0.843
FADS1	rs174546	C/T	12	0.08	0.03	9	0.08	0.04	0.490	8	0.05	0.02	20	0.08	0.03	0.066	**0.025†**	0.727
FADS1	rs174553	A/G	12	0.08	0.03	9	0.08	0.04	0.490	8	0.05	0.02	21	0.07	0.03	0.084	**0.025†**	0.815
FADS2	rs1535	A/G	10	0.09	0.02	8	0.07	0.05	0.152	8	0.05	0.02	19	0.08	0.03	**0.031***	**0.003†**	0.395
FADS2	rs174583	C/T	10	0.09	0.02	9	0.08	0.04	0.225	8	0.05	0.02	19	0.08	0.03	**0.031***	**0.003†**	0.559
FADS2	rs99780	C/T	6	0.09	0.02	11	0.08	0.04	0.226	8	0.05	0.02	17	0.08	0.03	**0.042***	**0.006†**	0.782
DHA:AA																		
FADS1	rs174545	C/G	12	0.73	0.27	9	0.57	0.14	0.098	8	0.50	0.09	20	0.60	0.21	0.250	**0.017†**	0.734
FADS1	rs174546	C/T	12	0.73	0.27	9	0.57	0.14	0.098	8	0.50	0.09	20	0.59	0.21	0.372	**0.017†**	0.914
FADS1	rs174553	A/G	12	0.73	0.27	9	0.57	0.14	0.098	8	0.50	0.09	21	0.59	0.21	0.332	**0.017†**	0.865
FADS2	rs1535	A/G	10	0.74	0.28	8	0.56	0.15	0.102	8	0.50	0.09	19	0.59	0.22	0.347	**0.020†**	0.782
FADS2	rs174583	C/T	10	0.74	0.28	9	0.57	0.14	0.103	8	0.50	0.09	19	0.59	0.22	0.347	**0.020†**	0.863
FADS2	rs99780	C/T	6	0.70	0.32	11	0.59	0.14	0.387	8	0.50	0.09	17	0.58	0.22	0.461	0.089	0.710
GLA:LA																		
FADS2	rs1535	A/G	10	0.01	0.00	8	0.01	0.00	0.151	8	0.01	0.00	19	0.01	0.01	0.098	0.151	0.076
FADS2	rs174583	C/T	10	0.01	0.00	9	0.01	0.00	0.109	8	0.01	0.00	19	0.01	0.01	0.098	0.151	0.051
FADS2	rs99780	C/T	6	0.01	0.00	11	0.01	0.00	0.824	8	0.01	0.00	17	0.01	0.01	0.143	0.561	0.214
DGLA:LA																		
FADS2	rs1535	A/G	10	0.04	0.01	8	0.05	0.01	0.060	8	0.05	0.02	19	0.05	0.02	0.398	0.655	0.719
FADS2	rs174583	C/T	10	0.04	0.01	9	0.05	0.01	0.091	8	0.05	0.02	19	0.05	0.02	0.398	0.655	0.917
FADS2	rs99780	C/T	6	0.04	0.01	11	0.05	0.01	0.118	8	0.05	0.02	17	0.05	0.02	0.551	0.930	0.643
AA:LA																		
FADS1	rs174545	C/G	12	0.05	0.02	9	0.05	0.02	0.563	8	0.05	0.02	20	0.05	0.02	0.951	0.807	0.724
FADS1	rs174546	C/T	12	0.05	0.02	9	0.05	0.02	0.563	8	0.05	0.02	20	0.05	0.02	0.945	0.807	0.639
FADS1	rs174553	A/G	12	0.05	0.02	9	0.05	0.02	0.563	8	0.05	0.02	21	0.05	0.02	0.894	0.807	0.587
FADS2	rs1535	A/G	10	0.05	0.02	8	0.05	0.02	0.678	8	0.05	0.02	19	0.05	0.02	0.873	0.964	0.525
FADS2	rs174583	C/T	10	0.05	0.02	9	0.05	0.02	0.728	8	0.05	0.02	19	0.05	0.02	0.873	0.964	0.579
FADS2	rs99780	C/T	6	0.05	0.01	11	0.05	0.02	0.772	8	0.05	0.02	17	0.05	0.02	0.938	0.727	0.977
AA:DGLA																		
FADS1	rs174545	C/G	12	1.24	0.27	9	0.97	0.38	**0.037***	8	1.15	0.32	20	1.02	0.25	0.279	0.408	0.425
FADS1	rs174546	C/T	12	1.24	0.27	9	0.97	0.38	**0.037***	8	1.15	0.32	20	1.03	0.26	0.337	0.408	0.379
FADS1	rs174553	A/G	12	1.24	0.27	9	0.97	0.38	**0.037***	8	1.15	0.32	21	1.03	0.25	0.326	0.408	0.365
EPA:ALA																		
FADS1	rs174545	C/G	12	0.09	0.04	9	0.07	0.04	0.212	8	0.07	0.05	20	0.09	0.05	0.083	0.087	0.209
FADS1	rs174546	C/T	12	0.09	0.04	9	0.07	0.04	0.212	8	0.07	0.05	20	0.10	0.05	0.057	0.087	0.142
FADS1	rs174553	A/G	12	0.09	0.04	9	0.07	0.04	0.212	8	0.07	0.05	21	0.10	0.05	0.062	0.087	0.156
FADS2	rs1535	A/G	10	0.10	0.04	8	0.07	0.04	0.075	8	0.07	0.05	19	0.11	0.05	**0.022***	0.060	**0.032†**
FADS2	rs174583	C/T	10	0.10	0.04	9	0.07	0.04	0.141	8	0.07	0.05	19	0.11	0.05	**0.022***	0.060	0.064
FADS2	rs99780	C/T	6	0.10	0.02	11	0.08	0.05	0.274	8	0.07	0.05	17	0.10	0.05	**0.039***	0.070	0.213
DHA:EPA																		
FADS2	rs1535	A/G	10	9.41	5.76	8	9.84	4.28	0.771	8	11.00	4.16	19	8.36	3.51	0.103	0.347	0.433
FADS2	rs174583	C/T	10	9.41	5.76	9	9.41	4.20	0.917	8	11.00	4.16	19	8.36	3.51	0.103	0.347	0.577
FADS2	rs99780	C/T	6	7.79	3.55	11	9.30	3.76	0.434	8	11.00	4.16	17	8.43	3.65	0.123	0.136	0.544
DHA:DPAn3																		
FADS2	rs1535	A/G	10	2.94	1.26	8	2.62	0.74	0.558	8	2.26	0.64	19	2.43	0.70	0.643	0.129	0.554
FADS2	rs174583	C/T	10	2.94	1.26	9	2.55	0.72	0.433	8	2.26	0.64	19	2.43	0.70	0.643	0.129	0.672
FADS2	rs99780	C/T	6	2.52	0.54	11	2.64	0.63	0.765	8	2.26	0.64	17	2.47	0.73	0.590	0.403	0.497

Data are means of PUFAs expressed as percentages of the total phospholipid profile (standard error).

[a]P-values derived from ANOVA comparing PUFAs between allele groups within each group of weight. Significant differences are marked by stars ($p<0.05$).

[b]P-values derived from ANOVA comparing PUFAs between groups of weight. Significant differences are marked by daggers ($p<0.05$).

Major allele: M; minor allele: m; LA: Linoleic Acid; GLA: γ-Linolenic Acid; DGLA: Dihomo-γ-Linolenic Acid; AA: Arachidonic Acid; AdA: Adrenic Acid; DPAn6: Docosapentaenoic acid n6; ALA: α-linolenic Acid; EPA: Eicosapentaenoic acid; DPAn3: Docosapentaenoic acid n3; DHA: Docosahexaenoic acid.

Supplemental Table 3. Associations between colostrum proportions of PUFAs and *FADS* polymorphisms.

Fatty acids and Genes	SNP	*M/m*	NORMAL-WEIGHT			OVERWEIGHT/OBESE		
			N	β	*P*	N	β	*P*
LA (C18:2n6)								
FADS1	rs174545	*C/G*	13	0.70	0.353	16	-0.28	0.465
FADS1	rs174546	*C/T*	13	0.70	0.353	17	-0.21	0.532
FADS1	rs174553	*A/G*	13	0.70	0.353	17	-0.21	0.532
FADS2	rs1535	*A/G*	11	1.59	0.109	17	-0.21	0.532
FADS2	rs174583	*C/T*	12	1.14	0.165	17	-0.21	0.532
FADS2	rs99780	*C/T*	11	1.11	0.130	17	-0.21	0.532
GLA (C18:3n6)								
FADS1	rs174545	*C/G*	13	0.17	0.650	16	0.21	0.409
FADS1	rs174546	*C/T*	13	0.17	0.650	17	0.37	0.166
FADS1	rs174553	*A/G*	13	0.17	0.650	17	0.37	0.166
FADS2	rs1535	*A/G*	11	0.45	0.398	17	0.37	0.166
FADS2	rs174583	*C/T*	12	0.11	0.807	17	0.37	0.166
FADS2	rs99780	*C/T*	11	0.46	0.135	17	0.37	0.166
DGLA (C20:3n6)								
FADS1	rs174545	*C/G*	13	1.02	**0.018***	16	0.03	0.936
FADS1	rs174546	*C/T*	13	1.02	**0.018***	17	0.22	0.492
FADS1	rs174553	*A/G*	13	1.02	**0.018***	17	0.22	0.492
FADS2	rs1535	*A/G*	11	1.29	**0.040***	17	0.22	0.492
FADS2	rs174583	*C/T*	12	1.01	0.051	17	0.22	0.492
FADS2	rs99780	*C/T*	11	0.94	**0.024***	17	0.22	0.492
AA (C20:4n6)								
FADS1	rs174545	*C/G*	13	0.37	0.618	16	-0.45	0.135
FADS1	rs174546	*C/T*	13	0.37	0.618	17	-0.05	0.883
FADS1	rs174553	*A/G*	13	0.37	0.618	17	-0.05	0.883
FADS2	rs1535	*A/G*	11	0.26	0.845	17	-0.05	0.883
FADS2	rs174583	*C/T*	12	0.44	0.641	17	-0.05	0.883
FADS2	rs99780	*C/T*	11	-0.18	0.853	17	-0.05	0.883
AdA (C22:4n6)								
FADS1	rs174545	*C/G*	13	0.17	0.834	16	-0.56	0.073
FADS1	rs174546	*C/T*	13	0.17	0.834	17	-0.17	0.625
FADS1	rs174553	*A/G*	13	0.17	0.834	17	-0.17	0.625
FADS2	rs1535	*A/G*	11	-0.48	0.713	17	-0.17	0.625
FADS2	rs174583	*C/T*	12	0.20	0.839	17	-0.17	0.625
FADS2	rs99780	*C/T*	11	-0.54	0.549	17	-0.17	0.625
DPAn6 (C22:5n6)								
FADS1	rs174545	*C/G*	13	1.05	0.104	16	-0.22	0.467
FADS1	rs174546	*C/T*	13	1.05	0.104	17	0.02	0.951
FADS1	rs174553	*A/G*	13	1.05	0.104	17	0.02	0.951
FADS2	rs1535	*A/G*	11	0.80	0.381	17	0.02	0.951
FADS2	rs174583	*C/T*	12	1.14	0.147	17	0.02	0.951
FADS2	rs99780	*C/T*	11	0.37	0.600	17	0.02	0.951
ALA (C18:3n3)								
FADS1	rs174545	*C/G*	13	0.05	0.946	16	0.09	0.754
FADS1	rs174546	*C/T*	13	0.05	0.946	17	0.04	0.859
FADS1	rs174553	*A/G*	13	0.05	0.946	17	0.04	0.859
FADS2	rs1535	*A/G*	11	1.04	0.274	17	0.04	0.859
FADS2	rs174583	*C/T*	12	0.46	0.590	17	0.04	0.859
FADS2	rs99780	*C/T*	11	0.37	0.640	17	0.04	0.859
EPA (C20:5n3)								
FADS1	rs174545	*C/G*	13	-0.23	0.428	16	0.82	**0.026***
FADS1	rs174546	*C/T*	13	-0.23	0.428	17	0.73	**0.011***
FADS1	rs174553	*A/G*	13	-0.23	0.428	17	0.73	**0.011***
FADS2	rs1535	*A/G*	11	0.17	0.367	17	0.73	**0.011***
FADS2	rs174583	*C/T*	12	-0.04	0.869	17	0.73	**0.011***
FADS2	rs99780	*C/T*	11	0.15	0.217	17	0.73	**0.011***

Supplemental Table 3. Continuation...

Fatty acids and Genes	SNP	*M/m*	NORMAL-WEIGHT			OVERWEIGHT/OBESE		
			N	β	*P*	N	β	*P*
DPAn3 (C22:5n3)								
FADS1	rs174545	*C/G*	13	-0.57	0.451	16	0.08	0.812
FADS1	rs174546	*C/T*	13	-0.57	0.451	17	0.20	0.506
FADS1	rs174553	*A/G*	13	-0.57	0.451	17	0.20	0.506
FADS2	rs1535	*A/G*	11	-0.51	0.670	17	0.20	0.506
FADS2	rs174583	*C/T*	12	-0.22	0.785	17	0.20	0.506
FADS2	rs99780	*C/T*	11	-0.50	0.545	17	0.20	0.506
DHA (C22:6n3)								
FADS1	rs174545	*C/G*	13	-0.65	0.354	16	0.18	0.666
FADS1	rs174546	*C/T*	13	-0.65	0.354	17	0.22	0.547
FADS1	rs174553	*A/G*	13	-0.65	0.354	17	0.22	0.547
FADS2	rs1535	*A/G*	11	-0.43	0.719	17	0.22	0.547
FADS2	rs174583	*C/T*	12	-0.52	0.538	17	0.22	0.547
FADS2	rs99780	*C/T*	11	-0.50	0.551	17	0.22	0.547
n6 PUFA								
FADS1	rs174545	C/G	13	0.72	0.233	16	-0.24	0.623
FADS1	rs174546	C/T	13	0.72	0.233	17	-0.08	0.859
FADS1	rs174553	A/G	13	0.72	0.233	17	-0.08	0.859
FADS2	rs1535	A/G	11	1.17	0.122	17	-0.08	0.859
FADS2	rs174583	C/T	12	0.93	0.106	17	-0.08	0.859
FADS2	rs99780	C/T	11	1.37	0.070	17	-0.08	0.859
n3 PUFA								
FADS1	rs174545	C/G	13	-0.42	0.507	16	0.39	0.391
FADS1	rs174546	C/T	13	-0.42	0.507	17	0.46	0.266
FADS1	rs174553	A/G	13	-0.42	0.507	17	0.46	0.266
FADS2	rs1535	A/G	11	-0.02	0.986	17	0.46	0.266
FADS2	rs174583	C/T	12	-0.34	0.644	17	0.46	0.266
FADS2	rs99780	C/T	11	0.17	0.875	17	0.46	0.266
n6 LC-PUFA								
FADS1	rs174545	C/G	13	0.68	0.298	16	-0.46	0.160
FADS1	rs174546	C/T	13	0.68	0.298	17	-0.08	0.856
FADS1	rs174553	A/G	13	0.68	0.298	17	-0.08	0.856
FADS2	rs1535	A/G	11	0.36	0.714	17	-0.08	0.856
FADS2	rs174583	C/T	12	0.72	0.351	17	-0.08	0.856
FADS2	rs99780	C/T	11	0.53	0.622	17	-0.08	0.856
n3 LC-PUFA								
FADS1	rs174545	C/G	13	-0.61	0.265	16	0.25	0.626
FADS1	rs174546	C/T	13	-0.61	0.265	17	0.34	0.452
FADS1	rs174553	A/G	13	-0.61	0.265	17	0.34	0.452
FADS2	rs1535	A/G	11	-0.57	0.545	17	0.34	0.452
FADS2	rs174583	C/T	12	-0.56	0.378	17	0.34	0.452
FADS2	rs99780	C/T	11	-0.56	0.598	17	0.34	0.452
n6:n3 PUFA								
FADS1	rs174545	C/G	13	0.70	0.194	16	-0.37	0.479
FADS1	rs174546	C/T	13	0.70	0.194	17	-0.30	0.524
FADS1	rs174553	A/G	13	0.70	0.194	17	-0.30	0.524
FADS2	rs1535	A/G	11	0.52	0.536	17	-0.30	0.524
FADS2	rs174583	C/T	12	0.69	0.272	17	-0.30	0.524
FADS2	rs99780	C/T	11	0.46	0.634	17	-0.30	0.524
n6:n3 LC-PUFA								
FADS1	rs174545	C/G	13	0.94	**0.022***	16	-0.59	0.241
FADS1	rs174546	C/T	13	0.94	**0.022***	17	-0.36	0.457
FADS1	rs174553	A/G	13	0.94	**0.022***	17	-0.36	0.457
FADS2	rs1535	A/G	11	0.71	0.201	17	-0.36	0.457
FADS2	rs174583	C/T	12	0.89	0.056	17	-0.36	0.457
FADS2	rs99780	C/T	11	0.77	0.211	17	-0.36	0.457

Supplemental Table 3. Continuation...

Fatty acids and Genes	SNP	*M/m*	NORMAL-WEIGHT			OVERWEIGHT/OBESE		
			N	β	*P*	N	β	*P*
EPA:AA								
FADS1	rs174545	*C/G*	13	-0.46	0.405	16	0.91	**0.005***
FADS1	rs174546	*C/T*	13	-0.46	0.405	17	0.72	**0.023***
FADS1	rs174553	*A/G*	13	-0.46	0.405	17	0.72	**0.023***
FADS2	rs1535	*A/G*	11	-0.03	0.977	17	0.72	**0.023***
FADS2	rs174583	*C/T*	12	-0.34	0.626	17	0.72	**0.023***
FADS2	rs99780	*C/T*	11	0.27	0.674	17	0.72	**0.023***
DHA:AA								
FADS1	rs174545	*C/G*	13	-1.04	**0.014***	16	0.46	0.201
FADS1	rs174546	*C/T*	13	-1.04	**0.014***	17	0.20	0.574
FADS1	rs174553	*A/G*	13	-1.04	**0.014***	17	0.20	0.574
FADS2	rs1535	*A/G*	11	-0.71	0.150	17	0.20	0.574
FADS2	rs174583	*C/T*	12	-0.96	**0.049***	17	0.20	0.574
FADS2	rs99780	*C/T*	11	-0.42	0.293	17	0.20	0.574
GLA:LA								
FADS2	rs1535	*A/G*	11	-0.25	0.443	17	0.52	0.122
FADS2	rs174583	*C/T*	12	-0.36	0.180	17	0.52	0.122
FADS2	rs99780	*C/T*	11	-0.03	0.904	17	0.52	0.122
DGLA:LA								
FADS2	rs1535	*A/G*	11	0.24	0.568	17	0.29	0.241
FADS2	rs174583	*C/T*	12	0.30	0.329	17	0.29	0.241
FADS2	rs99780	*C/T*	11	0.21	0.479	17	0.29	0.241
AA:LA								
FADS1	rs174545	*C/G*	13	-0.09	0.907	16	-0.12	0.700
FADS1	rs174546	*C/T*	13	-0.09	0.907	17	0.11	0.733
FADS1	rs174553	*A/G*	13	-0.09	0.907	17	0.11	0.733
FADS2	rs1535	*A/G*	11	-0.61	0.645	17	0.11	0.733
FADS2	rs174583	*C/T*	12	-0.25	0.796	17	0.11	0.733
FADS2	rs99780	*C/T*	11	-0.69	0.441	17	0.11	0.733
AA:DGLA								
FADS1	rs174545	*C/G*	13	-0.43	0.466	16	-0.47	0.202
FADS1	rs174546	*C/T*	13	-0.43	0.466	17	-0.34	0.304
FADS1	rs174553	*A/G*	13	-0.43	0.466	17	-0.34	0.304
EPA:ALA								
FADS1	rs174545	*C/G*	13	-0.23	0.625	16	0.58	0.085
FADS1	rs174546	*C/T*	13	-0.23	0.625	17	0.54	**0.041***
FADS1	rs174553	*A/G*	13	-0.23	0.625	17	0.54	**0.041***
FADS2	rs1535	*A/G*	11	-0.70	0.349	17	0.54	**0.041***
FADS2	rs174583	*C/T*	12	-0.37	0.500	17	0.54	**0.041***
FADS2	rs99780	*C/T*	11	-0.18	0.766	17	0.54	**0.041***
DHA:EPA								
FADS2	rs1535	*A/G*	11	-0.46	0.611	17	-0.64	**0.017***
FADS2	rs174583	*C/T*	12	-0.37	0.550	17	-0.64	**0.017***
FADS2	rs99780	*C/T*	11	-0.50	0.416	17	-0.64	**0.017***
DHA:DPAn3								
FADS2	rs1535	*A/G*	11	0.29	0.488	17	-0.11	0.691
FADS2	rs174583	*C/T*	12	-0.36	0.604	17	-0.11	0.691
FADS2	rs99780	*C/T*	11	0.18	0.566	17	-0.11	0.691

Associations between SNPs and fatty acids were analyzed using linear regression. SNPs were coded according to minor allele count and analyzed as a numeric variable. "β"= beta per minor allele standardized per the major allele. Significant associations after adjustment for potential confounders such as gestational weight gain, age, education, smoking, parity, dietary n6:n3 ratio and energy intake are denoted by stars ($p<0.05$). LA: Linoleic Acid; GLA: γ-Linolenic Acid; DGLA: Dihomo-γ-Linolenic Acid; AA: Arachidonic Acid; AdA: Adrenic Acid; DPAn6: Docosapentaenoic acid n6; ALA: α-linolenic Acid; EPA: Eicosapentaenoic acid; DPAn3: Docosapentaenoic acid n3; DHA: Docosahexaenoic acid.

5.3. EVALUATION OF LESS INVASIVE METHODS TO ASSESS FATTY ACIDS FROM PHOSPHOLIPID FRACTION: CHEEK CELL AND CAPILLARY BLOOD SAMPLING

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Abstract

Plasma is the most commonly employed matrix for analyzing fatty acids (FAs), but its extraction is not well accepted in the infant population. The objectives of this study were to evaluate cheek cells and capillary blood as alternatives to plasma sampling for FA analysis and to standardize the methodology. Samples were obtained from 20 children who underwent lipid extraction, phospholipid isolation by Solid Phase Extraction (SPE) in a 96-well plate, methylation, and analysis by fast gas chromatography (GC). A positive correlation was found for most of the FAs, especially long-chain polyunsaturated fatty acids (LC-PUFAs), in cheek cells and capillary blood versus plasma samples (r¼0.32–0.99). No differences were found in the levels of n-6: n-3 PUFA and n-6: n-3 LC-PUFA ratios between cheek cells and capillary blood. These two proposed samples can therefore be used as alternatives to plasma sampling for phospholipid FA analysis, especially LC-PUFAs.

Keywords: phospholipids, buccal mucosal cells, infants, fingertip blood, plasma.

Abbreviations: FA, fatty acid, **LC-PUFA,** long-chain polyunsaturated fatty acid, **DCM:MeOH,** dichloromethane methanol, **SPE,** solid phase extraction, **GC,** gas chromatography, **CVD,** cardiovascular disease, **DHA,** docosahexaenoic acid, **EPA,** eicosapentanoic acid, **BF_3,** boron triflouride, **MeOH,** methanol, **BHT,** butyl hydroxi toluene, **$NaOCH_3$,** sodium methylate, **FAME,** fatty acid methyl ester, **PUFA,** polyunsaturated fatty acid, **PC-C15:0,** 1,2-dipentadecanoyl-*sn*-glycero-3-phosphocholine, **IS,** internal standard, **TLC,** thin layer chromatography, **DBS,** drop blood spots, **NaCl,** sodium chloride, **CV,** coefficient of variation, **FID,** flame ionization detector, **AA,** arachidonic acid, **SFA,** saturated fatty acid, **MUFA,** monounsaturated fatty acid.

Introduction

Many epidemiological and interventional studies have reported that n-3 and n-6 long-chain polyunsaturated fatty acids (LC-PUFAs) play important roles in human health and common diseases (Calder & Yaqoob, 2009; Lopez-Huertas, 2010; Riediger et al., 2009). LC-PUFAs have a critical influence on neurodevelopment in childhood since they are implicated in the normal growth and functioning of the brain and central nervous system (Calder & Yaqoob, 2009; Lopez-Huertas, 2010; Riediger et al., 2009; Ryan et al., 2010). It is well known that imbalances in these nutrients, from either dietary intake or metabolism, may have significant negative effects on brain function in both the developmental phase and throughout the entire life span (Schuchardt et al., 2010) and there is increasing evidence regarding the positive effects of supplementation with LCPUFAs, especially with those involved in neuronal processes such as the n-3 fatty acids (FAs) docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Lopez-Huertas, 2010, Ryan et al., 2010, Schuchardt et al., 2010). Some of the positive effects of supplementation shown by controlled trials are improvement in visual, cognitive and motor functions (Calder & Yaqoob, 2009; Lopez-Huertas, 2010). Moreover, a link has been shown between developmental disorders in childhood and imbalances in the n6:n-3 ratio (Schuchardt et al., 2010), and several authors agree on the need to lower the n-6:n-3 ratio to 1–2:1 (Go´mez et al., 2011; Granados et al., 2006). Therefore, further research on newborns and infants is of special interest to evaluate how FAs influence health disorders.

Current studies use different biological compartments for FA analysis, such as whole blood, red blood cells, plasma, and adipose tissue. Of these, plasma is most commonly used to determine an individual's fatty acid status (Klingler & Koletzko, 2012). Separation of the phospholipid fraction is the best way to assess FAs since other lipid fractions, such as triglycerides or total FAs, are more influenced by recent diet. However, these approaches are invasive and not well accepted in population studies, particularly in infants and young children. For this reason, studies on FAs often rely on food intake questionnaires which do not allow accurate estimation of FA status, since they merely provide data about the type of diet consumed and therefore dietary fat intake (Klingler et al., 2011; Klingler & Koletzko, 2012).

Since the 1980s several studies have proposed the cheek cell sampling as an alternative matrix for evaluating FA status. However, the sampling and analytical methods applied in these studies have differed widely, thus hindering the comparison of results. A wide variety of materials has been used for sample collection, such as distilled water (Browning et al., 2012; Hodson et al., 2014), plastic spoons (Sampugna et al., 1988), sterile cotton swabs (Koletzko et al., 1999), sterile wooden spatulas (Hoffman et al., 1999), sterile gauzes (Connor et al., 2000) and endocervical brushes (Grindel et al., 2013; Kirby et al., 2010; Klingler et al., 2011; Laitinen et al., 2006). Furthermore, the lipid extraction methods proposed by Bligh & Dyer (1959), Folch et al. (1957) and Hoffman & Uauy (1992), have been previously used as references (Browning et al., 2012; Connor et al., 2000; Grindel et al., 2013; Hodson et al., 2014; Hoffman et al., 1999; Kirby et al., 2010; Klingler et al., 2011; Koletzko et al., 1999; Laitinen et al., 2006; McMurchie et al., 1984; Sampugna et al., 1988). Regarding the separation of phospholipids, two methods have commonly been used: thin-layer chromatography (TLC) (Connor et al., 2000; Grindel et al., 2013; Hodson et al., 2014; Hoffman et al., 1999; Koletzko et al., 1999; McMurchie et al., 1984; Sampugna et al., 1988) or solid phase extraction (SPE) with cartridges (Browning et al., 2012; Laitinen et al., 2006), the latter of which limits the quantity of samples that can be processed simultaneously. Another important obstacle to overcome is

small sample size, which may hinder proper analysis of FAs, as well as loss of lipid fractions during sample preparation (Klingler & Koletzko, 2012).

Meanwhile, capillary blood, commonly called drop blood spots (DBS), has also been proposed as alternative to plasma analysis (Armstrong et al., 2008; Bailey-Hall et al., 2008; Bicalho et al., 2008; Marangoni et al., 2004; Metherel et al., 2012; Min et al., 2011; Ryan & Nelson, 2008). Normally, the process consists of direct methylation of whole capillary blood from fingertips absorbed on a chromatographic paper (Armstrong et al., 2008; Bailey-Hall et al., 2008; Bicalho et al., 2008; Metherel et al., 2012; Ryan & Nelson, 2008). This method is much simpler and reduces the amount of analytical time required when compared with plasma samples (Min et al., 2011).

To date, plasma remains the most common matrix for FA analysis and yet there is no agreed standard procedure. However, plasma sampling is not recommended in studies involving infants and young children, or subjects with inaccessible veins. This presents an issue when recruiting volunteers for studies, especially if multiple blood sampling is necessary. Although attempts have been made to compare plasma sampling with other less invasive techniques, such as mucosal sampling (Klingler & Koletzko, 2012), to the best of our knowledge, no previous studies have compared the results of these three matrices: cheek cells, capillary blood and plasma.

The aim of this study was (1) to evaluate cheek cells and capillary blood as less invasive alternatives to traditional plasma sampling for FA analysis and (2) to contribute to standardization of sample preparation independently of the matrix. In addition, an assessment was conducted of correlations between FA status in these two biological matrices, cheek cells and capillary blood, versus plasma (as the standard matrix).

Methods

Subjects

Cheek cell, capillary blood and plasma samples were obtained from child volunteers at the "San Cecilio" Clinical University Hospital in Granada, Spain, who had fasted prior to the collection procedure. Complete sets of data were available from 20 subjects. Written informed consent was obtained from the parents of all study participants prior to sample collection. For method validation, 12 samples of each matrix from different adult volunteers belonging to our research group at the time were collected and mixed as explained below to create working pools.

Reagents and materials

Methanol (HPLC grade) and anhydrous sodium sulfate were purchased from Scharlab (Sentmenat, Spain). Sodium chloride, diethyl ether, 2-propanol, acetic acid, ethanol 96%, dichloromethane and chloroform were purchased from Panreac (Barcelona, Spain). The n-hexane was purchased from Merck (Darmstadt, Germany). A 14% boron trifluoride methanol solution (BF3/MeOH), 50 mg/mL butylated hydroxytoluene (BHT) in ethanol and sodium methylate in 0.5M methanol (NaOCH3/ MeOH) were purchased from Sigma Aldrich (St. Louis, MO).

Discovery DSC-NH2 SPE 96-well plates (100 mg/well) were purchased from Supelco (Bellefonte, PA). Cheek cells were collected using a Rovers EndoCervex-Brush supplied by Heinz Herenz (Hamburg, Germany) and a Fluka blood collection kit purchased from Sigma Aldrich was used to collect capillary blood samples (St. Louis, MO). Other mucosal cell extraction devices were also tested, such as sterile cotton swabs with a wooden handle, supplied by Deltalab (Barcelona, Spain), sterile cotton swabs with a plastic handle from COPAN Innovation (Brescia, Italy), sterile gauzes from the INDAS Laboratory (Portillo, Spain) and a circular-shaped Gynobrush from Heinz Herenz (Hamburg, Germany).

A supelco 37-component fatty acid methyl ester (FAME) mix and polyunsaturated fatty acid (PUFA) methyl ester mix (animal source) were used for peak identification, and 1,2- dipentadecanoyl-sn-glycero-3-phosphocholine (PC-C15:0) was used as the internal standard solution (IS), all purchased from Supelco (Bellefonte, PA). Stock standard solutions were prepared by dissolving the commercial standards in n-hexane in appropriate concentrations (200–1000 mg/mL) and stored at 20 ºC until use.

Sampling and lipid extraction

Plasma samples

Blood was obtained by arm venipuncture and plasma was separated by centrifugation and immediately frozen and stored at 80 ºC until analysis. Lipid extraction and phospholipid isolation were performed according to a modified version of the method described by Chisaguano et al. (2013). Prior to lipid extraction, 40 mL of IS (PC-C15:0) at a concentration of 0.4 mg/mL was added to 300 mL of plasma, and lipids were subsequently extracted with 3mL of dichloromethane/methanol (DCM:MeOH) (2:1). After sonication for 5 min, 500 mL of a saturated sodium chloride solution in water was added to the tube, shaken in vortex and centrifuged for 7 min at 3000 rpm and 25 ºC. After centrifugation, the organic phase was collected by aspiration, transferred into a conical-shaped tube and dried under a stream of N2 at 40 ºC in a multiple position evaporator.

Cheek Cell samples

Prior to cheek cell collection, the mouth was rinsed 3 times with tap water to eliminate possible food residues. Then, each inner cheek was gently scraped back and forth 25 times with a Rovers EndoCervex-Brush using one different side of the brush for each cheek. The tip of the brush was transferred into a 4mL cryotube and stored at 80 ºC until analysis.

For analysis, the brush was inserted into an assay glass tube. The remains in the cryotube were swept with 3mL of DCM:MeOH (2:1) and transferred to the assay glass tube along with the brush. The tube was vortexed for 1 min and sonicated for 5 min. The brush was removed and 750 mL of sodium chloride (NaCl) (0.9%) was added. The tube was shaken and centrifuged at 3000 rpm for 5 min. The supernatant was discarded and the organic phase was collected by aspiration and transferred into a conical-shaped tube. 20 mL of 0.4 mg/mL IS solution (PC-C15:0) was added and then vortexed for 30 s. The organic extract was then dried under a stream of N2 at 40 ºC.

Capillary blood samples

One drop of blood was obtained by punching the index finger with a lancet and, avoiding skin contact, the drop was absorbed on a chromatographic paper from the blood collection kit. 10 mL of BHT (50 mg/mL in ethanol) was added to the paper to prevent oxidation. The sample was stored at 80 ºC in a cellophane envelope with airtight closure until analysis.

For analysis, the filter paper was transferred to an assay tube with 3mL of DCM:MeOH (2:1). The tube was sonicated for 5 min and then 750 mL of NaCl (0.9%) was added. The tube was vortexed for 1 min and centrifuged at 3000 rpm for 5 min. The supernatant was discarded and the organic phase was collected by aspiration and transferred into a conical-shaped tube to which 20 mL of 0.4 mg/mL IS solution (PC-C15:0) was added and then vortexed for 30 s. The extract was then dried under a stream of N2 at 40 ºC.

Phospholipids isolation using Solid Phase Extraction (SPE)

The dry lipid extracts were dissolved in 400 mL of chloroform and loaded into a previously activated solid phase extraction (SPE) 96-well plate. Each well was activated with 2 x 1mL of hexane and 1mL of chloroform/2-propanol (2:1). After sample load, wells were washed with another 1mL of chloroform/2-propanol (2:1) to remove neutral lipids, followed by 1mL of 2% acetic acid in diethyl ether to remove the free FA and finally eluted with 1.5mL of methanol to collect the phospholipid fraction in the 96-well collection plate.

Fatty acids methylation

Methylation was performed according to a modified version of the method described by Chisaguano et al. (2013). Briefly, 2mL of sodium methylate in 0.5M methanol was added to the phospholipid fraction obtained from SPE. The mixture was vortexed for 30 s and heated (Thermoblock P-Selecta, Barcelona, Spain) at 80 ºC for 10 min. After subsequent cooling in an ice bath, 2mL of boron trifluoride methanolic solution (14% v/v) was added, vortexed, and the mixture was heated again at 80 ºC for 3 min. After that, the tubes were cooled down. Fatty acid methyl esters (FAMEs) were then extracted by adding 500 mL of n-hexane and 2mL of saturated sodium chloride solution in water was used to enhance phase separation. The mixture was centrifuged for 10 min at 3000 rpm. After this, the clear organic top layer was transferred into another tube and dried over anhydrous sodium sulfate. Finally, 300 mL of the extract was transferred into an automatic injector vial equipped with a volume adapter. The aliquot was evaporated to dryness under a stream of N2 and re-diluted with 50 mL of n-hexane.

Fatty Acids determination by Gas Chromatography (GC)

Separation of FAMEs was performed by fast gas chromatography following a modified version of the method developed by Chisaguano et al. (2013). The chromatographic system consisted of an HP-6890 Series GC System (Hewlett-Packard, Waldbronn, Germany) equipped with a split/splitless injector, an HP-7683B Series autoinjector, an electronic pressure control unit, a flame ionization detector (FID), and a

fused-silica capillary column RTX-2330 (40m x 0.18mm internal diameter, 0.10 mm film thickness) coated with a 10% cyanopropyl phenyl-90% biscyanopropyl polysiloxane non-bonded stationary phase from Restek (Souderton, UK). The chromatographic conditions used were: helium was used as a carrier gas at a constant pressure of 37.97 psi (equivalent to linear velocity of 26 cm/s at 110 ºC). The detector and injector temperatures were set at 300 ºC and 250 ºC, respectively; the split ratio was at 1:15 for capillary blood and mucosa, and 1:50 for plasma and standard analyzes. The injection volume was 1 mL. Oven temperatures were programed as follows: initial temperature was set at 110 ºC, which was held for 0.5 min, then the temperature was increased at a rate of 52ºC/min to 195 ºC. This temperature was held for 6min and finally increased to 230 ºC at a rate of 25ºC/min, and held for 6.5 min.

Identification of FAMEs from both biological matrices was performed by comparing peak retention times of FAME samples and standard FAME mix solutions. FAMEs were quantified by standard normalization (% total FA).

Method Validation

Cheek cells and capillary blood from 12 different individuals were used to prepare a pool from each matrix after lipid extraction. The pools were aliquoted in different samples which were then processed in groups to evaluate repeatability (n¼3) and reproducibility (n¼9 on 3 different days). Two blank samples from each process were prepared in order to identify possible contaminations.

The recovery of the method was assessed according to Chisaguano et al. (2013), adding known amounts of 1,2- dipentadecanoyl-sn-glycero-3-phosphocholine (10, 50 and 100 mg) to samples before and after the SPE stage. Although PC-C15:0 was used as the IS solution in the final methodology, in this specific evaluation of recovery, PC-C15:0 was selected to assess the yield of the method, and C13:0 was also added as IS. Triplicate determinations were performed for each level. All samples were subjected to the complete proposed procedure.

Statistical analysis

All statistical analyzes were performed using SPSS 20.0 for Windows (SPSS Inc., Chicago, IL). The Kolmogorov–Smirnov test was used to study the normal distribution of data and the nonnormal distributed were naturally log transformed. FA levels within the different matrices were compared by means of a univariate ANOVA applying Bonferroni's post-hoc correction. Correlations between different variables were determined by Pearson's correlation coefficient. The confidence level was established at 95% for all analyses. Thus, results obtaining a *p* value of below 0.05 were considered statistically significant.

Results

The same 19 FAs were analyzed from the phospholipid fraction and identified in the chromatograms of each different matrix, as shown in Figure 1.

The FA composition of cheek cell, plasma and capillary blood samples is presented in Table 1. Statistical differences were found in the mean of FAs from each matrix. Within the unsaturated FAs, the quantitatively dominant FAs in all matrices were oleic (C18:1,n -9), linoleic (C18:2, n-6) and arachidonic (C20:4, n-6) acids. As noted in the table, arachidonic acid (AA) and DHA contents were approximately 2–4 times higher in plasma than in cheek cells and capillary blood. The n-6 series PUFAs were more abundant than the n-3 series. The mean of the n-6: n-3 and n-6: n-3 LC-PUFA ratios were found to be similar between cheek cell and capillary blood samples, but both of these groups were statistically different from the plasma samples.

The precision of the method was calculated through the coefficients of variation (CV) which are shown in Table 2. The CV for cheek cell samples ranged from 1.3 to 5.6% (intra-day) and 3.7 to 9.3% (inter-day) while the CV from capillary blood samples ranged from 0.5 to 7.7% (intra-day) and 1.7 to 9.8% (inter-day). Relative recovery of the SPE process was 100.5%, expressed as an average (3 different addition levels) ratio of calculated and nominal spiked amount x 100.

Fatty acids of cheek cell and capillary blood samples were correlated with those of plasma samples. Most of the FAs showed a statistically significant association with values from r¼0.32 to r¼0.99, especially LC-PUFAs, where FA such as AA, EPA and DHA are included (Table 3).

Discussion

As plasma sampling status is not well accepted to assess FA, especially when dealing with newborn or child populations (Klingler et al., 2011; Klingler & Koletzko, 2012), it has become increasingly important to study alternative matrices. Cheek cells and capillary blood are the most commonly considered alternatives, but to our knowledge there are still no studies that have evaluated and compared the results obtained for these two different approaches with those of traditional plasma sampling.

In preliminary studies on cheek cell samples, we tested different devices commonly used in other studies for their extraction (Connor et al., 2000; Grindel et al., 2013; Hoffman et al., 1999; Kirby et al., 2010; Klingler et al., 2011; Koletzko et al., 1999; Laitinen et al., 2006), including sterile cotton swabs a wooden handle, sterile cotton swabs a plastic handle, sterile gauzes, a circular-shaped Gynobrush and a Rovers EndoCervex- Brush. First, the wooden spatula was rejected because of the unpleasant sensation it caused in comparison with the other options, and the sterile cotton swabs with a wooden handle and the sterile gauzes were ruled out because of high levels of contamination that interfered with the analyzes (data not shown). The sterile cotton swabs with a plastic handle were also found to be inadequate because the plastic was not resistant to the solvent used for lipid extraction. The circular-shaped Gynobrush was also dismissed because it had a wire and nylon bristle combination that could potentially hurt the patient if not used carefully. However, the Rovers EndoCervex-Brush was found to be a perfect sampling device for several reasons: it was made entirely of flexible, soft polypropylene, and it consisted of a brush head that could be detached from the handle after collection, making it easy to store in a small tube. Additionally, the use of a brush for scraping was selected in preference to the cotton swab with a wooden handle because it has been shown that the average cell yield obtained with a cotton swab is approximately two-thirds of that collected with an endocervical brush. Consequently, the use of a brush might be crucial to

collect an adequate amount of cells in young children for a reliable analysis of FAs with this method (Klingler et al., 2011).

Proper collection of cheek cells requires a strict sampling procedure. It has been proved that rinsing the mouth after scraping the inner cheeks and collecting this residue, leads to significantly higher cell yields (Devereux-Graminski & Sampugna, 1993). However, the rinsing residue may be discarded because it is not applicable when collecting cells in babies and we observed that the differences were negligible. It has also been demonstrated that cleaning the mouth prior to sampling reduces possible food residues (Devereux-Graminski & Sampugna, 1993), however this does not apply in babies with no solid food intake.

It is known that cheek cells are robust and hard to disrupt by isolated applications of detergents, ultrasound or polar solvents. Methanol and ultrasound were used in this study to disrupt cell membranes, as Klingler et al. (2011) have shown that this method has a recovery rate of >90%.

Capillary blood is another suitable matrix for assessing FA status instead of plasma, and represents a less invasive sampling technique. Collection only requires punching one finger to obtain one drop of blood. Nevertheless, capillary blood sampling for children is less recommended than cheek cells because extraction is more aggressive. Moreover, capillary blood requires the addition of BHT to prevent oxidation of the sample absorbed on the chromatographic paper, and this could lead to contamination issues.

The identification of false-positive peaks is important for accurate qualification of FAs: not only does this define the lower limit of the method, but also excludes FAs from analysis (Klingleret al., 2011). The systematic analysis of blanks may yield information about contamination derived from chemicals or materials. In the present study, four impurities were found to co-elute with the peaks of C16:0, C18:0, C20:0 and C22:0 and their presence was attributed to SPE well plate contamination. These data were taken into account in calculations, and C16:0 and C18:0 peak areas from blanks were subtracted from samples. Meanwhile, due to their negligible contribution to the global profile, since they were minority peaks, C20:0 and C22:0 were excluded from the study. Blanks were carried out to evaluate contamination from the Rovers EndoCervex-Brushand the chromatographic paper from the blood collection kit and peaks from contaminations did not co-elute with any FA peak.

Data on the precision of FA analysis have mainly been published for plasma, and only to a lesser extent for cheek cells and capillary blood. The CV reported for FA determination in cheek cells has ranged from 1.0% to 10.5% (Glaser et al., 2009; Klingler et al., 2011), and in the present study, the CV for cheek cell and capillary blood samples was lower than 10%. The recovery assessment results were considered satisfactory, as described by other authors (Browning et al., 2012; Chisaguano et al., 2013; Laitinen et al., 2006), and SPE represents a good approach in phospholipid FA analysis.

The levels of FAs found in all matrices differed considerably, but can only be compared with those found in other studies which evaluated the same FAs in order to conduct equivalent comparisons. Since the data presented by other authors are limited exclusively to PUFAs and exclude or summarize saturated and

monounsaturated FAs (Browning et al., 2012; Grindel et al., 2013; Hodson et al., 2014; Hoffman et al., 1999; Klingler et al., 2011; Laitinen et al., 2006), only the same FAs were considered in a comparison of results. Reported PUFA levels were comparable in all studies, whereas saturated acids differed, possibly because of dietary habits or contamination (Klingler et al., 2011). The n-6:n-3 ratios found in our study (11:1 for cheek cell samples, 10:1 for capillary blood samples and 7:1 for plasma samples) were considerably higher than the ratio of 1-2:1 reported by some authors (Go´mez et al., 2011; Granados et al., 2006). As is well known, excessive amounts of n-6 and a very high n-6: n-3 ratio can be caused by an imbalanced diet and they promote the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases, whereas increased levels of n-3 (a low n-6: n-3 ratio) exert suppressive effects (Simopoulos, 2006).

In accordance with previous studies (Grindel et al., 2013; Klingler et al., 2011; Marangoni et al., 2004), the dominant unsaturated FAs observed in this study were the oleic, linoleic and arachidonic acids for all 3 matrices. In addition, we observed differences in AA and DHA between cheek cell, capillary blood and plasma samples. This has also been reported in previous studies, where plasma levels of these FAs were approximately three times higher (Klingler et al., 2011).

A study of correlations between the three different matrices was useful to determine the suitability of capillary blood and cheek cells as alternatives to plasma for assessing FA status in infants. We focused on the determination of LC- PUFAs because several authors have recently found that an understanding of their metabolic synthesis routes is a key factor in the study of several diseases (Barman et al., 2013; Gottrand, 2008). As noted by other authors, we also observed good correlation coefficients for FAs in cheek cell (Grindel et al., 2013; Klingler et al., 2011) and capillary blood samples in relation to plasma, especially for LC-PUFAs such as EPA and DHA.

Therefore, in line with other studies, cheek cell (Connor et al., 2000; Grindel et al., 2013; Hoffman et al., 1999; Kirby et al., 2010; Klingler et al., 2011) and capillary blood samples (Marangoni et al., 2004; Metherel et al., 2012) reflected LC-PUFA status as well as plasma does, hence functioning as alternatives for LC-PUFA status evaluation in children. Moreover, further studies should be carried out to improve the lipid separation step in order to avoid contamination with saturated FAs from the SPE well plate. The use of a 96-well plate for phospholipid separation represents a marked improvement over the use of cartridges since it enables a much larger quantity of samples to be processed simultaneously. The SPE 96-well plate method is simpler and less time consuming than traditional methods, such as TLC.

Conclusions

Cheek cell and capillary blood samples are less invasive and reliable methods for FA analysis from phospholipid fraction. The modifications applied to sampling and sample preparation methodology of both matrices, resulted in a positive correlation for most of the FAs, especially LC-PUFAs, when compared with plasma samples. These two methods can therefore be used as alternatives to traditional plasma sampling for analysis of these FAs. In particular, their use does not necessitate the involvement of healthcare staff in sample collection and this markedly simplifies the analytical procedure.

Conflict of interest

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Figures and Tables

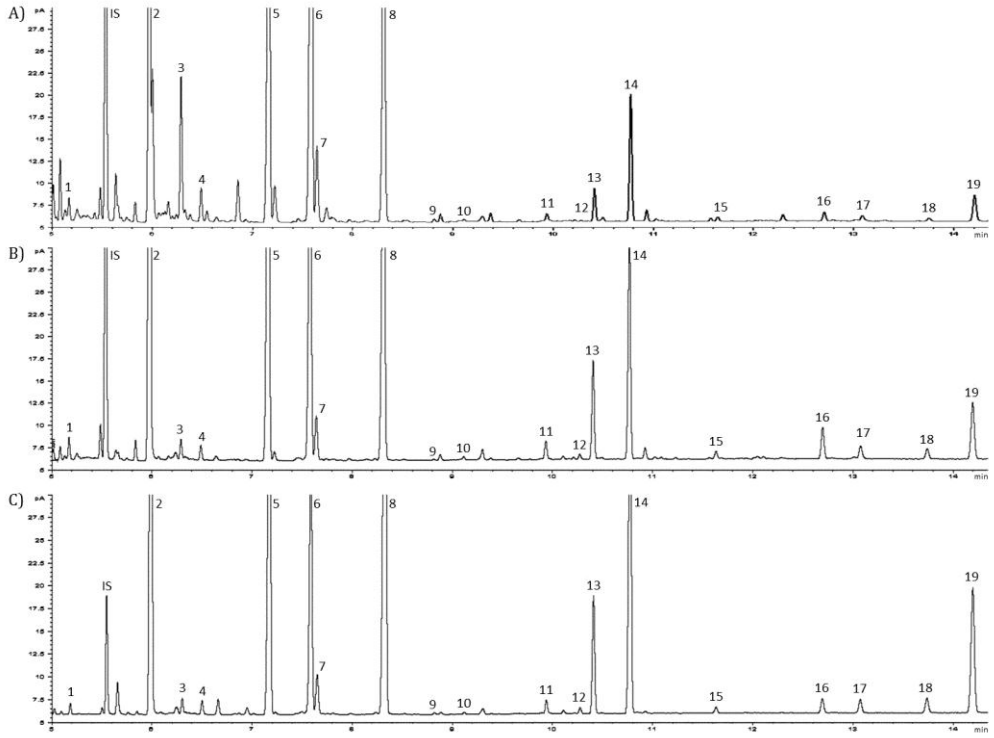

Figure 1. Chromatograms of the FAs profile from each matrix. A) Chromatogram from cheek cell sample. B) Chromatogram from capillary blood sample. C) Chromatogram from plasma sample. 1. C14:0, 2. C16:0, 3. C16:1, n-7, 4. C17:0, 5. C18:0, 6. C18:1, n-9, 7. C18:1, n-7, 8. C18:2, n-6, 9. C18:3, n-6, 10. C18:3, n-3, 11. C20:2, n-6, 12. C20:3, n-9, 13. C20:3, n-6, 14. AA, 15. EPA, 16. C22:4, n-6, 17. C22:5, n-6, 18. C22:5, n-3, 19. DHA, IS. C15:0.

Table 1. Fatty acids profile according to each different matrix.

Fatty Acid	Mucose Mean (%) ± SD	Plasma Mean (%) ± SD	Capillary blood Mean (%) ± SD	p
C14:0	1.12 ± 0.28 [a]	0.24 ± 0.06 [b]	0.66 ± 0.17 [c]	<0.0001
C16:0	24.96 ± 2.11 [a]	27.25 ± 1.54 [a]	34.61 ± 1.43 [b]	<0.0001
C16:1, n-7	3.94 ± 1.34 [a]	0.35 ± 0.07 [b]	0.19 ± 0.07 [b]	<0.0001
C17:0	1.39 ± 0.22 [a]	0.33 ± 0.05 [b]	0.40 ± 0.04 [b]	<0.0001
C18:0	25.82 ± 10.60 [a]	14.22 ± 1.23 [b]	40.13 ± 4.05 [c]	<0.0001
C18:1, n-9	21.29 ± 5.60 [a]	10.52 ± 1.33 [b]	7.45 ± 1.51 [c]	<0.0001
C18:1, n-7	3.00 ± 0.89 [a]	1.44 ± 0.19 [b]	0.70 ± 0.13 [c]	<0.0001
C18:2, n-6	10.22 ± 3.51 [a]	23.93 ± 3.53 [b]	8.37 ± 2.54 [a]	<0.0001
C18:3, n-6	0.27 ± 0.10 [a]	0.12 ± 0.05 [b]	0.05 ± 0.01 [c]	<0.0001
C18:3, n-3	0.18 ± 0.06 [a]	0.58 ± 0.56 [b]	0.06 ± 0.01 [a]	<0.0001
C20:2, n-6	0.57 ± 0.15 [a]	0.37 ± 0.04 [b]	0.13 ± 0.04 [c]	<0.0001
C20:3, n-9	0.15 ± 0.03 [a]	0.19 ± 0.04 [b]	0.08 ± 0.02 [c]	<0.0001
C20:3, n-6	0.98 ± 0.32 [a]	3.07 ± 0.77 [b]	0.84 ± 0.34 [a]	<0.0001
C20:4, n-6 (AA)	4.11 ± 1.66 [a]	11.14 ± 2.03 [b]	3.93 ± 1.13 [a]	<0.0001
C20:5, n-3 (EPA)	0.31 ± 0.12 [a]	0.66 ± 0.36 [b]	0.19 ± 0.07 [a]	<0.0001
C22:4, n-6	0.38 ± 0.19 [a]	0.39 ± 0.08 [a]	0.78 ± 0.36 [b]	<0.0001
C22:5, n-6	0.31 ± 0.15 [a]	0.31 ± 0.08 [b]	0.12 ± 0.04 [c]	<0.0001
C22:5, n-3	0.22 ± 0.03 [a]	0.66 ± 0.14 [a]	0.29 ± 0.11 [a]	<0.0001
C22:6, n-3 (DHA)	1.13 ± 0.58 [a]	4.24 ± 1.36 [b]	1.05 ± 0.38 [a]	<0.0001
SFAs	53.29 ± 11.37 [a]	42.03 ± 1.16 [b]	75.81 ± 5.09 [c]	<0.0001
MUFAs	28.22 ± 6.87 [a]	12.31 ± 1.37 [b]	8.34 ± 1.62 [c]	<0.0001
PUFAs	18.50 ± 5.54 [a]	45.66 ± 1.42 [b]	15.83 ± 3.81 [c]	<0.0001
n-3 PUFAs	1.70 ± 0.73 [a]	6.15 ± 1.86 [b]	1.58 ± 0.51 [a]	<0.0001
n-6 PUFAs	16.77 ± 5.05 [a]	39.33 ± 2.53 [b]	14.18 ± 3.56 [a]	<0.0001
n-3 LC-PUFAs	1.61 ± 0.71 [a]	5.57 ± 1.65 [b]	1.52 ± 0.52 [a]	<0.0001
n-6 LC-PUFAs	6.30 ± 2.05 [a]	15.29 ± 2.15 [b]	5.80 ± 1.56 [c]	<0.0001
n-6:n-3 PUFAs	11.25 ± 5.03 [a]	6.97 ± 2.48 [b]	9.97 ± 4.52 [a]	<0.0001
n-6: n-3 LC-PUFAs	4.48 ± 2.17 [a]	2.94 ± 0.84 [b]	4.08 ± 1.22 [a]	<0.0001

$p<0.05$ indicates statistical significant differences according to global ANOVA. Natural Log was used for non-normal variables. Means of FAs with different superscript letters indicate significant differences after applying the Bonferroni post-hoc correction.

Table 2. Precision of the method for cheek cell and capillary blood samples.

	Cheek Cells		Capillary Blood	
	Intra day (n=3)	Inter day (n=9)	Intra day (n=3)	Inter day (n=9)
Fatty Acid	CV (%)	CV (%)	CV (%)	CV (%)
C14:0	6.2	9.3	4.0	7.8
C16:0	3.0	3.7	0.5	1.7
C16:1, n-7	4.4	6.0	5.3	5.3
C17:0	3.1	6.9	3.4	9.8
C18:0	6.3	6.8	2.4	8.1
C18:1, n-9	3.0	6.0	5.3	9.3
C18:1, n-7	4.0	5.3	5.7	9.5
C18:2, n-6	3.5	7.1	6.3	5.5
C18:3, n-6	3.7	4.1	3.8	3.8
C20:0	1.3	6.6	5.0	9.2
C18:3, n-3	6.0	5.9	3.9	4.8
C20:2, n-6	2.5	5.8	6.3	6.7
C20:3, n-9	4.4	8.5	7.7	9.0
C20:3, n-6	3.1	5.7	6.3	6.6
C22:0	5.1	7.8	6.8	8.5
C20:4, n-6 (AA)	3.4	6.2	4.5	5.1
C20:5, n-3 (EPA)	5.0	9.3	5.3	9.6
C22:4, n-6	4.0	8.8	2.9	2.8
C22:5, n-6	4.3	5.7	3.5	5.3
C22:5, n-3	6.5	6.1	3.8	5.2
C22:6, n-3 (DHA)	1.5	5.6	3.1	5.0

Table 3. Correlation coefficients of each matrix referred to plasma.

	Mucose vs. Plasma		Capillary Blood vs. Plasma	
Fatty Acid	r	P	r	P
C14:0	0,09	0,610	0,49**	0,001
C16:0	0,37*	0,038	0,64**	0,000
C16:1, n-7	0,20	0,265	0,40*	0,011
C17:0	0,18	0,338	0,45**	0,004
C18:0	0,07	0,719	0,05	0,777
C18:1, n-9	0,38*	0,032	0,34*	0,030
C18:1, n-7	0,36*	0,041	0,32*	0,045
C18:2, n-6	0,36*	0,045	0,63**	0,000
C18:3, n-6	0,59**	0,001	0,58*	0,029
C18:3, n-3	0,76**	0,001	0,42**	0,007
C20:2, n-6	0,50**	0,004	0,32*	0,047
C20:3, n-9	0,99**	0,000	0,39*	0,013
C20:3, n-6	0,46**	0,008	0,73**	0,000
C20:4, n-6 (AA)	0,43*	0,015	0,32*	0,044
C20:5, n-3 (EPA)	0,68**	0,000	0,81**	0,000
C22:4, n-6	0,36*	0,049	0,32*	0,044
C22:5, n-6	0,62**	0,000	0,47**	0,002
C22:5, n-3	0,42*	0,040	0,36*	0,023
C22:6, n-3 (DHA)	0,59**	0,000	0,57**	0,000

*P < 0.05

† P < 0.01

5.4. IMPACT OF MATERNAL OBESITY ON FATTY ACIDS AND COGNITION IN EARLY LIFE - THE PREOBE FOLLOW-UP

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ABSTRACT

Background and aims: Fatty acid (FA) status in early life may influence child health outcomes and, consequently, health throughout adulthood. Pregnancy is a crucial period for health programming and thus pregnancy conditions influence child outcomes. Therefore, the aim of this study was to determine the impact of maternal BMI and/or infant feeding practice in infant FA concentrations, and analyze if these FAs associate with cognitive performance.

Methods: Mother-child pairs (n=155) were selected from the PREOBE cohort and grouped according to maternal pre-pregnancy body mass index (BMI): normal-weight (n=81) and overweight (n=42) and obese (n=32). Cheek cell samples were collected at 6 months, 1, 1.5, 2 and 3 years of age to analyze infant FA composition.

Results and Conclusion: Maternal pre-pregnancy BMI altered the infant FA behavior in evolution, feeding practice and cognition. A high maternal pre-pregnancy weight caused an increase of n6 FAs in infants up to 2 years of age. Infants of obese mothers had the lowest changes in FA evolution compared to infants of overweight and, especially, normal-weight mothers. In general, FA concentrations decreased towards the 3 years of life, except for the SFAs, n6:n3 and LC-n6:n3 ratios which were increased. Exclusive breastfeeding seems to increase crucial FAs (e.g. DHA) in infants at 6 months of age, which is a critical period for development. With increasing maternal BMI, the influence of feeding practice on infant FA status reduced. This suggests that breast milk quality of obese mothers could be compromised by their nutritional status and diet, and consequently breast milk FA content could be similar than formulas. In general, cognitive performance was improved in infants with high levels of PUFAs (e.g. DHA, AA) at 6 months of age, with the positive effect of the n3 FAs still present at 1.5 years of life. Our results highlight the importance of a healthy maternal pre-pregnancy weight and confirm that essential FAs, and consequently their products, are the influenced by diet. Since FAs are fundamental for growth and development, it is important to control maternal weight before, during and after pregnancy, and promote an adequate FA intake in mother and child to pursue better infant outcomes.

INTRODUCTION

Fatty acid (FA) status in early life may influence child health outcomes and, consequently, their health later in life. FAs are physiological important molecules that influence health and disease risk for playing important roles in metabolism; they can be used as energy, cell membrane components and cell signaling (1). Long-chain polyunsaturated fatty acids (LC-PUFAs), such as arachidonic acid (AA) of the n6 series and docosahexaenoic acid (DHA) of the n3 series, play a key role in growth and cognition. Studies have shown that AA, and particularly DHA, are the main PUFAs present in the grey matter of the brain. Fetal brain growth accentuates during the last trimester of pregnancy and the first year of life (2), which is when DHA conveniently accumulates in the brain and serves as a pool to satisfy the demand. After birth, this accumulation of DHA is also used for different growing organs and tissues (3). Furthermore, it has been observed that the dietary intake of n3 PUFAs contributes to weight loss, lowers the development of obesity related diseases (4), reduces neonatal deaths, reduces asthma and allergic asthma, increases immunity (5), reduces food allergies and reduces eczema (2). Although AA has a structural role in the brain, in general, increased n6 PUFAs in the diet are related to increased inflammatory factors that are associated to chronic diseases such as cardiovascular disease (CVD), diabetes mellitus type 2 (DM2), obesity, rheumatoid arthritis and irritable bowel syndrome (IBS) (6).

Excessive dietary fat intake is associated to a high weight status (5), but the sum of n6 PUFAs (6), and mainly saturated fatty acids (SFAs) are associated to an increased risk for obesity (7), while LC-PUFAs (7), DHA and n3 PUFAs (4) are negatively associated to it. Obesity is linked to the development of complications such as hypertension, dyslipidemia, DM2, coronary heart disease, stroke, gallbladder disease, osteoarthritis, sleep apnea and other breathing problems, cancer, mental illness (e.g. depression, anxiety), low quality of life, body pain and difficulty physical functioning (8). SFAs are also positively associated to inflammation, insulin resistance, cholesterol, triacylglycerols (TGs), coagulation, risk for CVD, coronary heart disease (CHD) and DM2, but not all SFAs have the same power. C16:0, C12:0 and C14:0 are associated to an increased risk while C15:0 and C17:0 are associated to lowering it. Regarding MUFAs (e.g. C18:1,n-9 and C16:1,n-7), results are not yet conclusive but they seem to have a protective role in the development of high blood pressure, insulin resistance, CHD, cardiovascular events and mortality, cholesterol, TGs and DM2 (1).

FAs are obtained through the diet or endogenous production (1), thus any condition altering FA dietary intake, production or metabolism, would compromise the FA concentrations and therefore their physiological functions. Current dietary habits vary widely, but they may correspond to a 10-20:1 n6:n3 ratio, which is a lot higher than 1-4:1 that some authors recommend (6, 9). However, there is no agreed recommendation for dietary n6:n3 ratio (5).

Pregnancy and breastfeeding are crucial periods for health programming, consequently maternal conditions during those periods influence the child outcomes in different regards (10). Maternal obesity is a disease that has gained a lot of attention due to its increasing rates and relationship with the health of future generations (11). This condition could alter maternal FA status compromising fetal and neonatal supply through the placenta and breastmilk, respectively. Additionally, maternal obesity increases the likelihood of children to be obese (8), and while obese children are related to underachievement in school and lower self-esteem (12), they are also more likely to stay obese into adulthood with a high risk of

developing obesity related diseases (8). Therefore, it is of interest to investigate if maternal obesity affects infant FA composition since it could impair their growth and development and associate to future health issues.

To our knowledge, there are no studies analyzing the effect of maternal weight in child FA composition and evolution. One possible explanation is that plasma is the most common matrix to analyze FAs and it is considered invasive and not recommended in studies involving infants and young children. To address this issue, other matrices such as cheek cells and capillary blood have been recently proposed as alternative sampling methods (13).

The aim of this study was to determine the impact of maternal BMI and/or infant feeding practice in infant FA concentrations, and analyze if these FAs associate with cognitive performance.

METHODS

Ethics statement

The study was carried out following the ethical standards recognized by the Declaration of Helsinki (2004), the Good Clinical Practice recommendations of the EEC (document 111/3976/88 July 1990) and the current Spanish legislation governing clinical research in humans (Royal Decree 561/1993 on clinical trials). Moreover, the study was approved by the Ethics Committee of San Cecilio University Hospital and the Faculty of Medicine at the University of Granada.

Study population and design

Mother-child pairs were selected from the participants of the observational PREOBE cohort study (Role of Nutrition and Maternal Genetics on the Programming of Development of Fetal Adipose Tissue). The information regarding the PREOBE study has been published elsewhere (14) and it was registered at www.ClinicalTrials.gov (NCT01634464).

Briefly, participants were recruited at San Cecilio University Hospital and Mother-Infant Hospital in the city of Granada, Spain, where samples and information were also collected. Study design and information about PREOBE participants are exhibited in Supplemental Figure 1. The inclusion criteria were singleton pregnancy, gestation between 12 and 20 weeks at enrollment, and intention to deliver at the centers involved in the study. Women were excluded if they were participating in other research studies, receiving drug treatment or supplements of DHA or folate for more than the first 3 months of pregnancy, suffering from disorders such as hypertension, pre-eclampsia, fetal intrauterine growth retardation, infections, hypo- or hyperthyroidism and hepatic renal diseases or those following an extravagant or vegan diet. Using questionnaires and medical records, some baseline and background characteristics were recorded such as maternal age, pre-pregnancy BMI, parity, smoking status, diet, alcohol drinking habits, socio-demographic information, educational level, and weight gain during pregnancy.

For the present study, 155 mother-child pairs were selected and divided according to maternal pre-pregnancy BMI, normal-weight (BMI=18.5-24.9, n=81), overweight (BMI=25-29.99, n=42) and obese women (BMI>30, n=32).

Cheek cell samples

Infant cheek cells were collected at 6 months, 1, 1.5, 2 and 3 years of age. Prior to cheek cell collection, the mouth was rinsed 3 times with tap water to eliminate possible food residues. Then, each inner cheek was gently scraped back and forth 25 times with a Rovers EndoCervex-Brush using one different side of the brush for each cheek. The tip of the brush was transferred into a cryotube and stored at 80º C until analysis.

Fatty acid analysis

The cheek cell FA composition was assessed by glycerophospholipid determination using a modified version of the method described by de la Garza et al (13). Lipid extraction was performed using methanol with butylated hydroxytoluene (BHT). FA methyl esters (FAMEs) were obtained by the reaction of FAs with sodium methylate in methanol (25 wt % in methanol) and boron trifluoride methanol solution (14% v/v), before being separated and quantified by fast gas chromatography (GC) with flame ionization detection (FID). The relative amount of each FA was quantified and expressed as the percentage of the total amount of FAs.

Cognitive development analysis

Infant cognitive development was analyzed at 6 months and 1.5 years of life using the Bayley III Cognitive Scale. Cognition was finally expressed as the scores of the test.

Statistical analysis

Statistical analyses were performed using the SPSS statistical software package for Windows (SPSS Inc., Chicago, IL). The Kolmogorov-Smirnov test was used to study the normal distribution of the data, with non-normally distributed data log transformed. FA percentages were expressed as mean ± standard deviation and compared by univariate ANOVA applying Bonferroni's post-hoc correction. The analyses are focused in PUFA and total FA levels, however the complete FA profile is found under the Supplementary information. Total FA levels were derived by adding the single FAs to SFAs, MUFAs, PUFAs, n6 PUFAs, n3 PUFAs, n6 LC-PUFAs, n3 LC-PUFAs, n6:n3 PUFAs and n6:n3 LC-PUFAs. The associations between FAs and maternal pre-pregnancy BMI were determined using linear regression and corrected for potential confounders such as maternal smoking, education, gestational weight gain and parity, and children characteristics such as sex, BMI z-score and infant feeding practices. The associations between cognition scores and infant FA levels were determined with a linear regression and binary logistic regression analyses; correction for potential confounders such as maternal smoking, maternal education, maternal diet (ratio n6:n3, n3 PUFAs) and infant feeding practice was performed. For the logistic regression analysis, we classified the infants into low and high cognition scores, and used the high score

group as reference. The confidence level was established at 95% for all the tests. Thus, results obtaining a P value of below 0.05 were considered statistically significant.

RESULTS

Population characteristics

The baseline characteristics of mother-child pairs participating in the PREOBE cohort study are shown in Table 1. The mean of gestational weight gain was significantly higher for the normal-weight mothers when compared to the obese (according to the Institute of Medicine (IOM) parameters (15), all women had an adequate gestational weight gain for their BMI group). Obese and overweight mothers had a lower energy intake compared to those with normal weight. However, the dietary intake of n6:n3 ratio was significantly higher in obese mothers compared to overweight and normal-weight groups. No significant differences were found in maternal dietary intake of the remaining FA.

50% of the infants were males. No significant differences were shown in their characteristics, but some tendencies were observed. According to the Lubchenco curves classification (16), only in the group of infants born to normal-weight mothers 1 infant (1.3%) was found small for gestational age. On the other hand, the overweight and obese mothers had the most infants considered large for gestational age (19.4% and 29.6%, respectively). Obese mothers were more likely (48.3%) to feed their infants with formula, while normal-weight were the more likely to exclusively breastfeed.

Infant fatty acid evolution

Table 2 shows the FA evolution in children during their first 3 years of life. When all children were analyzed, some FAs were found to decrease towards the 3 years of age with a significant decrease at 1.5 (DHA, n3 PUFAs), 2 (AA, DHA, n3 PUFAs, n3 LC-PUFAs, n6 LC-PUFAs) and 3 years of age (C18:2n6, AA, DHA, MUFAs, PUFAs, n3 PUFAs, n6 PUFAs, n3 LC-PUFAs and n6 LC-PUFAs). On the other hand, SFAs, n6:n3 and LC-n6:n3 ratios were found increased at the age of 3, with n6:n3 ratio starting to rise at 2.

By the 3 years of age, the same tendencies remained when children were separated according to maternal pre-pregnancy BMI, but not all results were significant. Infants with normal-weight mothers showed significant changes at 1 (↓SFA, ↑C18:2n6, ↑PUFAs), 2 (↓n3 PUFAs, ↓n3 LC-PUFAs, ↑n6:n3 ratio) and at 3 years of age (↓C18:2n6, ↓AA, ↓DHA, ↑SFA, ↓MUFAs, ↓PUFAs, ↓n3 PUFAs, ↓n6 PUFAs, ↓n3 LC-PUFAs, ↓n6 LC-PUFAs, ↑n6:n3, ↑LCn6:n3 ratios). Infants of overweight mothers had a similar behavior too but with less significant results; at 2 (↓AA, ↓DHA, ↓n3 PUFAs, ↓n3 LC-PUFAs, ↓n6 LC-PUFAs, ↑n6:n3 ratio) and at 3 years of age (↓AA, ↓DHA, ↑SFA, ↓MUFAs, ↓n3 PUFAs, ↓n3 LC-PUFAs, ↑n6:n3, ↑LCn6:n3 ratios). Additionally, infants with obese mothers had the fewest significant changes in evolution and only until the 3 years of age (↓DHA, ↑SFA, ↓M1FAs, ↓n3 PUFAs, ↓n3 LC-PUFAs, ↓n6 LC-PUFAs).

Supplementary table SS1 shows the analysis of the complete FA profile.

Infant fatty acid composition according to maternal weight

At 6 months of age, infants born to normal-weight mothers had lower levels of C18:2n6, PUFAs and n6 PUFAs and higher levels of SFA than children of obese women. Also at 6 months of age, infants of overweight mothers had lower LC-n6:n3 ratio than those with obese mothers. By the age of 1, infants of normal-weight mothers showed higher levels of C18:3n3 than infants of overweight women. Infants at 1.5 year of age, those born to overweight mothers had higher levels of DHA than infants with normal-weight mothers. And finally, when infants were 2 years old, AA was found higher in infants of obese mothers compared to infants of overweight mothers; MUFAs were found higher in infants of normal-weight mothers than infants of overweight mothers; and n6 LC-PUFAs were found lower in infants of overweight mothers compared to those born to obese women.

Supplementary table SS1 shows the analysis of the complete FA profile.

Infant fatty acid levels according to feeding practice

Table 3 shows the infant FA concentrations during the first 1.5 year of life according to the feeding practice performed. The complete profile can be found in supplementary table SS2.

At 6 months of age, all infants (regardless of maternal BMI group) who were exclusively breast fed had higher levels of LA, DHA, PUFAs, n6 PUFAs and n6:n3, and lower levels of ALA compared to infants who were partially breast fed. Additionally, infants of partial breastfeeding had lower MUFAs compared to formula fed infants.

This analysis at 6 months of age was also performed dividing infants per maternal weight group. Infants of normal-weight mothers with exclusive breastfeeding had significantly higher levels of LA, PUFAs, n6 PUFAs, n6 LC-PUFAs, n3 LC-PUFAs and n6:n3, and lower levels of ALA, SFAs than partially breastfed infants. Similar results were found in the group of infants born to overweight mothers, but with less significant results. Only in the group of infants with overweight mothers, exclusive breastfeeding resulted in higher DHA levels compared to infants who were partially breastfed; and partially breast fed infants had lower levels of MUFAs than those fed with formula. On the other hand, FAs in infants with obese mothers were not altered by the type of feeding practice. No differences were found in n3 PUFA and AA levels according to maternal pre-pregnancy BMI or infant feeding practice.

The only significant difference by 1.5 year of age was found for the n6:n3 ratio where regardless of maternal pre-pregnancy weight, exclusively breast fed infants had lower ratio than infants fed with formula.

Maternal pre-pregnancy BMI did not affect FA levels according to the type of feeding practice.

Association between maternal pre-pregnancy BMI and infant fatty acid levels

When analyzing the association between maternal BMI and infant FA levels we found that the levels of LA (at 6 months and 1.5 year), PUFAs, n6 PUFAs (at 6 months, 1.5 and 2 years) and n6:n3 (at 3 years) were positively associated with the increase of maternal BMI. No associations were found with n3 FAs or LC-n6:n3 ratio.

Child cognition and cheek cell fatty acids levels

The cognitive performance Bayley III test was administered in order to explore whether an association between cognitive performance and PUFA levels exists. Relationships between PUFA levels and cognition scores were analyzed using logistic regression and linear regression analyses correcting for potential confounders (Table 3). When analyzing all infants regardless of maternal group of weight, according to the binary logistic regression analysis, we found that at 6 months of age, DHA, AA, n6 PUFAs and n6 LC-PUFAs have a protective role on cognition. Moreover, the linear regression analyses showed that higher AA and n6 PUFA levels were associated with increased cognitive performance, confirming these FAs have a protective role.

On the other hand, positive associations between cognition and n6 FAs were lost by 1.5 years of age. Nonetheless, applying the binary logistic regression analysis, we still found that n3 PUFAs had a protective role on cognition, and using the linear regression test we found higher levels of DHA, n3 PUFAs and n3 LC-PUFAs and lower n6:n3 ratio associated with an increased cognitive score.

Supplementary Table SS3 and SS4 show the analyses performed for infants according to maternal group of weight. Infants were divided into two groups for this analysis; 1) born to normal-weight mothers and 2) born to overweight/obese mothers (these infants were included in the same group to enhance the statistical power of the analysis). All significant associations in the binary logistic regression test were lost in this analysis. Meanwhile, with the linear regression analysis we found that infants born to normal-weight women had a positive association between n6:n3 ratio and cognition scores at 6 months of age; and when infants reached 1.5 years of age we found that n3 PUFAs in infants of both normal-weight and obese mothers were positively associated to cognition (infants of obese women had a higher significance). These last results are similar to those obtained without dividing the infants per maternal weight group.

DISCUSSION

In this population-based, prospective PREOBE- cohort study, we analyzed the FA composition of infant cheek cells according to maternal pre-pregnancy BMI, and their relationship with the type of feeding practice and cognitive performance during the first 3 years of life. To our knowledge, there are no studies analyzing the effect of maternal weight on infant FA status during the first years of life, and this is one of the few studies that uses a biological sample that is easy to extract and reflects the dietary FA content (13, 17). The PUFAs, particularly LC-PUFAs (AA, DHA), play an important and beneficial physiologic role in the offspring who especially requires these FAs for critical periods of development (first 1000 days of life). Therefore, the maternal nutritional status and FA dietary intake during pregnancy and breastfeeding

are critical factors that are strongly associated with normal fetal and postnatal development. Alterations in this regard could result in modifications of fetal programming and in increased risk for developing diseases later in life (18). Considering the physiological importance of FAs, this study was emphasized in the analysis of essential PUFAs (LA, ALA), LC-PUFAs (AA, EPA, DHA) and the sum of individual FAs (SFAs, MUFAs, MCFA, n6 and n3 PUFAs, n6 and n3 LC-PUFAs, n6:n3 and LC-n6:n3 ratios).

When we analyzed the infant evolution of FA concentrations, we observed that, especially, the FA in the n3 series, such as DHA, n3 PUFAs and n3 LC-PUFAs decreased towards the 3 years of life, which would be explained by the infant high demand for an adequate development of the central nervous system, and these results were confirmed when we stratified by type of infant feeding practice. At 6 months of age, the infants of mothers (not considering maternal BMI) who were exclusively breastfed had higher levels of DHA than those partially breastfed; this effect disappeared when the child reached 1.5 years.

Contrary to the age related drop of n3 FAs, we found that the SFAs, n6:n3 and LC-n6:n3 ratios were increased by 3 years of life. Evidently, this could be related to the quality and increase of complementary feeding. Different studies suggest that higher n6:n3 ratios have been associated with adverse effects in the child, especially with an increased risk of obesity. High dietary intake of n6 FAs leads to increased n6 FA levels in plasma, red cells and white adipose tissue, and chronic inflammation, which are all hallmarks of obesity (19, 20). On the other hand, it is known that the increased maternal BMI increases the incidence of labor induction, caesarean section, preterm labor and macrosomia (21, 22). Our results show that even in postnatal life, infants born to obese woman (despite having an adequate weight gain during pregnancy) may be affected by the pre-pregnancy weight of the mother, by showing significant increases in some FA of the n6 family. The major FA of the n6 series is LA, and, at 6 months of life, infants of obese mothers had higher LA levels compared to those of normal-weight mothers, and higher PUFAs and LC-n:6:n3 ratio compared to infants of overweight mothers.

Furthermore, we analyze the effect of feeding practices on infant FAs during their first 1.5 years of life. Exclusively breastfed infants had higher levels of LA, DHA, PUFAs, n6 PUFAs and n6:n3n and lower level of ALA at 6 months compared to those who received partial breastfeeding. Since the ALA is an essential FA, the decreased level in the infant suggests a lack this FA in breast milk as a consequence of poor maternal dietary intake. At 1.5 years of life, infants fed with formula showed higher n6:n3 ratio than infants who were exclusively breastfed. These results were independent of the maternal pre-pregnancy BM and confirm that exclusive breastfeeding is the best option for the infants, since they require adequate amounts of crucial FAs to protect them from diseases later in life (23, 24). When infants were separated by maternal pre-pregnancy BMI, similar results were found for infants of overweight and, especially, normal-weight mothers (these infants also showed lower SFAs after an exclusive breastfeeding). Meanwhile, infants of obese mothers showed no impact by type of feeding practice received. One possible explanation for this is that the breast milk quality of obese mothers could be compromised by their nutritional status and diet, and consequently having a similar FA content than formulas. These results are evidence that essential FAs, and consequently their products, are the influenced by diet, so exclusively breastfed infants had higher levels of PUFAs, especially in infants born to normal-weight women.

Finally, the risk of having a low cognitive score was analyzed at 6 months and 1.5 years of life in spite of the maternal BMI. At 6 months of age, higher levels of DHA, AA and n6 PUFAs were associated with higher cognitions scores. Additionally, our results showed that when the infants reached the age of 1.5 years, the protective effect of n3 FAs was continued, but the protective effect of n6 FAs was lost, and we even found a negative association between n6:n3 ratio and cognitive scores. These results are in accordance with observational, cross-sectional and longitudinal, studies in healthy children, which suggest that there might be a positive relationship between n3 FA and cognitive outcomes (25-27). Kirby et al., investigated the association between children's learning and behavior and cheek cell PUFA levels and found that higher n3 FA levels were associated with decreased levels of inattention, hyperactivity, emotional and conduct difficulties and increased levels of prosocial behavior (26). When analyzing the infants according to normal-weight and overweight/obese mothers, we found that infants born to normal-weight women had a positive association between n6:n3 ratio and cognition scores at 6 months of age; and when infants turned 1.5 years old we found that n3 PUFAs were positively associated to cognition in infants of both normal-weight and obese women. Infants of obese women had a higher significance in this result, which suggests that they could benefit the most from a high n3 FA dietary intake. These results indicate the importance of a healthy diet for the infant, since it influences cognitive development and could have consequences in adolescence and adulthood.

There are some limitations in the present study. The most important is not having the information on the infant diet, however the sample used allowed us to indirectly measure the FA intake of the children since it has been demonstrated that circulating FAs reflect their dietary intake (17). Moreover, we were able to perform multivariate analyses to establish the relationship between the increase of maternal pre-pregnancy BMI and infant FA levels up to 3 years of age, and the association between infant cognitive performance and FA levels. It is important to emphasize that infant analyses independently of maternal pre-pregnancy BMI, showed more and higher significances compared to the analyses performed to infants classified in maternal groups of weight. One possible explanation for this could be the relatively small sample size within each group.

CONCLUSION

Maternal pre-pregnancy BMI altered the infant FA behavior in evolution, feeding practice and cognition. A high maternal pre-pregnancy weight caused an increase of n6 FAs in infants up to 2 years of age. Infants of obese mothers had the lowest changes in FA evolution compared to infants of overweight and, especially, normal-weight mothers. In general, FA concentrations decreased towards the 3 years of life, except for the SFAs, n6:n3 and LC-n6:n3 ratios which were increased. Exclusive breastfeeding seems to increase crucial FAs (e.g. DHA) in infants at 6 months of age, which is a critical period for development. With increasing maternal BMI, the influence of feeding practice on infant FA status reduced. This suggests that breast milk quality of obese mothers could be compromised by their nutritional status and diet, and consequently breast milk FA content could be similar than formulas. In general, cognitive performance was improved in infants with high levels of PUFAs (e.g. DHA, AA) at 6 months of age, with the positive effect of the n3 FAs still present at 1.5 years of life. Our results highlight the importance of a healthy maternal pre-pregnancy weight and confirm that essential FAs, and consequently their products, are the influenced by diet. Since FAs are fundamental for growth and development, it is important to

control maternal weight before, during and after pregnancy, and promote an adequate FA intake in mother and child to pursue better infant outcomes.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Table 1. Characteristics of the population.

	Normal-weight				Overweight				Obese				*p*
	n	**Mean**	**±**	**SD**	**n**	**Mean**	**±**	**SD**	**n**	**Mean**	**±**	**SD**	
Maternal Characteristics													
Age (years)	81	31.09	±	3.78	42	32.38	±	4.01	32	30.50	±	4.53	0.10
Pre-pregnancy BMI (kg/m2)	81	22.09	±	1.68[a]	42	27.19	±	1.26[b]	32	33.13	±	2.55[c]	**<0.01**
Weight Gain (kg)	67	12.94	±	5.90[a]	38	10.69	±	4.93[b]	26	7.61	±	6.50[b]	**<0.01**
Parity (%)													0.29
0	47	58.8%			23	54.8%			15	46.9%			
1	30	37.5%			15	35.7%			12	37.5%			
2+	3	3.8%			4	9.5%			5	15.6%			
Education (%)													0.66
>Highschool	61	76.3%			28	66.7%			21	65.6%			
Highschool	10	12.5%			9	21.4%			6	18.8%			
<Highschool	9	11.3%			5	11.9%			5	15.6%			
Smoking during pregnancy (%)													0.67
No. never	34	63.0%			18	69.2%			16	69.6%			
Quit	6	11.1%			2	7.7%			4	17.4%			
Yes	14	25.9%			6	23.1%			3	13.0%			
Dietary intakes													
Energy intake (kcal/d)	49	2166.31	±	345.97[a]	28	2153.11	±	649.90[a]	15	1795.27	±	628.05[b]	**0.01**
AA intake (g/d)	49	0.12	±	0.07[a]	28	0.19	±	0.13[b]	15	0.13	±	0.08[ab]	**0.02**
EPA intake (g/d)	49	0.14	±	0.11	28	0.10	±	0.10	15	0.13	±	0.13	0.43
DHA intake (g/d)	49	0.27	±	0.19	28	0.23	±	0.19	15	0.24	±	0.22	0.27
n3 from fish intake (g/d)	49	0.40	±	0.32	28	0.29	±	0.30	15	0.35	±	0.36	0.15
n6:n3 intake (g/d)	49	12.94	±	2.71[a]	28	13.78	±	3.26[a]	15	18.69	±	8.41[b]	**<0.01**
Child characteristics													
Birth weight (g)	80	3283.63	±	380.26	42	3360.95	±	451.92	31	3510.97	±	504.34	0.07
Birth length (cm)	74	50.51	±	1.61	40	50.64	±	1.49	30	51.37	±	2.25	0.09
Birth head Circumference (cm)	63	34.51	±	1.26	36	34.44	±	1.25	27	34.50	±	1.61	0.98
Placenta (g)	71	484.37	±	115.71	37	523.78	±	117.69	26	555.00	±	138.46	0.06
Sex. male (%)	40	49.4%			19	45.2%			18	58.1%			0.55
Newborn according Lubchencko curves# (%)													0.15
SGA	1	1.3%			0	0.0%			0	0.0%			
AGA	71	88.8%			29	80.6%			19	70.4%			
LGA	8	10.0%			7	19.4%			8	29.6%			
Breastfeeding													0.11
Exclusive	41	58.6%			21	54.1%			10	34.5%			
Mixt	14	20.0%			6	16.2%			5	17.2%			
Artificial	15	21.4%			11	29.7%			14	48.3%			

Natural logarithms were used for non-normal variables and quantitative variables were analyzed with ANOVA and Bonferroni Post-hoc test while qualitative variables were analyzed with chi-square test. Different superscript letters show significant differences between groups of maternal weight (p-value <0.05). #The newborns were divided into three groups according to the Lubchenko curves as: small for gestational age (SGA), appropriate for gestational age (AGA) and large for gestational age (LGA).

Table 2. Infant fatty acid profile and evolution according to maternal pre-pregnancy BMI.

Fatty acids	6 m (n=108)			1 y (n=128)			1.5 y (n=96)			2 y (n=86)			3 y (n=76)		
	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
C18:2 n6 (LA)															
1	13.41	±	3.24^{*ac}	15.27	±	2.33^{b}	13.36	±	2.64^{ac}	14.44	±	4.07^{ab}	11.91	±	3.32^{c}
2	15.05	±	2.84*†	15.08	±	2.55	14.32	±	3.16	14.84	±	2.76	13.80	±	4.17
3	15.21	±	2.12†	14.96	±	3.44	14.64	±	2.84	15.91	±	2.67	13.73	±	2.80
All	14.23	±	3.03^{a}	15.16	±	2.61^{a}	13.95	±	2.88^{a}	14.88	±	3.48^{a}	12.62	±	3.53^{b}
C18:3 n3 (ALA)															
1	0.30	±	0.19	0.33	±	0.18*	0.30	±	0.13	0.26	±	0.08	0.27	±	0.14
2	0.25	±	0.15	0.23	±	0.11†	0.32	±	0.21	0.27	±	0.13	0.24	±	0.10
3	0.27	±	0.10	0.24	±	0.10*†	0.27	±	0.12	0.26	±	0.11	0.31	±	0.10
All	0.28	±	0.16	0.29	±	0.16	0.30	±	0.15	0.26	±	0.10	0.27	±	0.13
C20:4 n6 (AA)															
1	2.29	±	0.85^{a}	2.32	±	0.65^{a}	1.95	±	0.51^{ab}	1.89	±	0.66$^{*\dagger ab}$	1.78	±	0.72^{b}
2	2.56	±	0.81^{a}	2.33	±	0.62^{ac}	2.22	±	0.63^{ab}	1.76	±	0.85^{*b}	1.83	±	0.74^{bc}
3	2.52	±	0.70	2.30	±	0.66	2.24	±	0.73	2.34	±	0.56†	1.98	±	0.51
All	2.41	±	0.81^{a}	2.32	±	0.64^{a}	2.10	±	0.61^{ab}	1.95	±	0.72^{bc}	1.82	±	0.69^{c}
C22:5 n6 (DHA)															
1	0.74	±	0.38^{a}	0.74	±	0.34^{a}	0.52	±	0.18^{*a}	0.53	±	0.24^{a}	0.41	±	0.22^{b}
2	0.87	±	0.31^{a}	0.79	±	0.30^{a}	0.66	±	0.24$^{\dagger a}$	0.50	±	0.30^{b}	0.41	±	0.17^{b}
3	0.83	±	0.33^{a}	0.75	±	0.27^{a}	0.65	±	0.24$^{*\dagger a}$	0.60	±	0.26^{a}	0.39	±	0.24^{b}
All	0.79	±	0.36^{a}	0.76	±	0.32^{a}	0.59	±	0.22^{b}	0.54	±	0.26^{b}	0.40	±	0.21^{c}
SFA															
1	40.84	±	9.21^{*a}	35.95	±	4.37^{b}	38.12	±	6.01^{ab}	37.29	±	5.44^{ab}	46.50	±	10.83^{c}
2	34.93	±	4.48$^{\dagger a}$	†	±	4.84^{a}	36.93	±	4.91^{a}	35.89	±	7.42^{a}	45.02	±	7.22^{b}
3	35.14	±	4.41$^{\dagger a}$	37.06	±	5.51^{a}	35.96	±	4.18^{a}	37.50	±	4.10^{a}	46.01	±	7.60^{b}
All	38.04	±	7.84^{a}	36.60	±	4.74^{a}	37.26	±	5.32^{a}	36.94	±	5.79^{a}	46.09	±	9.59^{b}
MUFA															
1	39.71	±	6.17^{a}	41.84	±	4.53^{a}	42.17	±	5.14^{a}	43.27	±	4.18^{*a}	35.70	±	9.05^{b}
2	41.90	±	4.04^{a}	41.64	±	3.75^{a}	42.21	±	5.81^{a}	40.31	±	5.87$^{\dagger a}$	34.62	±	8.80^{b}
3	42.38	±	4.16^{a}	42.32	±	3.95^{a}	43.28	±	3.69^{a}	41.48	±	3.29$^{*\dagger a}$	36.00	±	5.34^{b}
All	40.86	±	5.37^{a}	41.88	±	4.21^{a}	42.44	±	5.05^{a}	42.05	±	4.68^{a}	35.50	±	8.44^{b}
PUFA															
1	18.81	±	3.99^{*ab}	20.64	±	3.05^{b}	18.02	±	3.08^{a}	18.75	±	4.47^{ab}	15.91	±	4.30^{c}
2	20.66	±	3.45*†	20.47	±	3.35	19.50	±	3.86	18.81	±	3.67	18.06	±	5.02
3	21.27	±	2.75†	20.30	±	3.97	19.80	±	3.55	20.97	±	2.92	17.98	±	3.35
All	19.82	±	3.75^{a}	20.53	±	3.29^{a}	18.89	±	3.51^{a}	19.26	±	4.02^{a}	16.72	±	4.41^{b}
n3 PUFA															
1	1.33	±	0.38^{a}	1.32	±	0.39^{a}	1.10	±	0.27^{ab}	1.01	±	0.32^{bc}	0.93	±	0.30^{c}
2	1.37	±	0.27^{a}	1.25	±	0.34^{a}	1.28	±	0.35^{a}	0.96	±	0.35^{b}	0.90	±	0.21^{b}
3	1.33	±	0.34^{a}	1.26	±	0.26^{a}	1.18	±	0.29^{ab}	1.10	±	0.28^{ab}	0.95	±	0.25^{b}
All	1.34	±	0.34^{a}	1.29	±	0.35^{ab}	1.18	±	0.31^{b}	1.02	±	0.32^{c}	0.92	±	0.28^{c}
n6 PUFA															
1	17.47	±	3.81^{*ab}	19.33	±	2.87^{a}	16.92	±	3.00^{b}	17.74	±	4.39^{ab}	14.98	±	4.11^{c}
2	19.29	±	3.37*†	19.22	±	3.17	18.21	±	3.71	17.85	±	3.56	17.16	±	4.83
3	19.94	±	2.53†	19.04	±	3.88	18.62	±	3.39	19.86	±	2.90	17.03	±	3.22
All	18.48	±	3.60^{a}	19.25	±	3.13^{a}	17.71	±	3.37^{a}	18.24	±	3.94^{a}	15.79	±	4.24^{b}
n3 LC-PUFA															
1	1.03	±	0.39^{a}	0.99	±	0.38^{ab}	0.80	±	0.25^{ab}	0.76	±	0.30^{bc}	0.66	±	0.28^{c}
2	1.12	±	0.33^{a}	1.01	±	0.34^{a}	0.96	±	0.30^{a}	0.69	±	0.36^{b}	0.66	±	0.20^{b}
3	1.06	±	0.37^{a}	1.02	±	0.29^{a}	0.90	±	0.29^{ab}	0.84	±	0.30^{ab}	0.64	±	0.24^{b}
All	1.06	±	0.37^{a}	1.00	±	0.36^{a}	0.87	±	0.28^{a}	0.76	±	0.32^{b}	0.65	±	0.26^{b}
n6 LC-PUFA															
1	3.88	±	1.15^{a}	3.89	±	0.95^{a}	3.41	±	0.89^{ab}	3.18	±	1.02$^{*\dagger ab}$	2.97	±	1.09^{b}
2	4.07	±	1.15^{a}	3.99	±	0.93^{a}	3.72	±	0.83^{a}	2.92	±	1.35^{*b}	3.26	±	0.95^{ab}
3	4.55	±	0.85^{a}	3.91	±	0.93^{ab}	3.80	±	1.10^{ab}	3.82	±	0.87$^{\dagger ab}$	3.20	±	0.78^{b}
All	4.07	±	1.12^{a}	3.92	±	0.93^{a}	3.60	±	0.93^{ab}	3.25	±	1.12^{bc}	3.07	±	1.01^{c}

Table2. Continuation

Fatty acids	6 m (n=108)			1 y (n=128)			1.5 y (n=96)			2 y (n=86)			3 y (n=76)		
	Mean	**±**	**SD**	**Mean**	**±**	**SD**	**Mean**	**±**	**SD**	**Mean**	**±**	**SD**	**Mean**	**±**	**SD**
n6:n3															
1	13.91	±	4.22[a]	15.74	±	4.21[ab]	16.08	±	4.12[ab]	18.81	±	6.35[b]	17.63	±	6.27[b]
2	14.68	±	4.20[a]	16.18	±	3.82[ac]	15.00	±	4.34[a]	21.19	±	9.91[bc]	18.84	±	3.10[c]
3	15.78	±	3.82	15.65	±	4.09	16.40	±	3.68	19.24	±	6.00	18.84	±	4.93
All	14.51	±	4.16[a]	15.84	±	4.06[a]	15.81	±	4.09[a]	19.57	±	7.42[b]	18.09	±	5.48[b]
LC-n6:n3															
1	4.00	±	1.00[*†a]	4.29	±	1.30[ab]	4.54	±	1.43[ab]	4.54	±	1.43[ab]	4.97	±	1.77[b]
2	3.74	±	0.92[*a]	4.17	±	1.05[ab]	4.08	±	1.05[ab]	4.62	±	1.52[ab]	5.12	±	1.47[b]
3	4.87	±	2.37[†]	4.08	±	1.33	4.46	±	1.36	5.09	±	2.28	5.59	±	2.25
All	4.11	±	1.42[a]	4.22	±	1.24[a]	4.38	±	1.31[a]	4.68	±	1.67[ab]	5.10	±	1.78[b]

Maternal BMI groups: 1. normal-weight; 2. overweight; 3. obese. Natural logarithms were used for non-normal variables. Different symbols indicate significant differences between maternal BMI groups. Different superscript letters indicate significant differences among timing of sample collection. Significant differences were established according to according to ANOVA and the Bonferroni post-hoc test with ($p < 0.05$). LA: Linoleic Acid; AA: Arachidonic Acid; ALA: α-linolenic Acid; DHA: Docosahexaenoic Acid; SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids.

Table3. Fatty acids levels of cheek cells glycerophospholipids according to maternal BMI and infant feeding practice.

	6 m									1.5 y								
	Exclusive breastfeeding (n=52)			Mixt (n=18)			Formula (n=32)			Exclusive breastfeeding (n=52)			Mixt (n=18)			Formula (n=32)		
	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
C18:2 n6 (LA)																		
1	14.68	±	2.62^{a}	11.17	±	3.75^{b}	12.23	±	3.40^{ab}	13.83	±	2.53	12.46	±	2.12	13.90	±	2.02
2	16.10	±	2.10^{a}	11.79	±	3.59^{b}	15.09	±	2.39^{a}	14.59	±	3.29	14.44	±	1.20	14.20	±	3.27
3	15.49	±	2.73	15.69	±	1.30	14.70	±	1.92	14.87	±	2.09	13.78	±	3.78	14.69	±	3.38
All	15.20	±	2.53^{a}	12.05	±	3.68^{b}	14.03	±	2.83^{a}	14.26	±	2.72	13.45	±	2.34	14.29	±	2.83
C18:3 n3 (ALA)																		
1	0.25	±	0.15^{a}	0.41	±	0.27^{b}	0.37	±	0.16^{b}	0.30	±	0.14	0.23	±	0.04	0.30	±	0.06
2	0.17	±	0.09^{a}	0.41	±	0.24^{b}	0.27	±	0.11^{b}	0.29	±	0.17	0.28	±	0.13	0.21	±	0.04
3	0.29	±	0.13	0.20	±	0.03	0.28	±	0.09	0.33	±	0.15	0.18	±	0.01	0.25	±	0.07
All	0.23	±	0.13^{a}	0.38	±	0.24^{b}	0.31	±	0.13^{b}	0.30	±	0.15	0.24	±	0.08	0.26	±	0.07
C20:4 n6 (AA)																		
1	2.53	±	0.85	2.01	±	0.51	1.84	±	1.06	1.97	±	0.58	1.74	±	0.38	2.00	±	0.36
2	2.93	±	0.81	2.03	±	0.88	2.27	±	0.51	2.24	±	0.69	2.34	±	0.64	2.40	±	0.49
3	2.27	±	0.95	2.54	±	0.19	2.64	±	0.63	2.34	±	0.61	1.71	±	0.43	2.30	±	0.87
All	2.61	±	0.87	2.10	±	0.60	2.27	±	0.81	2.12	±	0.63	1.93	±	0.54	2.22	±	0.64
C22:5 n6 (DHA)																		
1	0.82	±	0.35	0.58	±	0.36	0.57	±	0.44	0.55	±	0.19	0.44	±	0.08	0.43	±	0.15
2	1.02	±	0.28^{a}	0.60	±	0.33^{b}	0.78	±	0.24^{ab}	0.71	±	0.23	0.69	±	0.30	0.58	±	0.27
3	0.73	±	0.33	1.09	±	0.23	0.81	±	0.36	0.66	±	0.22	0.64	±	0.26	0.64	±	0.28
All	0.87	±	0.34^{a}	0.66	±	0.37^{b}	0.72	±	0.36^{ab}	0.62	±	0.22	0.58	±	0.23	0.55	±	0.25
SFA																		
1	37.97	±	6.31^{a}	47.61	±	11.23^{b}	42.87	±	12.31^{ab}	36.36	±	5.80	43.17	±	5.76	39.11	±	4.01
2	35.58	±	3.18	31.91	±	7.56	35.48	±	4.17	35.56	±	3.95	38.08	±	5.56	38.56	±	2.67
3	36.27	±	5.34	33.66	±	1.98	34.99	±	4.59	37.60	±	2.60	33.82	±	1.69	35.13	±	5.40
All	37.05	±	5.48	41.27	±	11.91	37.69	±	8.42	36.33	±	4.78	39.14	±	6.04	37.39	±	4.62
MUFA																		
1	41.20	±	4.89	36.14	±	8.00	37.96	±	7.24	42.77	±	4.30	40.01	±	5.33	42.48	±	3.01
2	41.65	±	2.96^{ab}	38.69	±	6.05^{a}	43.88	±	3.53^{b}	42.61	±	6.55	41.71	±	4.72	41.59	±	3.94
3	39.20	±	4.05	44.67	±	1.51	43.70	±	3.94	42.08	±	3.43	48.05	±	2.63	42.93	±	3.25
All	41.06	±	4.30^{ab}	38.15	±	7.32^{a}	41.91	±	5.68^{b}	42.59	±	4.92	42.59	±	5.40	42.45	±	3.24
PUFA																		
1	20.47	±	3.30^{a}	16.37	±	4.25^{b}	16.57	±	4.16^{b}	18.50	±	3.09	16.82	±	2.10	18.41	±	2.36
2	22.23	±	2.40^{a}	16.66	±	4.46^{b}	20.29	±	2.71^{a}	19.79	±	4.12	19.70	±	1.92	19.38	±	3.80
3	21.03	±	3.61	21.67	±	1.26	21.10	±	2.68	20.28	±	2.10	17.92	±	4.57	19.92	±	4.40
All	21.05	±	3.15^{a}	17.28	±	4.30^{b}	19.38	±	3.71^{ab}	19.23	±	3.34	18.06	±	2.84	19.25	±	3.55
n3 PUFA																		
1	1.36	±	0.35	1.37	±	0.32	1.18	±	0.51	1.13	±	0.30	0.92	±	0.16	1.00	±	0.21
2	1.43	±	0.31	1.26	±	0.15	1.32	±	0.24	1.33	±	0.36	1.24	±	0.44	1.06	±	0.27
3	1.21	±	0.31	1.55	±	0.25	1.33	±	0.39	1.28	±	0.32	1.05	±	0.33	1.12	±	0.24
All	1.36	±	0.34	1.37	±	0.28	1.28	±	0.39	1.22	±	0.33	1.06	±	0.32	1.06	±	0.23
n6 PUFA																		
1	19.11	±	3.15^{a}	15.01	±	4.13^{b}	15.38	±	3.80^{b}	17.37	±	2.99	15.90	±	2.00	17.41	±	2.23
2	20.81	±	2.29^{a}	15.40	±	4.39^{b}	18.97	±	2.72^{a}	18.46	±	3.90	18.46	±	1.88	18.33	±	3.63
3	19.82	±	3.37	20.12	±	1.21	19.77	±	2.40	19.00	±	1.95	16.87	±	4.32	18.80	±	4.25
All	19.69	±	3.00^{a}	15.92	±	4.18^{b}	18.09	±	3.49^{a}	18.01	±	3.18	17.00	±	2.67	18.19	±	3.40

Table 3. Continuation

Fatty acids	6 m									1.5 y								
	Exclusive breastfeeding (n=52)			Mixt (n=18)			Exclusive breastfeeding (n=52)			Mixt (n=18)			Exclusive breastfeeding (n=52)			Mixt (n=18)		
	Mea	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
n3 LC-PUFA																		
1	1.11	±	0.37[a]	0.95	±	0.30[ab]	0.81	±	0.50[b]	0.84	±	0.27	0.69	±	0.13	0.70	±	0.23
2	1.26	±	0.32	0.85	±	0.32	1.05	±	0.28	1.04	±	0.32	0.96	±	0.35	0.84	±	0.27
3	0.92	±	0.36	1.35	±	0.23	1.05	±	0.40	0.96	±	0.33	0.86	±	0.33	0.87	±	0.27
All	1.13	±	0.36	0.99	±	0.33	0.97	±	0.41	0.92	±	0.31	0.82	±	0.27	0.80	±	0.26
n6 LC-PUFA																		
1	4.25	±	1.08 [a]	3.62	±	0.72[ab]	3.01	±	1.50[b]	3.40	±	1.00	3.29	±	0.55	3.37	±	0.52
2	4.53	±	1.23	3.41	±	1.10	3.71	±	0.76	3.70	±	0.94	3.89	±	0.68	3.95	±	0.67
3	4.16	±	1.16	4.23	±	0.16	4.89	±	0.67	3.96	±	0.94	2.98	±	0.68	3.91	±	1.29
All	4.32	±	1.13	3.66	±	0.80	3.90	±	1.28	3.59	±	0.97	3.41	±	0.68	3.73	±	0.94
n6:n3																		
1	14.80	±	3.91[a]	11.38	±	3.63[b]	14.39	±	5.42[ab]	16.13	±	4.38	17.39	±	2.15	17.84	±	2.97
2	15.39	±	4.47	12.19	±	3.28	14.86	±	4.07	14.41	±	3.05	16.70	±	7.80	17.79	±	3.69
3	16.73	±	2.32	13.18	±	2.36	15.97	±	4.84	15.45	±	3.21	16.48	±	2.87	17.23	±	4.36
All	15.23	±	3.91[a]	11.88	±	3.28[b]	15.10	±	4.69[a]	15.46	±	3.81[a]	16.93	±	4.47[ab]	17.58	±	3.61[b]
LC-n6:n3																		
1	4.01	±	0.99	4.02	±	1.09	4.02	±	1.13	4.37	±	1.55	4.87	±	1.23	5.15	±	1.32
2	3.66	±	0.94	4.11	±	0.90	3.69	±	0.95	3.72	±	0.88	4.45	±	1.83	4.83	±	0.62
3	4.81	±	1.39	3.20	±	0.64	5.48	±	3.02	4.52	±	1.57	3.61	±	0.63	4.65	±	1.34
All	4.02	±	1.07	3.92	±	1.00	4.43	±	2.09	4.19	±	1.38	4.42	±	1.35	4.87	±	1.18

Maternal BMI groups: 1. normal-weight; 2. overweight; 3. obese. Natural logarithms were used for non-normal variables. Different symbols indicate significant differences between maternal BMI groups. Different superscript letters indicate significant differences among type of infant feeding practice. Significant differences were established according to according to ANOVA and the Bonferroni post-hoc test with ($p < 0.05$). LA: Linoleic Acid; AA: Arachidonic Acid; ALA: α-linolenic Acid; DHA: Docosahexaenoic Acid; SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids.

Table 4. Association between maternal pre-pregnancy BMI and infant fatty acid levels.

Fatty Acid	6 m (n=64)				1 y (n=67)				1.5 y (n=53)				2 y (n=50)				3 y (n=33)			
	B	**p**	**β^c**	**p^c**	**β**	**p**	**β^c**	**p^c**	**β**	**p**	**β^c**	**p^c**	**β**	**p**	**β^c**	**p^c**	**β**	**p**	**β^c**	**p^c**
C18:2 n6 (LA)	0.26	**0.040**	0.33	**0.016**	-0.12	0.339	0.03	0.841	0.32	**0.019**	0.32	**0.027**	0.34	**0.016**	0.29	0.061	0.16	0.377	0.32	0.176
C18:3 n3 (ALA)	0.09	0.488	-0.02	0.864	-0.12	0.331	-0.03	0.825	0.10	0.462	0.15	0.337	-0.16	0.261	-0.13	0.411	-0.06	0.740	0.09	0.712
C20.4 n6 (AA)	0.13	0.322	0.15	0.301	-0.01	0.915	0.07	0.633	0.28	**0.043**	0.27	0.083	0.31	**0.029**	0.28	0.073	0.16	0.372	0.00	0.990
C22:6 n3 (DHA)	0.02	0.858	0.09	0.465	-0.02	0.865	0.07	0.643	0.25	0.072	0.17	0.204	0.15	0.306	0.13	0.410	0.11	0.551	0.00	0.994
SFA	-0.26	**0.037**	-0.25	0.086	0.08	0.496	0.07	0.620	-0.33	**0.016**	-0.28	**0.049**	-0.09	0.553	-0.07	0.684	0.00	0.986	-0.05	0.833
MUFA	0.20	0.113	0.23	0.117	0.06	0.647	0.09	0.540	0.05	0.707	-0.02	0.905	-0.24	0.100	-0.28	0.092	0.00	0.993	0.05	0.843
PUFA	0.27	**0.032**	0.33	**0.017**	-0.09	0.489	0.06	0.660	0.36	**0.009**	0.35	**0.017**	0.36	**0.009**	0.33	**0.032**	0.13	0.488	0.22	0.365
n3 PUFA	0.00	0.989	0.03	0.833	-0.04	0.725	0.11	0.450	0.23	0.094	0.18	0.201	0.12	0.407	0.12	0.436	-0.10	0.567	-0.20	0.431
n6 PUFA	0.28	**0.023**	0.34	**0.012**	-0.09	0.491	0.06	0.700	0.35	**0.011**	0.34	**0.020**	0.36	**0.010**	0.32	**0.036**	0.14	0.437	0.24	0.307
n3 LC-PUFA	-0.03	0.810	0.02	0.893	0.00	0.975	0.12	0.427	0.17	0.216	0.08	0.536	0.13	0.383	0.11	0.482	-0.02	0.916	-0.39	0.274
n6 LC-PUFA	0.22	0.084	0.23	0.101	0.02	0.872	0.10	0.513	0.20	0.142	0.19	0.230	0.23	0.110	0.24	0.119	0.08	0.652	-0.05	0.843
n6:n3	0.22	0.086	0.23	0.085	-0.02	0.904	-0.08	0.604	0.03	0.820	0.08	0.572	0.11	0.453	0.09	0.580	0.32	0.074	0.57	**0.011**
LC-n6:n3	0.25	0.051	0.20	0.143	0.01	0.925	-0.06	0.690	0.00	0.996	0.07	0.597	0.06	0.682	0.08	0.563	0.19	0.293	0.21	0.369

Natural logarithms were used for non-normal variables. Associations were analyzed using a multivariate lineal regression analysis. β^c and P^c= are corrected values after adjustment for potential confounders. Significant differences with a p value <0.05 are highlighted in bold. LA: Linoleic Acid; AA: Arachidonic Acid; ALA: α-linolenic Acid; DHA: Docosahexaenoic Acid; SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids.
6m and 1 y: all values were adjusted for maternal smoking, parity, maternal education, pregnancy weight gain, child sex, child BMI z-score and type of infant feeding practice.
1.5 y – 3 y: all values were adjusted for maternal smoking, parity, maternal education, pregnancy weight gain, child sex and child BMI z-score.

Table 5. Association between cheek cell fatty acid levels and cognitive development score.

Fatty acids	FA levels *vs* low and high cognition score†						FA levels *vs* cognition score≠			
	OR	**95%-CI**	**P**	**OR^c**	**95%-CI ^{c}**	**P^c**	**β**	**p**	**β ^{c}**	**p^c**
DHA 6m	0.37	(0.11 - 1.25)	0.110	0.13	(0.02 - 0.75)	**0.023**	0.23	0.097	0.21	0.161
DHA 1.5y	0.36	(0.07 - 1.93)	0.233	1.10	(0.97 - 1.26)	0.147	0.27	0.077	0.38	**0.026**
AA 6m	0.09	(0.01 - 0.75)	**0.027**	0.01	(0.00 - 0.24)	**0.005**	0.33	**0.017**	0.28	**0.047**
AA 1.5y	0.15	(0.01 - 2.01)	0.153	0.05	(0.00 - 1.45)	0.082	0.23	0.129	0.22	0.192
n3 PUFA 6m	0.66	(0.08 - 5.77)	0.705	0.17	(0.01 - 3.34)	0.245	0.08	0.582	0.04	0.791
n3 PUFA 1.5y	0.14	(0.01 - 1.97)	0.144	<0.01	(0.00 - 0.73)	**0.038**	0.25	0.104	0.40	**0.031**
n3 LC-PUFA 6m	0.60	(0.12 - 2.95)	0.529	0.19	(0.02 - 1.67)	0.133	0.10	0.486	0.08	0.575
n3 LC-PUFA 1.5y	0.38	(0.05 - 2.92)	0.356	0.24	(0.01 - 4.98)	0.357	0.24	0.124	0.35	**0.049**
n6 PUFA 6m	0.01	(0.00 - 0.32)	**0.010**	0.00	(0.00 - 0.14)	**0.006**	0.38	**0.005**	0.32	**0.034**
n6 PUFA 1.5y	0.95	(0.78 - 1.15)	0.600	0.93	(0.73 - 1.19)	0.567	0.14	0.367	0.02	0.914
n6LC-PUFA 6m	0.60	(0.32 - 1.14)	0.117	0.42	(0.19 - 0.95)	**0.036**	0.25	0.074	0.14	0.351
n6 LC-PUFA 1.5y	0.65	(0.31 - 1.36)	0.248	0.65	(0.27 - 1.56)	0.337	0.27	0.079	0.22	0.187
n6:n3 6m	0.06	(0.00 - 0.97)	**0.048**	0.06	(0.00 - 1.68)	0.097	0.25	0.073	0.20	0.153
n6:n3 1.5y	1.05	(0.90 - 1.23)	0.526	1.07	(0.85 - 1.34)	0.574	-0.16	0.312	-0.39	**0.036**
LC-n6:n3 6m	0.45	(0.06 - 3.76)	0.463	0.58	(0.05 - 6.41)	0.657	0.10	0.484	0.01	0.923
LC-n6:n3 1.5y	0.95	(0.60 - 1.51)	0.827	0.82	(0.41 - 1.63)	0.568	-0.05	0.747	-0.18	0.321

Natural logarithms were used for non-normal variables. Associations were analyzed using †binary logistic regression and ≠lineal regression analyses. OR^c, β^c and P^c= are corrected values after adjustment for maternal smoking, maternal education, maternal diet (ratio n6:n3, n3 PUFAs) and type of infant feeding practice. Significant differences with a p value <0.05 are highlighted in bold. LA: Linoleic Acid; AA: Arachidonic Acid; ALA: α-linolenic Acid; DHA: Docosahexaenoic Acid; SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids.

Table SS1 Infant fatty acid profile and evolution according to maternal pre-pregnancy BMI.

Fatty acids	6 m (n=108)			1 y (n=128)			1.5 y (n=96)			2 y (n=86)			3 y (n=76)		
	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
C15:1															
1	2.86	±	1.69[a]	2.82	±	1.30[a]	2.54	±	1.39[*ab]	2.20	±	1.25[ab]	2.01	±	1.20[b]
2	3.51	±	1.41[a]	3.13	±	1.13[a]	3.27	±	1.21[a]	2.36	±	1.39[b]	2.66	±	1.02[ab]
3	3.22	±	1.45	3.09	±	1.34	2.89	±	1.15[*]	2.86	±	1.07	1.97	±	1.19
All	3.12	±	1.58[a]	2.95	±	1.26[a]	2.85	±	1.31[ab]	2.39	±	1.26[bc]	2.15	±	1.18[c]
C16:0															
1	19.46	±	4.03[*a]	17.34	±	3.06[b]	19.52	±	3.36[a]	20.03	±	4.57[a]	20.44	±	4.19[a]
2	16.03	±	4.36[*†a]	18.86	±	4.21[b]	18.74	±	3.12[b]	19.68	±	5.60[b]	20.58	±	3.88[b]
3	15.85	±	3.69[†a]	18.76	±	5.37[ab]	18.34	±	1.98[ab]	19.83	±	3.78[b]	20.57	±	2.54[b]
All	17.77	±	4.39[a]	18.00	±	3.93[ab]	19.01	±	3.03[bc]	19.89	±	4.67[c]	20.49	±	3.86[c]
C16:1 n-7															
1	3.30	±	2.60[*a]	3.98	±	1.50[b]	4.23	±	1.37[b]	4.39	±	1.92[b]	3.30	±	1.70[a]
2	3.24	±	1.11[*†]	3.83	±	1.38	4.28	±	1.48	3.56	±	1.53	3.56	±	1.56
3	4.16	±	1.12[†ab]	4.57	±	1.98[a]	4.83	±	1.61[a]	4.72	±	1.33[a]	3.03	±	1.06[b]
All	3.46	±	2.04[a]	4.05	±	1.58[b]	4.38	±	1.47[b]	4.23	±	1.74[b]	3.32	±	1.58[a]
C17:0															
1	0.94	±	0.24	1.03	±	0.27	0.95	±	0.25	0.86	±	0.26	0.97	±	0.31
2	1.09	±	0.33	1.09	±	0.29	1.00	±	0.24	0.91	±	0.38	1.04	±	0.18
3	1.12	±	0.30	1.04	±	0.26	0.94	±	0.23	0.89	±	0.23	0.97	±	0.17
All	1.02	±	0.29[a]	1.05	±	0.27[a]	0.96	±	0.24[ab]	0.88	±	0.29[b]	0.99	±	0.26[ab]
C18:0															
1	19.29	±	6.66[a]	16.49	±	1.90[ab]	16.55	±	3.51[ab]	15.36	±	3.66[b]	23.96	±	8.18[c]
2	16.73	±	2.88[a]	16.61	±	1.63[a]	16.12	±	2.80[ab]	14.35	±	3.89[b]	22.33	±	5.53[c]
3	17.07	±	3.46[a]	16.18	±	1.96[a]	15.63	±	2.92[a]	15.69	±	1.93[a]	23.29	±	5.84[b]
All	18.12	±	5.38[a]	16.46	±	1.84[a]	16.21	±	3.16[ab]	15.15	±	3.43[b]	23.49	±	7.28[c]
C18:1 n-9															
1	29.54	±	6.52[ab]	30.56	±	3.51[a]	30.73	±	4.55[a]	32.38	±	4.04[*a]	26.73	±	7.10[b]
2	31.11	±	3.74[a]	30.53	±	2.78[a]	30.39	±	4.17[a]	30.45	±	4.23[*†a]	24.77	±	6.03[b]
3	30.78	±	2.67[a]	30.41	±	2.79[ab]	31.05	±	3.64[a]	29.52	±	2.71[†ab]	27.75	±	5.21[b]
All	30.23	±	5.25[a]	30.53	±	3.19[a]	30.70	±	4.21[a]	31.21	±	3.99[a]	26.45	±	6.61[b]
C18:1 n-7															
1	3.31	±	0.9[ac]	3.97	±	0.93[b]	4.15	±	1.06[b]	3.85	±	1.01[ab]	3.27	±	1.17[c]
2	3.44	±	0.57	3.66	±	0.82	3.78	±	0.72	3.57	±	0.70	3.27	±	1.10
3	3.77	±	0.82[a]	3.79	±	0.98[a]	4.00	±	0.76[a]	3.95	±	0.65[a]	2.87	±	0.99[b]
All	3.44	±	0.82[ac]	3.85	±	0.92[b]	4.00	±	0.91[b]	3.79	±	0.86[ab]	3.21	±	1.12[c]
C18:2 n6 (LA)															
1	13.41	±	3.24[*ac]	15.27	±	2.33[b]	13.36	±	2.64[ac]	14.44	±	4.07[ab]	11.91	±	3.32[c]
2	15.05	±	2.84[*†]	15.08	±	2.55	14.32	±	3.16	14.84	±	2.76	13.80	±	4.17
3	15.21	±	2.12[†]	14.96	±	3.44	14.64	±	2.84	15.91	±	2.67	13.73	±	2.80
All	14.23	±	3.03[a]	15.16	±	2.61[a]	13.95	±	2.88[a]	14.88	±	3.48[a]	12.62	±	3.53[b]
C18:3 n6															
1	0.18	±	0.07[a]	0.17	±	0.08[a]	0.15	±	0.07[ab]	0.12	±	0.05[*bc]	0.11	±	0.07[c]
2	0.17	±	0.08[a]	0.15	±	0.08[ac]	0.17	±	0.07[a]	0.09	±	0.05[†bc]	0.10	±	0.07[c]
3	0.17	±	0.06[a]	0.18	±	0.08[a]	0.17	±	0.06[a]	0.13	±	0.05[*ab]	0.10	±	0.06[b]
All	0.18	±	0.07[a]	0.16	±	0.08[a]	0.16	±	0.07[a]	0.12	±	0.05[b]	0.10	±	0.07[b]
C18:3 n3 (ALA)															
1	0.30	±	0.19	0.33	±	0.18[*]	0.30	±	0.13	0.26	±	0.08	0.27	±	0.14
2	0.25	±	0.15	0.23	±	0.11[†]	0.32	±	0.21	0.27	±	0.13	0.24	±	0.10
3	0.27	±	0.1	0.24	±	0.10[*†]	0.27	±	0.12	0.26	±	0.11	0.31	±	0.10
All	0.28	±	0.16	0.29	±	0.16	0.30	±	0.15	0.26	±	0.1	0.27	±	0.13
C20:0															
1	0.58	±	0.21[ab]	0.50	±	0.12[a]	0.52	±	0.12[ab]	0.51	±	0.09[*a]	0.65	±	0.27[b]
2	0.50	±	0.10[ab]	0.48	±	0.10[ab]	0.50	±	0.10[ab]	0.45	±	0.10[†a]	0.55	±	0.13[b]
3	0.50	±	0.12[a]	0.50	±	0.09[a]	0.49	±	0.10[a]	0.52	±	0.16[*†ab]	0.69	±	0.28[b]
All	0.54	±	0.17[a]	0.49	±	0.11[a]	0.51	±	0.11[a]	0.49	±	0.11[a]	0.63	±	0.25[b]

Table SS1. Continuation

Fatty acids	6 m (n=108)			1 v (n=128)			1.5 v (n=96)			2 v (n=86)			3 v (n=76)		
	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
C20:1 n-9															
1	0.37	±	0.25^{*a}	0.25	±	0.10^{b}	0.24	±	0.08^{b}	0.22	±	0.09^{b}	0.17	±	0.07^{c}
2	0.32	±	0.20$^{*\dagger a}$	0.22	±	0.11^{ab}	0.20	±	0.06^{abc}	0.20	±	0.11^{bc}	0.15	±	0.09^{c}
3	0.21	±	0.08$^{\dagger ab}$	0.21	±	0.10^{ab}	0.23	±	0.06^{a}	0.19	±	0.04^{ab}	0.15	±	0.04^{b}
All	0.32	±	0.22^{a}	0.23	±	0.10^{b}	0.23	±	0.07^{b}	0.21	±	0.09^{b}	0.16	±	0.07^{c}
C20:2 n6															
1	0.20	±	0.10^{a}	0.19	±	0.08^{a}	0.15	±	0.06^{ab}	0.16	±	0.07^{ab}	0.17	±	0.12^{b}
2	0.21	±	0.07^{a}	0.16	±	0.06^{ab}	0.17	±	0.06^{ab}	0.14	±	0.08^{b}	0.24	±	0.13^{a}
3	0.18	±	0.04	0.17	±	0.06	0.17	±	0.05	0.17	±	0.04	0.21	±	0.13
All	0.20	±	0.08^{a}	0.18	±	0.07^{ab}	0.16	±	0.06^{ab}	0.16	±	0.07^{b}	0.19	±	0.13^{b}
C20:3 n6															
1	0.73	±	0.30^{ac}	0.90	±	0.25^{b}	0.80	±	0.29^{bc}	0.77	±	0.27^{bc}	0.68	±	0.31^{c}
2	0.79	±	0.27^{ab}	0.88	±	0.26^{a}	0.91	±	0.24^{a}	0.70	±	0.37^{b}	0.79	±	0.34^{ab}
3	1.09	±	0.52^{a}	0.93	±	0.35^{ab}	0.91	±	0.31^{ab}	0.88	±	0.34^{ab}	0.65	±	0.27^{b}
All	0.82	±	0.37^{ab}	0.90	±	0.27^{a}	0.86	±	0.28^{ab}	0.77	±	0.32^{bc}	0.70	±	0.31^{c}
C20:4 n6															
1	2.29	±	0.85^{a}	2.32	±	0.65^{a}	1.95	±	0.51^{ab}	1.89	±	0.66$^{*\dagger ab}$	1.78	±	0.72^{b}
2	2.56	±	0.81^{a}	2.33	±	0.62^{ac}	2.22	±	0.63^{ab}	1.76	±	0.85^{*b}	1.83	±	0.74^{bc}
3	2.52	±	0.70	2.30	±	0.66	2.24	±	0.73	2.34	±	0.56†	1.98	±	0.51
All	2.41	±	0.81^{a}	2.32	±	0.64^{a}	2.10	±	0.61^{ab}	1.95	±	0.72^{bc}	1.82	±	0.69^{c}
C22:0															
1	0.58	±	0.15^{a}	0.59	±	0.12^{a}	0.59	±	0.10^{a}	0.52	±	0.10^{ab}	0.47	±	0.15^{b}
2	0.58	±	0.15^{a}	0.60	±	0.10^{a}	0.57	±	0.10^{ab}	0.49	±	0.18^{b}	0.52	±	0.09^{ab}
3	0.59	±	0.13	0.58	±	0.12	0.57	±	0.11	0.56	±	0.17	0.49	±	0.17
All	0.58	±	0.14^{a}	0.59	±	0.12^{a}	0.58	±	0.10^{a}	0.52	±	0.14^{b}	0.49	±	0.14^{b}
C22:1 n-9															
1	0.25	±	0.16^{a}	0.17	±	0.08^{ab}	0.17	±	0.07^{ab}	0.12	±	0.05$^{*\dagger bc}$	0.13	±	0.08^{c}
2	0.20	±	0.12^{a}	0.16	±	0.06^{ac}	0.17	±	0.06^{ac}	0.10	±	0.05^{*b}	0.12	±	0.06^{bc}
3	0.16	±	0.05	0.16	±	0.05	0.18	±	0.06	0.14	±	0.06†	0.17	±	0.13
All	0.22	±	0.14^{a}	0.16	±	0.07^{b}	0.17	±	0.07^{ab}	0.12	±	0.05^{c}	0.13	±	0.08^{c}
C20:5 n3															
1	0.14	±	0.10	0.12	±	0.08	0.15	±	0.12	0.12	±	0.08	0.16	±	0.12
2	0.11	±	0.05^{a}	0.11	±	0.04^{a}	0.17	±	0.08^{b}	0.11	±	0.06^{a}	0.16	±	0.09^{ab}
3	0.10	±	0.05	0.11	±	0.03	0.14	±	0.10	0.13	±	0.05	0.19	±	0.11
All	0.12	±	0.08	0.12	±	0.07	0.16	±	0.11	0.12	±	0.07	0.17	±	0.11
C22:4 n6															
1	0.60	±	0.52^{a}	0.42	±	0.24^{ab}	0.44	±	0.26^{ab}	0.28	±	0.15^{bc}	0.28	±	0.22^{c}
2	0.44	±	0.23^{a}	0.55	±	0.38^{a}	0.35	±	0.18^{ab}	0.28	±	0.19^{b}	0.34	±	0.25^{ab}
3	0.67	±	0.57^{a}	0.44	±	0.26^{ab}	0.40	±	0.19^{ab}	0.36	±	0.21^{ab}	0.30	±	0.16^{b}
All	0.57	±	0.47^{a}	0.45	±	0.29^{a}	0.40	±	0.22^{a}	0.30	±	0.17^{b}	0.30	±	0.21^{b}
C22:5 n6															
1	0.06	±	0.03^{*ab}	0.07	±	0.03^{ab}	0.08	±	0.04^{a}	0.07	±	0.04^{*ab}	0.06	±	0.04^{b}
2	0.07	±	0.03$^{*\dagger a}$	0.07	±	0.03^{a}	0.08	±	0.03^{a}	0.05	±	0.03$^{\ddagger b}$	0.07	±	0.04^{ab}
3	0.08	±	0.03$^{\dagger a}$	0.07	±	0.03^{ab}	0.09	±	0.05^{a}	0.06	±	0.03$^{*\ddagger ab}$	0.06	±	0.04^{b}
All	0.07	±	0.03^{ab}	0.07	±	0.03^{ab}	0.08	±	0.04^{a}	0.06	±	0.04^{bc}	0.06	±	0.04^{c}
C24:1															
1	0.09	±	0.05	0.10	±	0.06	0.11	±	0.06	0.11	±	0.06*	0.09	±	0.05
2	0.08	±	0.05^{ab}	0.11	±	0.08^{ab}	0.11	±	0.05^{a}	0.07	±	0.04$^{\dagger b}$	0.08	±	0.06^{ab}
3	0.08	±	0.04	0.09	±	0.05	0.10	±	0.06	0.10	±	0.06*†	0.07	±	0.03
All	0.08	±	0.05a	0.10	±	0.06^{ab}	0.11	±	0.06^{b}	0.09	±	0.06^{ab}	0.08	±	0.05^{a}
C22:5 n3															
1	0.15	±	0.09^{a}	0.13	±	0.08^{abc}	0.13	±	0.05^{ab}	0.10	±	0.07$^{*\ddagger bc}$	0.09	±	0.06^{c}
2	0.14	±	0.07^{a}	0.11	±	0.06^{a}	0.12	±	0.04^{a}	0.08	±	0.06^{*b}	0.09	±	0.04^{ab}
3	0.14	±	0.06^{a}	0.16	±	0.09^{a}	0.12	±	0.05^{a}	0.11	±	0.04$^{\dagger a}$	0.06	±	0.04^{b}
All	0.15	±	0.08^{a}	0.13	±	0.07^{a}	0.12	±	0.05^{a}	0.10	±	0.06^{b}	0.08	±	0.06^{b}

Table SS1. Continuation

Fatty acids	6 m (n=108)			1 y (n=128)			1.5 y (n=96)			2 y (n=86)			3 y (n=76)		
	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
C22:5 n6 (DHA)															
1	0.74	±	0.38^{a}	0.74	±	0.34^{a}	0.52	±	0.18^{*a}	0.53	±	0.24^{a}	0.41	±	0.22^{b}
2	0.87	±	0.31^{a}	0.79	±	0.30^{a}	0.66	±	0.24^{†a}	0.50	±	0.30^{b}	0.41	±	0.17^{b}
3	0.83	±	0.33^{a}	0.75	±	0.27^{a}	0.65	±	0.24^{*†a}	0.60	±	0.26^{a}	0.39	±	0.24^{b}
All	0.79	±	0.36^{a}	0.76	±	0.32^{a}	0.59	±	0.22^{b}	0.54	±	0.26^{b}	0.40	±	0.21^{c}
SFA															
1	40.84	±	9.21^{*a}	35.95	±	4.37^{b}	38.12	±	6.01^{ab}	37.29	±	5.44^{ab}	46.50	±	10.83^{c}
2	34.93	±	4.48^{†a}	37.65	±	4.84^{a}	36.93	±	4.91^{a}	35.89	±	7.42^{a}	45.02	±	7.22^{b}
3	35.14	±	4.41^{†a}	37.06	±	5.51^{a}	35.96	±	4.18^{a}	37.50	±	4.10^{a}	46.01	±	7.60^{b}
All	38.04	±	7.84^{a}	36.60	±	4.74^{a}	37.26	±	5.32^{a}	36.94	±	5.79^{a}	46.09	±	9.59^{b}
MUFA															
1	39.71	±	6.17^{a}	41.84	±	4.53^{a}	42.17	±	5.14^{a}	43.27	±	4.18^{*a}	35.70	±	9.05^{b}
2	41.90	±	4.04^{a}	41.64	±	3.75^{a}	42.21	±	5.81^{a}	40.31	±	5.87^{†a}	34.62	±	8.80^{b}
3	42.38	±	4.16^{a}	42.32	±	3.95^{a}	43.28	±	3.69^{a}	41.48	±	3.29^{*†a}	36.00	±	5.34^{b}
All	40.86	±	5.37^{a}	41.88	±	4.21^{a}	42.44	±	5.05^{a}	42.05	±	4.68^{a}	35.50	±	8.44^{b}
PUFA															
1	18.81	±	3.99^{*ab}	20.64	±	3.05^{b}	18.02	±	3.08^{a}	18.75	±	4.47^{ab}	15.91	±	4.30^{c}
2	20.66	±	3.45*†	20.47	±	3.35	19.50	±	3.86	18.81	±	3.67	18.06	±	5.02
3	21.27	±	2.75†	20.30	±	3.97	19.8	±	3.55	20.97	±	2.92	17.98	±	3.35
All	19.82	±	3.75^{a}	20.53	±	3.29^{a}	18.89	±	3.51^{a}	19.26	±	4.02^{a}	16.72	±	4.41^{b}
n3 PUFA															
1	1.33	±	0.38^{a}	1.32	±	0.39^{a}	1.10	±	0.27^{ab}	1.01	±	0.32^{bc}	0.93	±	0.30^{c}
2	1.37	±	0.27^{a}	1.25	±	0.34^{a}	1.28	±	0.35^{a}	0.96	±	0.35^{b}	0.90	±	0.21^{b}
3	1.33	±	0.34^{a}	1.26	±	0.26^{a}	1.18	±	0.29^{ab}	1.10	±	0.28^{ab}	0.95	±	0.25^{b}
All	1.34	±	0.34^{a}	1.29	±	0.35^{ab}	1.18	±	0.31^{b}	1.02	±	0.32^{c}	0.92	±	0.28^{c}
n6 PUFA															
1	17.47	±	3.81^{*ab}	19.33	±	2.87^{a}	16.92	±	3.00^{b}	17.74	±	4.39^{ab}	14.98	±	4.11^{c}
2	19.29	±	3.37*†	19.22	±	3.17	18.21	±	3.71	17.85	±	3.56	17.16	±	4.83
3	19.94	±	2.53†	19.04	±	3.88	18.62	±	3.39	19.86	±	2.90	17.03	±	3.22
All	18.48	±	3.60^{a}	19.25	±	3.13^{a}	17.71	±	3.37^{a}	18.24	±	3.94^{a}	15.79	±	4.24^{b}
n3 LC-PUFA															
1	1.03	±	0.39^{a}	0.99	±	0.38^{ab}	0.80	±	0.25^{ab}	0.76	±	0.30^{bc}	0.66	±	0.28^{c}
2	1.12	±	0.33^{a}	1.01	±	0.34^{a}	0.96	±	0.30^{a}	0.69	±	0.36^{b}	0.66	±	0.20^{b}
3	1.06	±	0.37^{a}	1.02	±	0.29^{a}	0.90	±	0.29^{ab}	0.84	±	0.30^{ab}	0.64	±	0.24^{b}
All	1.06	±	0.37^{a}	1.00	±	0.36^{a}	0.87	±	0.28^{a}	0.76	±	0.32^{b}	0.65	±	0.26^{b}
n6 LC-PUFA															
1	3.88	±	1.15^{a}	3.89	±	0.95^{a}	3.41	±	0.89^{ab}	3.18	±	1.02^{*†ab}	2.97	±	1.09^{b}
2	4.07	±	1.15^{a}	3.99	±	0.93^{a}	3.72	±	0.83^{a}	2.92	±	1.35^{*b}	3.26	±	0.95^{ab}
3	4.55	±	0.85^{a}	3.91	±	0.93^{ab}	3.80	±	1.10^{ab}	3.82	±	0.87^{†ab}	3.20	±	0.78^{b}
All	4.07	±	1.12^{a}	3.92	±	0.93^{a}	3.60	±	0.93^{ab}	3.25	±	1.12^{bc}	3.07	±	1.01^{c}
n6:n3															
1	13.91	±	4.22^{a}	15.74	±	4.21^{ab}	16.08	±	4.12^{ab}	18.81	±	6.35^{b}	17.63	±	6.27^{b}
2	14.68	±	4.20^{a}	16.18	±	3.82^{ac}	15.00	±	4.34^{a}	21.19	±	9.91^{bc}	18.84	±	3.10^{c}
3	15.78	±	3.82	15.65	±	4.09	16.40	±	3.68	19.24	±	6.00	18.84	±	4.93
All	14.51	±	4.16^{a}	15.84	±	4.06^{a}	15.81	±	4.09^{a}	19.57	±	7.42^{b}	18.09	±	5.48^{b}
LC-n6:n3															
1	4.00	±	1.00^{*†a}	4.29	±	1.30^{ab}	4.54	±	1.43^{ab}	4.54	±	1.43^{ab}	4.97	±	1.77^{b}
2	3.74	±	0.92^{*a}	4.17	±	1.05^{ab}	4.08	±	1.05^{ab}	4.62	±	1.52^{ab}	5.12	±	1.47^{b}
3	4.87	±	2.37†	4.08	±	1.33	4.46	±	1.36	5.09	±	2.28	5.59	±	2.25
All	4.11	±	1.42^{a}	4.22	±	1.24^{a}	4.38	±	1.31^{a}	4.68	±	1.67^{ab}	5.10	±	1.78^{b}

Maternal BMI groups: 1. normal-weight; 2. overweight; 3. obese. Natural logarithms were used for non-normal variables. Different symbols indicate significant differences between maternal BMI groups. Different superscript letters indicate significant differences among timing of sample collection. Significant differences were established according to according to ANOVA and the Bonferroni post-hoc test with ($p < 0.05$). LA: Linoleic Acid; AA: Arachidonic Acid; ALA: α-linolenic Acid; DHA: Docosahexaenoic Acid; SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids.

Table SS2. Fatty acids levels of cheek cells glycerophospholipids according to maternal BMI and infant feeding practice.

Fatty acids	6 m									1.5 y								
	Exclusive breastfeeding (n=52)			Mixt (n=18)			Formula (n=32)			Exclusive breastfeeding (n=52)			Mixt (n=18)			Formula (n=32)		
	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
C15:1																		
1	2.64	±	1.75	3.81	±	1.91	2.43	±	1.22	2.45	±	1.66	2.19	±	0.94	2.59	±	0.93
2	3.56	±	1.73	2.86	±	0.82	3.77	±	1.07	3.19	±	1.14	3.15	±	0.27	3.69	±	1.81
3	3.23	±	0.87	3.51	±	1.94	3.36	±	1.61	3.12	±	0.77	2.92	±	1.10	2.67	±	1.49
All	2.98	±	1.68	3.51	±	1.66	3.19	±	1.41	2.81	±	1.40	2.69	±	0.88	2.88	±	1.42
C16:0																		
1	18.29	±	3.78	21.56	±	5.03	21.18	±	2.48	18.60	±	2.90	22.33	±	3.27	19.79	±	3.56
2	16.23	±	3.02^{a}	11.88	±	8.12^{b}	17.82	±	2.13^{a}	18.29	±	3.08	18.70	±	3.24	19.54	±	1.99
3	15.86	±	6.37	14.29	±	0.46	16.43	±	1.68	18.69	±	1.95	17.60	±	2.18	18.24	±	2.10
All	17.36	±	4.07	17.86	±	7.01	18.41	±	2.88	18.52	±	2.77	19.94	±	3.50	19.11	±	2.69
C16:1																		
1	2.86	±	1.42	4.74	±	5.07	2.89	±	1.54	4.18	±	1.26	3.70	±	0.93	4.49	±	1.21
2	2.85	±	0.86	2.87	±	1.20	4.00	±	1.08	4.50	±	1.39	3.66	±	0.86	4.78	±	1.85
3	3.39	±	0.90	4.65	±	0.92	4.43	±	1.15	5.03	±	1.70	4.47	±	1.40	4.76	±	1.71
All	2.93	±	1.22^{a}	4.24	±	3.92^{ab}	3.80	±	1.39^{b}	4.44	±	1.39	3.88	±	1.00	4.67	±	1.52
C17:0																		
1	0.94	±	0.22	0.99	±	0.16	0.80	±	0.34	0.88	±	0.25	1.04	±	0.23	0.91	±	0.15
2	1.13	±	0.36	1.01	±	0.31	1.07	±	0.30	1.04	±	0.29	0.99	±	0.13	0.90	±	0.08
3	1.27	±	0.14	1.21	±	0.73	1.03	±	0.18	1.09	±	0.19	0.89	±	0.12	0.82	±	0.22
All	1.04	±	0.29	1.03	±	0.32	0.97	±	0.29	0.97	±	0.26	0.99	±	0.17	0.87	±	0.17
C18:0																		
1	17.59	±	5.01	23.80	±	6.83	19.77	±	10.13	15.82	±	3.70	18.60	±	2.82	17.27	±	2.34
2	17.14	±	3.37	17.90	±	1.63	15.51	±	2.28	15.19	±	2.60	17.37	±	2.86	16.98	±	1.59
3	18.10	±	2.84	17.20	±	0.90	16.35	±	4.34	16.83	±	1.13	14.44	±	0.46	14.92	±	4.02
All	17.53	±	4.29	21.20	±	6.04^{a}	17.18	±	6.49^{b}	15.80	±	3.05	17.15	±	2.85	16.26	±	3.11
C18:1																		
1	31.70	±	5.39^{a}	23.38	±	6.29 ^{a}	28.87	±	7.52	31.31	±	4.23	30.23	±	4.99	30.54	±	4.00
2	31.27	±	3.89	29.27	±	4.67	31.79	±	3.07	30.70	±	4.49	30.66	±	3.80	28.44	±	1.95
3	28.85	±	2.93	31.99	±	2.01	31.40	±	2.16	29.43	±	1.91^{a}	36.29	±	3.93^{b}	30.95	±	3.53^{a}
All	31.19	±	4.75^{a}	26.29	±	6.35^{b}	30.71	±	4.80^{a}	30.77	±	4.00	31.89	±	4.78	30.20	±	3.44
C18:1																		
1	3.32	±	0.87	3.25	±	1.28	3.16	±	0.77	4.33	±	1.04	3.31	±	0.62	4.31	±	0.94
2	3.47	±	0.46	2.94	±	0.86^{a}	3.64	±	0.47^{b}	3.75	±	0.71	3.72	±	0.43	4.18	±	0.99
3	3.32	±	0.88	4.03	±	0.80	4.04	±	0.73	3.99	±	0.88	3.79	±	0.49	4.07	±	0.77
All	3.37	±	0.76	3.29	±	1.13	3.63	±	0.75	4.08	±	0.94	3.57	±	0.54	4.18	±	0.85
C18:2 n6 (LA)																		
1	14.68	±	2.62^{a}	11.17	±	3.75^{b}	12.23	±	3.40^{ab}	13.83	±	2.53	12.46	±	2.12	13.90	±	2.02
2	16.10	±	2.10^{a}	11.79	±	3.59^{b}	15.09	±	2.39^{a}	14.59	±	3.29	14.44	±	1.20	14.20	±	3.27
3	15.49	±	2.73	15.69	±	1.30	14.70	±	1.92	14.87	±	2.09	13.78	±	3.78	14.69	±	3.38
All	15.20	±	2.53^{a}	12.05	±	3.68^{b}	14.03	±	2.83^{a}	14.26	±	2.72	13.45	±	2.34	14.29	±	2.83
C18:3																		
1	0.18	±	0.06	0.22	±	0.09	0.15	±	0.07	0.13	±	0.06	0.15	±	0.05	0.14	±	0.05
2	0.17	±	0.05	0.19	±	0.11	0.17	±	0.10	0.17	±	0.07	0.13	±	0.06	0.18	±	0.07
3	0.16	±	0.06	0.19	±	0.01	0.18	±	0.07	0.17	±	0.04	0.11	±	0.05	0.20	±	0.07
All	0.17	±	0.06	0.21	±	0.09	0.16	±	0.08	0.15	±	0.06	0.14	±	0.05	0.17	±	0.07
C18:3 n3 (ALA)																		
1	0.25	±	0.15^{a}	0.41	±	0.27^{b}	0.37	±	0.16^{b}	0.30	±	0.14	0.23	±	0.04	0.30	±	0.06
2	0.17	±	0.09^{a}	0.41	±	0.24^{b}	0.27	±	0.11^{b}	0.29	±	0.17	0.28	±	0.13	0.21	±	0.04
3	0.29	±	0.13	0.20	±	0.03	0.28	±	0.09	0.33	±	0.15	0.18	±	0.01	0.25	±	0.07
All	0.23	±	0.13^{a}	0.38	±	0.24^{b}	0.31	±	0.13^{b}	0.30	±	0.15	0.24	±	0.08	0.26	±	0.07
C20:0																		
1	0.56	±	0.14	0.66	±	0.27	0.63	±	0.30	0.50	±	0.10	0.55	±	0.15	0.58	±	0.16
2	0.49	±	0.10	0.54	±	0.15	0.51	±	0.10	0.48	±	0.08	0.48	±	0.03	0.54	±	0.14
3	0.52	±	0.14	0.42	±	0.07	0.52	±	0.10	0.48	±	0.04	0.42	±	0.12	0.52	±	0.14
All	0.53	±	0.13	0.59	±	0.24	0.55	±	0.19	0.49	±	0.08	0.50	±	0.12	0.54	±	0.14

Table SS2. Continuation

Fatty acids	6 m									1.5 y								
	Exclusive breastfeeding (n=52)			Mixt (n=18)			Formula (n=32)			Exclusive breastfeeding (n=52)			Mixt (n=18)			Formula (n=32)		
	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
C20:1 n-9																		
1	0.34	±	0.24	0.51	±	0.26	0.37	±	0.28	0.23	±	0.08	0.31	±	0.12	0.26	±	0.07
2	0.28	±	0.18	0.38	±	0.27	0.35	±	0.21	0.19	±	0.07	0.20	±	0.08	0.23	±	0.03
3	0.20	±	0.06	0.20	±	0.10	0.23	±	0.10	0.24	±	0.06	0.27	±	0.05	0.21	±	0.05
All	0.30	±	0.21	0.43	±	0.26	0.31	±	0.21	0.22	±	0.08	0.26	±	0.10	0.23	±	0.06
C20:2 n6																		
1	0.19	±	0.07	0.26	±	0.17	0.16	±	0.07	0.16	±	0.06	0.14	±	0.06	0.17	±	0.05
2	0.21	±	0.07	0.22	±	0.12	0.20	±	0.06	0.16	±	0.05	0.18	±	0.09	0.15	±	0.04
3	0.21	±	0.05	0.18	±	0.02	0.17	±	0.03	0.18	±	0.05	0.14	±	0.05	0.17	±	0.06
All	0.20	±	0.07	0.24	±	0.14	0.18	±	0.05	0.16	±	0.06	0.15	±	0.06	0.16	±	0.05
C20:3 n6																		
1	0.79	±	0.32	0.62	±	0.23	0.56	±	0.28	0.78	±	0.31	0.78	±	0.15	0.77	±	0.16
2	0.84	±	0.24	0.77	±	0.43	0.73	±	0.22	0.88	±	0.17	1.05	±	0.20	0.91	±	0.20
3	0.81	±	0.42	1.12	±	0.29	1.26	±	0.59	0.90	±	0.30	0.87	±	0.30	0.94	±	0.34
All	0.81	±	0.31	0.74	±	0.34	0.87	±	0.50	0.83	±	0.27	0.89	±	0.22	0.87	±	0.26
C20:4 n6 (AA)																		
1	2.53	±	0.85	2.01	±	0.51	1.84	±	1.06	1.97	±	0.58	1.74	±	0.38	2.00	±	0.36
2	2.93	±	0.81	2.03	±	0.88	2.27	±	0.51	2.24	±	0.69	2.34	±	0.64	2.40	±	0.49
3	2.27	±	0.95	2.54	±	0.19	2.64	±	0.63	2.34	±	0.61	1.71	±	0.43	2.30	±	0.87
All	2.61	±	0.87	2.10	±	0.60	2.27	±	0.81	2.12	±	0.63	1.93	±	0.54	2.22	±	0.64
C22:0																		
1	0.60	±	0.14	0.60	±	0.16	0.49	±	0.14	0.56	±	0.09	0.64	±	0.08*	0.57	±	0.08
2	0.59	±	0.15	0.58	±	0.22	0.58	±	0.11	0.57	±	0.12	0.54	±	0.07*†	0.60	±	0.07
3	0.53	±	0.13	0.54	±	0.10	0.65	±	0.12	0.52	±	0.07^{ab}	0.47	±	0.09†^{a}	0.63	±	0.11^{a}
All	0.59	±	0.14	0.59	±	0.16	0.57	±	0.14	0.56	±	0.10	0.57	±	0.10	0.60	±	0.10
C22:1 n-9																		
1	0.25	±	0.16	0.32	±	0.18	0.17	±	0.10	0.16	±	0.06	0.18	±	0.04	0.16	±	0.08
2	0.15	±	0.06	0.27	±	0.13	0.23	±	0.16	0.17	±	0.08	0.19	±	0.04	0.18	±	0.04
3	0.12	±	0.04	0.21	±	0.02	0.16	±	0.06	0.17	±	0.04	0.17	±	0.03	0.19	±	0.09
All	0.21	±	0.14^{a}	0.29	±	0.15^{b}	0.19	±	0.11^{a}	0.16	±	0.06	0.18	±	0.04	0.18	±	0.07
C20:5 n3 (EPA)																		
1	0.13	±	0.09^{ab}	0.18	±	0.09^{a}	0.09	±	0.08^{b}	0.15	±	0.14	0.16	±	0.09	0.15	±	0.12
2	0.09	±	0.03	0.12	±	0.03	0.12	±	0.07	0.18	±	0.09	0.16	±	0.04	0.15	±	0.08
3	0.07	±	0.03	0.10	±	0.01	0.11	±	0.06	0.15	±	0.12	0.14	±	0.04	0.13	±	0.10
All	0.11	±	0.08	0.15	±	0.08	0.11	±	0.07	0.16	±	0.12	0.16	±	0.06	0.14	±	0.10
C22:4 n6																		
1	0.67	±	0.59	0.67	±	0.42	0.39	±	0.35	0.43	±	0.24	0.54	±	0.40	0.36	±	0.19
2	0.48	±	0.22	0.33	±	0.15	0.43	±	0.28	0.36	±	0.17	0.26	±	0.16	0.39	±	0.20
3	0.79	±	0.52	0.31	±	0.15	0.73	±	0.66	0.46	±	0.23	0.20	±	0.06	0.40	±	0.15
All	0.63	±	0.51	0.52	±	0.37	0.52	±	0.48	0.41	±	0.22	0.36	±	0.30	0.39	±	0.17
C22:5 n6																		
1	0.06	±	0.03	0.06	±	0.02	0.06	±	0.02	0.07	±	0.03	0.09	±	0.04	0.08	±	0.03
2	0.08	±	0.03	0.06	±	0.01	0.07	±	0.02	0.07	±	0.02	0.08	±	0.02	0.09	±	0.04
3	0.08	±	0.03	0.08	±	0.02	0.08	±	0.03	0.08	±	0.04	0.06	±	0.02	0.11	±	0.06
All	0.07	±	0.03	0.06	±	0.02	0.07	±	0.03	0.07	±	0.03^{a}	0.08	±	0.03^{ab}	0.09	±	0.04^{b}
C24:1																		
1	0.08	±	0.04	0.12	±	0.07	0.08	±	0.05	0.11	±	0.06	0.08	±	0.06	0.13	±	0.03
2	0.07	±	0.06	0.10	±	0.07	0.08	±	0.04	0.11	±	0.05	0.13	±	0.07	0.10	±	0.05
3	0.09	±	0.03	0.08	±	0.05	0.08	±	0.04	0.11	±	0.06	0.15	±	0.08	0.08	±	0.05
All	0.08	±	0.05	0.11	±	0.07	0.08	±	0.04	0.11	±	0.06	0.11	±	0.07	0.10	±	0.05
C22:5 n3																		
1	0.16	±	0.07	0.19	±	0.14	0.14	±	0.08	0.14	±	0.06	0.08	±	0.03	0.12	±	0.06
2	0.14	±	0.06	0.13	±	0.11	0.14	±	0.06	0.14	±	0.04	0.11	±	0.04	0.11	±	0.02
3	0.13	±	0.04	0.16	±	0.02	0.13	±	0.08	0.14	±	0.04	0.08	±	0.03	0.10	±	0.05
All	0.15	±	0.06	0.17	±	0.12	0.14	±	0.07	0.14	±	0.05^{a}	0.09	±	0.03^{b}	0.11	±	0.05^{b}

Table SS2. Continuation

Fatty acids		6 m									1.5 y								
		Exclusive breastfeeding (n=52)			Mixt (n=18)			Exclusive breastfeeding (n=52)			Mixt (n=18)			Exclusive breastfeeding (n=52)			Mixt (n=18)		
		Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
C22:5 n6 (DHA)																			
	1	0.82	±	0.35	0.58	±	0.36	0.57	±	0.44	0.55	±	0.19	0.44	±	0.08	0.43	±	0.15
	2	1.02	±	0.28^{a}	0.60	±	0.33^{b}	0.78	±	0.24^{ab}	0.71	±	0.23	0.69	±	0.30	0.58	±	0.27
	3	0.73	±	0.33	1.09	±	0.23	0.81	±	0.36	0.66	±	0.22	0.64	±	0.26	0.64	±	0.28
	All	0.87	±	0.34^{a}	0.66	±	0.37^{b}	0.72	±	0.36^{ab}	0.62	±	0.22	0.58	±	0.23	0.55	±	0.25
SFA																			
	1	37.9	±	6.31^{a}	47.61	±	11.23^{b}	42.87	±	12.31	36.36	±	5.80	43.17	±	5.76	39.11	±	4.01
	2	35.5	±	3.18	31.91	±	7.56	35.48	±	4.17	35.56	±	3.95	38.08	±	5.56	38.56	±	2.67
	3	36.2	±	5.34	33.66	±	1.98	34.99	±	4.59	37.60	±	2.60	33.82	±	1.69	35.13	±	5.40
	All	37.0	±	5.48	41.27	±	11.91	37.69	±	8.42	36.33	±	4.78	39.14	±	6.04	37.39	±	4.62
MUFA																			
	1	41.2	±	4.89	36.14	±	8.00	37.96	±	7.24	42.77	±	4.30	40.01	±	5.33	42.48	±	3.01
	2	41.6	±	2.96^{ab}	38.69	±	6.05^{a}	43.88	±	3.53^{b}	42.61	±	6.55	41.71	±	4.72	41.59	±	3.94
	3	39.2	±	4.05	44.67	±	1.51	43.70	±	3.94	42.08	±	3.43	48.05	±	2.63	42.93	±	3.25
	All	41.0	±	4.30	38.15	±	7.32^{a}	41.91	±	5.68^{b}	42.59	±	4.92	42.59	±	5.40	42.45	±	3.24
PUFA																			
	1	20.4	±	3.30^{a}	16.37	±	4.25^{b}	16.57	±	4.16^{b}	18.50	±	3.09	16.82	±	2.10	18.41	±	2.36
	2	22.2	±	2.40^{a}	16.66	±	4.46^{b}	20.29	±	2.71^{a}	19.79	±	4.12	19.70	±	1.92	19.38	±	3.80
	3	21.0	±	3.61	21.67	±	1.26	21.10	±	2.68	20.28	±	2.10	17.92	±	4.57	19.92	±	4.40
	All	21.0	±	3.15^{a}	17.28	±	4.30^{b}	19.38	±	3.71^{ab}	19.23	±	3.34	18.06	±	2.84	19.25	±	3.55
n3 PUFA																			
	1	1.36	±	0.35	1.37	±	0.32	1.18	±	0.51	1.13	±	0.30	0.92	±	0.16	1.00	±	0.21
	2	1.43	±	0.31	1.26	±	0.15	1.32	±	0.24	1.33	±	0.36	1.24	±	0.44	1.06	±	0.27
	3	1.21	±	0.31	1.55	±	0.25	1.33	±	0.39	1.28	±	0.32	1.05	±	0.33	1.12	±	0.24
	All	1.36	±	0.34	1.37	±	0.28	1.28	±	0.39	1.22	±	0.33	1.06	±	0.32	1.06	±	0.23
n6 PUFA																			
	1	19.1	±	3.15^{a}	15.01	±	4.13^{b}	15.38	±	3.80^{b}	17.37	±	2.99	15.90	±	2.00	17.41	±	2.23
	2	20.8	±	2.29^{a}	15.40	±	4.39^{b}	18.97	±	2.72^{a}	18.46	±	3.90	18.46	±	1.88	18.33	±	3.63
	3	19.8	±	3.37	20.12	±	1.21	19.77	±	2.40	19.00	±	1.95	16.87	±	4.32	18.80	±	4.25
	All	19.6	±	3.00^{a}	15.92	±	4.18^{b}	18.09	±	3.49^{a}	18.01	±	3.18	17.00	±	2.67	18.19	±	3.40
n3 LC-PUFA																			
	1	1.11	±	0.37^{a}	0.95	±	0.30^{ab}	0.81	±	0.50^{b}	0.84	±	0.27	0.69	±	0.13	0.70	±	0.23
	2	1.26	±	0.32	0.85	±	0.32	1.05	±	0.28	1.04	±	0.32	0.96	±	0.35	0.84	±	0.27
	3	0.92	±	0.36	1.35	±	0.23	1.05	±	0.40	0.96	±	0.33	0.86	±	0.33	0.87	±	0.27
	All	1.13	±	0.36	0.99	±	0.33	0.97	±	0.41	0.92	±	0.31	0.82	±	0.27	0.80	±	0.26
n6 LC-PUFA																			
	1	4.25	±	1.08^{a}	3.62	±	0.72^{ab}	3.01	±	1.50^{b}	3.40	±	1.00	3.29	±	0.55	3.37	±	0.52
	2	4.53	±	1.23	3.41	±	1.10	3.71	±	0.76	3.70	±	0.94	3.89	±	0.68	3.95	±	0.67
	3	4.16	±	1.16	4.23	±	0.16	4.89	±	0.67	3.96	±	0.94	2.98	±	0.68	3.91	±	1.29
	All	4.32	±	1.13	3.66	±	0.80	3.90	±	1.28	3.59	±	0.97	3.41	±	0.68	3.73	±	0.94
n6:n3																			
	1	14.8	±	3.91^{a}	11.38	±	3.63^{b}	14.39	±	5.42^{ab}	16.13	±	4.38	17.39	±	2.15	17.84	±	2.97
	2	15.3	±	4.47	12.19	±	3.28	14.86	±	4.07	14.41	±	3.05	16.70	±	7.80	17.79	±	3.69
	3	16.7	±	2.32	13.18	±	2.36	15.97	±	4.84	15.45	±	3.21	16.48	±	2.87	17.23	±	4.36
	All	15.2	±	3.91^{a}	11.88	±	3.28^{b}	15.10	±	4.69^{a}	15.46	±	3.81^{a}	16.93	±	4.47^{ab}	17.58	±	3.61^{b}
LC-n6:n3																			
	1	4.01	±	0.99	4.02	±	1.09	4.02	±	1.13	4.37	±	1.55	4.87	±	1.23	5.15	±	1.32
	2	3.66	±	0.94	4.11	±	0.90	3.69	±	0.95	3.72	±	0.88	4.45	±	1.83	4.83	±	0.62
	3	4.81	±	1.39	3.20	±	0.64	5.48	±	3.02	4.52	±	1.57	3.61	±	0.63	4.65	±	1.34
	All	4.02	±	1.07	3.92	±	1.00	4.43	±	2.09	4.19	±	1.38	4.42	±	1.35	4.87	±	1.18

Maternal BMI groups: 1. normal-weight; 2. overweight; 3. obese. Natural logarithms were used for non-normal variables. Different symbols indicate significant differences between maternal BMI groups. Different superscript letters indicate significant differences among type of infant feeding practice. Significant differences were established according to according to ANOVA and the Bonferroni post-hoc test with ($p < 0.05$). LA: Linoleic Acid; AA: Arachidonic Acid; ALA: α-linolenic Acid; DHA: Docosahexaenoic Acid; SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids

Table SS3. Association between cheek cell fatty acid levels and cognitive development score.

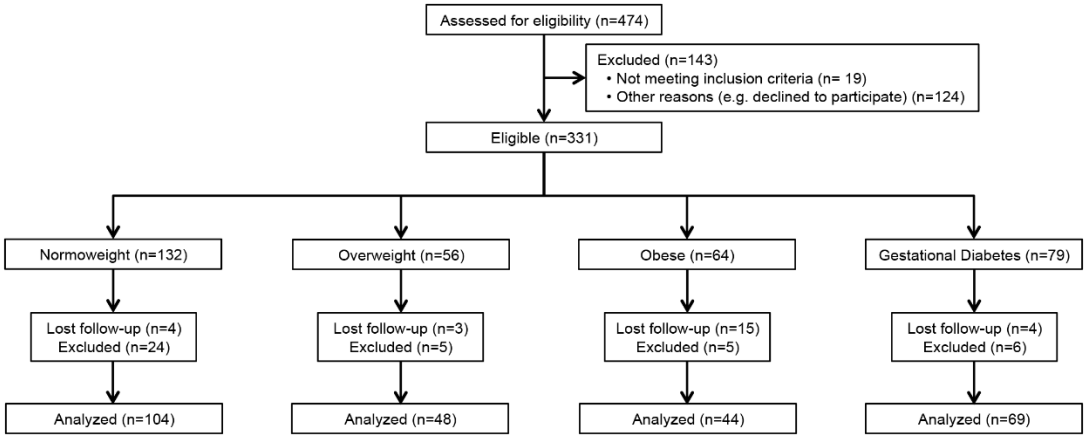
Fatty Acid	All				Normal-weight				Overweight				Obese			
	β	p	β^c	p^c	β	p	β^c	p^c	β	p	β^c	p^c	β	p	β^c	p^c
DHA 6m	0.23	0.097	0.21	0.161	0.13	0.536	0.10	0.745	0.14	0.589	0.28	0.340	0.34	0.314	1.25	0.066
DHA 1.5y	0.27	0.077	0.38	**0.026**	0.37	0.130	0.31	0.400	-0.12	0.664	0.05	0.906	0.32	0.369	0.89	0.065
AA 6m	0.33	0.017	0.28	**0.047**	0.33	0.113	0.33	0.206	-0.09	0.729	-0.03	0.949	0.56	0.071	0.62	0.161
AA 1.5y	0.23	0.129	0.22	0.192	0.27	0.287	0.14	0.616	0.21	0.429	0.31	0.367	-0.09	0.801	0.15	0.859
n3 PUFA 6m	0.08	0.582	0.04	0.791	-0.08	0.694	-0.19	0.496	0.24	0.344	0.12	0.667	0.08	0.821	1.02	0.268
n3 PUFA 1.5y	0.25	0.104	0.40	**0.031**	0.54	0.020	0.72	**0.041**	-0.23	0.387	-0.10	0.821	0.44	0.209	1.24	**0.004**
n3 LC-PUFA 6m	0.10	0.486	0.08	0.575	-0.05	0.821	-0.14	0.629	0.13	0.613	0.22	0.472	0.24	0.480	1.31	0.090
n3 LC-PUFA 1.5y	0.24	0.124	0.35	**0.049**	0.38	0.116	0.28	0.440	-0.15	0.572	0.00	0.992	0.31	0.379	0.89	0.073
n6 PUFA 6m	0.38	**0.005**	0.32	**0.034**	0.37	0.078	0.47	0.076	0.07	0.804	0.17	0.471	0.31	0.346	0.57	0.407
n6 PUFA 1.5y	0.14	0.367	0.02	0.914	0.21	0.410	-0.05	0.885	-0.17	0.518	-0.23	0.527	0.05	0.898	0.31	0.583
n6 LC-PUFA 6m	0.25	0.074	0.14	0.351	0.18	0.399	0.14	0.606	-0.10	0.693	-0.06	0.893	0.47	0.140	0.63	0.201
n6 LC-PUFA 1.5y	0.27	0.079	0.22	0.187	0.37	0.130	0.17	0.581	0.26	0.336	0.29	0.404	-0.11	0.754	0.01	0.992
n6:n3 6m	0.25	0.073	0.20	0.153	0.42	0.039	0.60	**0.015**	-0.17	0.518	0.09	0.737	0.09	0.801	-0.37	0.647
n6:n3 1.5y	-0.16	0.312	-0.39	**0.036**	-0.40	0.100	-0.49	0.083	0.13	0.638	-0.19	0.763	-0.43	0.219	-0.73	0.233
LC-n6:n3 6m	0.10	0.484	0.01	0.923	0.25	0.248	0.35	0.221	-0.29	0.257	-0.20	0.383	-0.05	0.894	-1.34	0.286
LC-n6:n3 1.5y	-0.05	0.747	-0.18	0.321	-0.19	0.443	-0.12	0.710	0.32	0.231	0.44	0.393	-0.39	0.265	-0.60	0.208

Natural logarithms were used for non-normal variables. Associations were analyzed using lineal regression analyses. β^c and P^c= are corrected values after adjustment for maternal smoking, maternal education, maternal diet (ratio n6:n3, n3 PUFAs) and type of infant feeding practice. Significant differences with a p value <0.05 are highlighted in bold. LA: Linoleic Acid; AA: Arachidonic Acid; ALA: α-linolenic Acid; DHA: Docosahexaenoic Acid; SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids.
All: 6mo (n= 52). 1.5y (n= 44)
Normal-weight: 6mo (n= 24). 1.5y (n= 18)
Overweight: 6mo (n= 17). 1.5y (n= 16)
Obesity: 6mo (n= 11). 1.5y (n= 10)

Table SS4. Association between cheek cell fatty acid levels and high or low cognition.

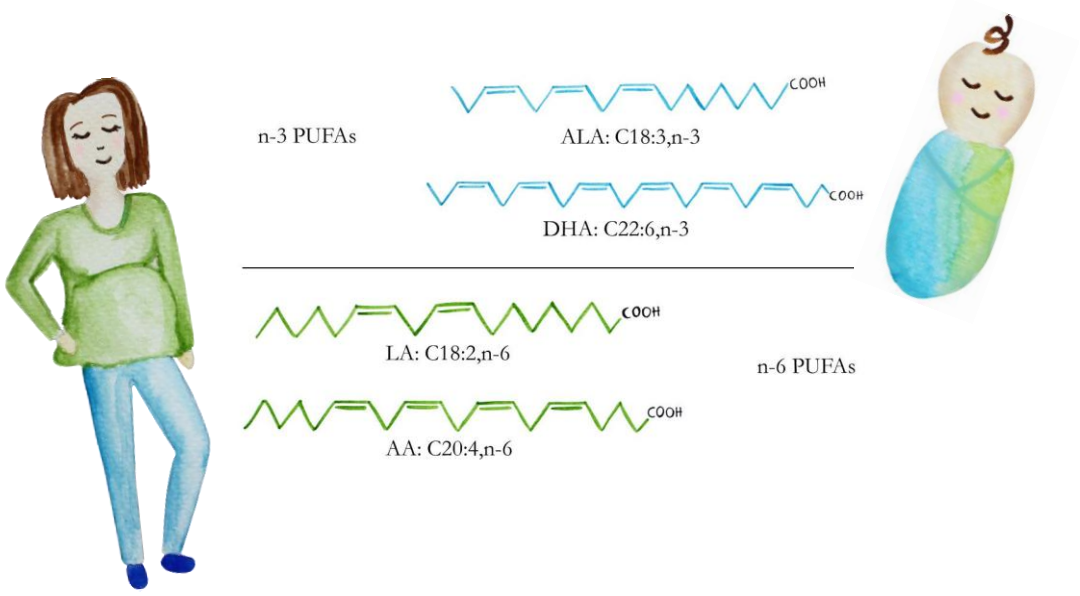
Fatty Acid	All						Normal-weight						Overweight/Obese					
	OR	95%-CI	P	OR^c	95%-CI ^{c}	P^c	OR	95%-CI	P	OR^c	95%-CI ^{c}	P^c	OR	95%-CI	P	OR^c	95%-CI ^{c}	P^c
DHA 6m	0.37	(0.11 - 1.25)	0.110	0.13	(0.02 - 0.75)	**0.023**	0.50	(0.09 - 2.74)	0.424	0.01	(0.00 - 5.06)	0.136	0.43	(0.06 - 3.34)	0.421	0.00	(0.00 - 2.11)	0.065
DHA 1.5y	0.36	(0.07 - 1.93)	0.233	1.10	(0.97 - 1.26)	0.147	0.10	(0.00 - 2.53)	0.163	0.25	(0.00 - 15.52)	0.511	1.01	(0.10 - 10.14)	0.992	0.49	(0.00 - 2.11)	0.734
AA 6m	0.09	(0.01 - 0.75)	**0.027**	0.01	(0.00 - 0.24)	**0.005**	0.08	(0.00 - 1.83)	0.115	0.00	(0.00 - 3.37)	0.096	0.32	(0.00 - 12.85)	0.546	0.00	(0.00 - 11.47)	0.126
AA 1.5y	0.15	(0.01 - 2.01)	0.153	0.05	(0.00 - 1.45)	0.082	0.01	(0.00 - 8.14)	0.186	0.03	(0.00 - 27.71)	0.322	0.38	(0.02 - 8.25)	0.538	0.06	(0.00 - 26.89)	0.363
n3 PUFA 6m	0.66	(0.08 - 5.77)	0.705	0.17	(0.01 - 3.34)	0.245	1.37	(0.15 - 12.18)	0.777	0.49	(0.02 - 12.40)	0.665	0.33	(0.01 - 8.86)	0.508	0.00	(0.00 - 18.62)	0.148
n3 PUFA 1.5y	0.14	(0.01 - 1.97)	0.144	0.00	(0.00 - 0.73)	**0.038**	0.00	(0.00 - 0.87)	**0.046**	0.00	(0.00 - 1.68)	0.057	0.77	(0.06 - 9.98)	0.8411	0.19	(0.00 - 87.04)	0.598
n3 LC-PUFA 6m	0.60	(0.12 - 2.95)	0.529	0.19	(0.02 - 1.67)	0.133	1.08	(0.12- 9.40)	0.946	0.07	(0.00 - 30.69)	0.397	0.45	(0.03 - 7.57)	0.582	0.00	(0.00 - 4.18)	0.077
n3 LC-PUFA 1.5y	0.38	(0.05 - 2.92)	0.356	0.24	(0.01 - 4.98)	0.357	0.07	(0.00 - 4.22)	0.200	0.42	(0.00 - 228.26)	0.786	1.15	(0.09 - 15.40)	0.916	0.80	(0.00 - 92.03)	0.925
n6 PUFA 6m	0.01	(0.00 - 0.32)	**0.010**	0.00	(0.00 - 0.14)	**0.006**	0.74	(0.54 - 1.01)	0.055	0.44	(0.15 - 1.31)	0.139	0.95	(0.70 - 1.29)	0.738	0.93	(0.61 - 1.42)	0.724
n6 PUFA 1.5y	0.95	(0.78 - 1.15)	0.600	0.93	(0.73 - 1.19)	0.567	0.92	(0.66 - 1.29)	0.618	1.07	(0.65 - 1.77)	0.795	1.03	(0.78 - 1.35)	0.862	1.07	(0.63 - 1.82)	0.797
n6LC-PUFA 6m	0.60	(0.32 - 1.14)	0.117	0.42	(0.19 - 0.95)	**0.036**	0.57	(0.23 - 1.41)	0.226	0.44	(0.14 - 1.43)	0.171	0.99	(0.34 - 2.86)	0.984	1.11	(0.23 - 5.41)	0.896
n6 LC-PUFA 1.5y	0.65	(0.31 - 1.36)	0.248	0.65	(0.27 - 1.56)	0.337	0.34	(0.05 - 2.28)	0.269	0.44	(0.04 - 5.26)	0.515	0.81	(0.35 - 1.88)	0.616	0.60	(0.13 - 2.83)	0.518
n6:n3 6m	0.06	(0.00 - 0.97)	**0.048**	0.06	(0.00 - 1.68)	0.097	0.01	(0.00 - 0.95)	**0.048**	0.00	(0.00 - 2.00)	0.064	1.04	(0.78 - 1.38)	0.800	1.15	(0.74 - 1.178)	0.539
n6:n3 1.5y	1.05	(0.90 - 1.23)	0.526	1.07	(0.85 - 1.34)	0.574	1.58	(0.97 - 2.58)	0.067	1.84	(0.95 - 3.56)	0.072	0.96	(0.80 - 1.16)	0.691	1.15	(0.64 - 2.08)	0.636
LC-n6:n3 6m	0.45	(0.06 - 3.76)	0.463	0.58	(0.05 - 6.41)	0.657	0.61	(0.26 - 1.43)	0.253	0.63	(0.15 - 2.68)	0.529	127	(0.60 - 2.71)	0.530	5.71	(0.69 - 46.12)	0.106
LC-n6:n3 1.5y	0.95	(0.60 - 1.51)	0.827	0.82	(0.41 - 1.63)	0.568	1.57	(0.53 - 4.33)	0.379	0.73	(0.10 - 5.09)	0.746	0.81	(0.456 - 1.42)	0.455	0.78	(0.27 - 2.26)	0.651

Natural logarithms were used for non-normal variables. Associations were analyzed using †binary logistic regression analyses. OR^c, β^c and P^c= are corrected values after adjustment for maternal smoking, maternal education, maternal diet (ratio n6:n3, n3 PUFAs) and type of infant feeding practice. Significant differences with a p value <0.05 are highlighted in bold. LA: Linoleic Acid; AA: Arachidonic Acid; ALA: α-linolenic Acid; DHA: Docosahexaenoic Acid; SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids.



SUPPLEMENTAL FIGURE 1. **PARTICIPANTS IN PREOBE STUDY AND CLASSIFICATION FOLLOWING BMI AND GESTATIONAL DIABETES CRITERIA.**

6. DISCUSSION



DISCUSSION

The following section discusses the results obtained through our different analyses and appear in the order stablished in the Hypothesis & Aims chapter 3. Therefore, the first section discusses the analyses related to maternal weight and FADS and ELOVL SNPs on plasma FA levels during pregnancy; the second section comments on the effect of the maternal FADS SNPS and weight on breast milk PUFA composition; the third part discusses about the method developed to validate a less invasive sampling procedure for children FA analysis; and then the last discussion is about the study of infant FAs and cognition according to the maternal weight and feeding practice.

Association of maternal weight with FADS and ELOVL genetic variants and fatty acid levels- The PREOBE follow-up

The effect of FADS and ELOVL genetic variants on a broad FA profile was performed. To the best of our knowledge, this is the first study to explore associations between FADS and ELOVL SNPs, FA levels and maternal pre-pregnancy weight. Despite a lack of studies analyzing the association between obesity risk and FADS or ELOVL polymorphisms, some authors have observed that minor alleles of rs174547 (FADS1) confer a higher risk of obesity-related conditions, such as increased triglyceride levels and decreased high-density lipoprotein cholesterol concentrations, as well as an increased risk of coronary disease. In the present study, we found that women who carried at least one minor allele of the FADS1 and FADS2 SNPs, were associated with a higher risk of having a BMI>25 than homozygotes for the major allele. This association could explain why most of the overweight/obese women carried one minor allele copy and normal-weight women were mostly homozygous for the major alleles. Both weight groups showed very similar distributions within the allele groups of ELOVL SNPs; thus, we did not expect to find any associations between ELOVL genotypes and weight.

In line with other studies, we found that the FADS1 and FADS2 SNPs were associated with FAs, mainly from the n6 series, and less with those from the n3 series. The only significant association (p-value <0.004) was found in the normal-weight group, where minor alleles of FADS1 were associated with a lower AA:DGLA index. Several nominal associations were also found; nevertheless, they were mainly in normal-weight women and the n6 series. Regarding the overweight/obese group, minor allele carriers of rs2236212 and rs3798713 (ELOVL2) were nominally associated with a lower DHA:n3 docosapentaenoic acid (DPAn3) index. Barman et al. observed results similar to this last association, but their significance did not persist after correction either.

We also observed that FADS genetic variants affected FA concentrations. Normal-weight women who were minor allele carriers of FADS1 SNPs had significantly lower levels of product (AA) and indexes (AA:LA and AA:DGLA) than major homozygotes. Consistently with this, nominal differences were also observed; minor allele carriers of FADS1 SNPs showed nominally lower substrates (DGLA and ALA). This was previously reported by other authors, who observed that minor allele carriers in FADS displayed lower FA indexes and products, and increased amounts of substrates. Overweight/obese women with FADS1 SNPs showed the same trends and some nominal differences in DGLA and AA:DGLA, but

none of them with a p-value <0.004. Other studies have also found that FADS2 SNPs were related to lower levels of AA and lower AA:LA index. This supports our findings for the normal-weight group, where minor allele carriers of FADS2 SNPs had significantly lower levels of AA and higher DGLA. We also found nominal differences consistent with the previous results (lower AA:LA index and higher ALA and DPAn3). Similarly to other studies, the DGLA:LA index was nominally higher in minor allele carriers. This could be because the DGLA:LA index precedes the AA:LA index and, therefore, a lower AA:LA index would cause an accumulation of DGLA, thereby "increasing" the DGLA:LA index. Overweight/obese women who were minor allele carriers of FADS2 SNPs only showed nominally higher levels of DGLA than major homozygotes.

As shown previously, the FADS1 and FADS2 genetic variants were found to affect mainly the n6 FAs, even though desaturases and elongases work on both the n6 and n3 series. Several studies have observed that the DHA status, or that of the n3 series, is less influenced by genetic variants in the FADS genes. One possible explanation for this is that the final conversion step from DPA to DHA requires translocation to the peroxisomes (which is not performed in the endoplasmic reticulum where the other reactions occur), making DHA the least efficiently synthesized n3 LC-PUFA. Therefore, the influence of SNPs might not ultimately affect DHA levels. It has also been postulated that DHA supplementation during pregnancy could reduce dependence on endogenous DHA synthesis. However, previous studies have reported that increased dietary intake of EPA and DHA is linked to higher FADS1 and lower FADS2 activities, suggesting that endogenous LC-PUFA production remains active and is possibly enhanced, despite additional dietary intake of LC-PUFAs. Moreover, in our study, we observed no differences in dietary intake of EPA, DHA or AA between the two BMI groups; and although normal-weight pregnant women were more likely to take DHA supplementation, it was very unusual and this supplementation did not affect DHA level in plasma.

Our results suggest that BMI modifies genotype responses. According to FADS SNPs, n6 FAs showed the same effects in the 2 groups studied, but significant effects were only found in the group of normal-weight women. This suggests that overweight/obese women are less affected. This could explain why women in the normal-weight group had significantly higher n3 substrate (ALA) levels and nominally lower n6 product (AA) levels than overweight/obese women, only when we compared minor allele carriers of SNPs in FADS1 and FADS2. Additionally, among the minor allele carriers of FADS2, overweight/obese women showed significantly higher DHA:EPA index than the normal-weight group (the DHA:DPAn3 index showed the same trend). This suggests that minor alleles of FADS2 SNPS in overweight/obese women could have a positive impact on n3 LC-PUFA production. Furthermore, among major homozygotes, overweight/obese women also showed nominally higher DHA:EPA index than those in the normal-weight group. Perhaps, regardless of genetic variants in the FADS gene, a high BMI could be linked to increased activity of enzymes involved in n3 FA synthesis. Nevertheless, there were no differences in amounts of DHA between the weight groups, suggesting that even if enzymatic activity in n3 FA production is increased in overweight/obese individuals, it is not enough to elicit greater n3 LC-PUFA levels than in normal-weight subjects.

We analyzed 3 ELOVL2 SNPs (rs2236212, rs3798713 and rs953413) and found that their genetic variants only affected n3 FA levels in overweight/obese women. Overweight/obese women who were minor allele carriers of rs2236212 and rs3798713 had nominally lower DHA:DPAn3 index compared with

major homozygotes. This led to nominally higher amounts of the substrate DPAn3. Lemaitre et al. found decreased DHA levels in the presence of at least one minor allele of rs2236212, which is consistent with the tendency observed in our results. The normal-weight group generally showed the same trends. Moreover, among minor allele carriers of ELOVL2 SNPs, overweight/obese women showed significantly higher DHA:EPA index than normal-weight women (DPA:EPA and DHA:DPA indexes showed the same tendency). This is in accordance with FADS2 results. Therefore, minor alleles of FADS2 and ELOVL2 SNPs in overweight/obese women, could increase n3 LC-PUFA production indexes, but without surpassing levels in normal-weight women.

Estrogen facilitates LC-PUFA synthesis, probably by regulating delta-6 desaturase. During pregnancy, estrogen levels are higher, leading to increased amounts of DHA and AA until delivery, when the release of prolactin inhibits estrogen activity. Since estrogen is produced in adipocytes, obesity is linked to high estrogen levels which increase proportionally to total body adiposity. This could be an alternative explanation of the increase in n3 LC-PUFA production observed in overweight/obese individuals. In this case, more studies introducing measurements of estrogen in both populations are needed.

We also studied n3:n6 ratios (EPA:AA and DHA:AA), which are associated with obesity risk. Among minor allele carriers of the FADS and ELOVL genes, normal-weight women showed nominally higher plasma ratios of EPA:AA and DHA:AA than women who were overweight/obese. The same trend was observed when comparing major homozygotes, but with a weaker (or no) association. This suggests that a high BMI leads to increased levels of AA and/or lower levels of DHA and EPA, more importantly in women who carry at least one copy of the minor allele of FADS and ELOVL SNPs.

To the best of our knowledge, this is the first report to directly associate FADS SNPs with obesity risk and to analyze how weight affects the impact of variations in genes involved in FA metabolism. Our results further justify the need for personalized nutrition by showing that metabolism is affected by nutritional status and genes.

Obesity and FADS Polymorphisms Affect PUFAs in Breast Milk – The PREOBE Follow-up

The relationship between colostrum PUFA content and maternal weight and FADS genotype was analyzed. There are few studies analyzing breast milk PUFA content according to maternal weight. Although some authors have studied the influence of FADS SNPs on breast milk PUFA composition, our study is the first to demonstrate the effect of both maternal weight and FADS genotype on colostrum PUFA profiles.

To the best of our knowledge, only four studies have evaluated the FA composition of breast milk according to maternal BMI. Marin et al. compared the FA composition of mature breast milk (collected 1-3 months postpartum) from normal, overweight and obese mothers. Regarding PUFAs, they reported a significant increase in LA and total n6 PUFAs at a high BMI; while patterns of n3 PUFAs, including DHA, did not differ significantly between the three groups. A recent study found that overweight/obese women had a higher n6:n3 ratio and lower amounts of EPA, DHA and total n3 PUFAs in mature milk (collected at 3 months postpartum) than normal-weight women. Another study collected breast milk

between 4 and 10 weeks postpartum and compared its FA composition between normal-weight and obese mothers. They showed that obese women presented lower levels of PUFAn3, ALA, EPA, DPAn3, and DHA and a higher n6:n3 ratio than normal-weight women. By contrast, Storck Lindholm et al. comparing the colostrum (collected at 3 days postpartum) from normal-weight and obese mothers, revealed that the obese group showed lower LA, ALA, EPA, DHA, total n6 and n3 PUFA levels and higher DGLA and AA levels. Thus, existing results in the literature are not entirely consistent and the studies differ on the groups of weight tested and the timing of sample collection. Our colostrum samples were collected 2-4 days postpartum, similar to Storck Lindholm et al. (2013), but our results are not entirely alike. In accordance with the other study, we found that a high BMI was associated with high DGLA and AA levels and a low DHA:AA ratio; however, we also observed associations between a high BMI and high levels of AdA, DPAn6 and total n6 LC-PUFAs (see Table 2).

We also compared PUFA levels between normal-weight and overweight/obese women taking into account their genotype. Among the major homozygotes of FADS SNPs, normal-weight women had higher levels of EPA, DHA, EPA:AA and DHA:AA and a lower n6:n3 LC-PUFA ratio than overweight/obese women, which is consistent with the results of previous studies. However, these differences were not observed among the minor allele carriers (see Table 3).

Based on our results, the effects of the FADS genetic variants on PUFA levels differed in their extent depending on maternal weight. Previous studies have reported that minor alleles of the FADS SNPs are associated with lower enzymatic activity and, therefore, lower amounts of FA products and higher levels of substrates. Within our normal-weight population, FADS minor allele carriers had higher DGLA levels and lower DHA concentrations and AA:DGLA index compared to major allele carriers, which is consistent with the studies that have additionally reported lower EPA concentrations. We also observed that normal-weight minor allele carriers presented higher n6:n3 LC-PUFA ratio. The overweight/obese group did not show the same behavior; minor allele carriers presented higher levels of EPA, EPA:ALA and EPA:AA than major allele carriers (but not higher than normal-weight women). This could explain why normal-weight women did not have higher EPA levels than overweight/obese women amongst the minor allele carriers (see Table 3).

We also tried to confirm the impact of FADS genetic variation by testing the associations between PUFAs and minor alleles of the FADS SNPs (Table 4). We used linear regression analysis that was adjusted for several potential confounders. Although we found few significant associations, all the results followed the same trend. The significant results that persisted in the normal-weight population were that the minor alleles were positively associated with the DGLA concentration and n6:n3 LC-PUFA ratio and negatively associated with the DHA:AA ratio. In overweight women, the minor alleles of FADS SNPs were positively associated with EPA levels, EPA:ALA index and EPA:AA ratio and negatively associated with the DHA:EPA index. Perhaps, this last negative association (FADS minor alleles with DHA:EPA) is a direct cause of the positive association with EPA, which is the substrate of the enzymatic reaction. When the enzymatic reactions are affected by genotype, the substrates can be accumulated in comparison with the products of the reactions. Thus, overweight/obese women with FADS minor alleles would accumulate EPA.

Our results suggest that FADS polymorphisms might have a positive effect in overweight and obese lactating women by regulating the PUFA composition of breast milk. However, estrogen could also influence these results. Estrogen is a hormone that facilitates LC-PUFA synthesis (probably by regulating delta-6 desaturase) and increases during a regular pregnancy and also in obese individuals. Thus, this could be an alternative explanation for the increased LC-PUFA production observed in the overweight/obese group. In this case, more studies are needed to assess the effect of estrogen in both populations.

Our analyses also confirmed that the DHA content of breast milk was affected by maternal diet during the third trimester of pregnancy. As mentioned before, we found reduced DHA concentrations in two different situations: (1) normal-weight women carrying FADS minor alleles showed lower DHA levels than those with two major alleles; and (2) overweight/obese women had lower DHA amounts than normal-weight participants among the FADS major allele carriers. It would be interesting to follow this line of research as it could develop into a clinical recommendation for these subgroups of women (FADS minor allele carriers and obese women) to optimize the colostrum DHA concentration and improve the supply of this crucial FA to the neonate.

To our knowledge, this is the first study to analyze breast milk PUFA composition according to maternal weight and FADS genotype. Our study supports the personalization of nutrition given that breast milk PUFA profiles are affected by maternal nutritional status and genes.

Evaluation of Less invasive methods to assess fatty acids from phospholipid fraction: cheek cell and capillary blood sampling

As plasma sampling status is not well accepted to assess FA, especially when dealing with newborn or child populations, it has become increasingly important to study alternative matrices. Cheek cells and capillary blood are the most commonly considered alternatives, but to our knowledge there are still no studies that have evaluated and compared the results obtained for these two different approaches with those of traditional plasma sampling.

In preliminary studies on cheek cell samples, we tested different devices commonly used in other studies for their extraction, including sterile cotton swabs a wooden handle, sterile cotton swabs a plastic handle, sterile gauzes, a circular-shaped Gynobrush and a Rovers EndoCervex- Brush. First, the wooden spatula was rejected because of the unpleasant sensation it caused in comparison with the other options, and the sterile cotton swabs with a wooden handle and the sterile gauzes were ruled out because of high levels of contamination that interfered with the analyzes (data not shown). The sterile cotton swabs with a plastic handle were also found to be inadequate because the plastic was not resistant to the solvent used for lipid extraction. The circular-shaped Gynobrush was also dismissed because it had a wire and nylon bristle combination that could potentially hurt the patient if not used carefully. However, the Rovers EndoCervex-Brush was found to be a perfect sampling device for several reasons: it was made entirely of flexible, soft polypropylene, and it consisted of a brush head that could be detached from the handle after collection, making it easy to store in a small tube. Additionally, the use of a brush for scraping was selected in preference to the cotton swab with a wooden handle because it has been shown that the average cell yield obtained with a cotton swab is approximately two-thirds of that collected with an

endocervical brush. Consequently, the use of a brush might be crucial to collect an adequate amount of cells in young children for a reliable analysis of FAs with this method.

Proper collection of cheek cells requires a strict sampling procedure. It has been proved that rinsing the mouth after scraping the inner cheeks and collecting this residue, leads to significantly higher cell yields. However, the rinsing residue may be discarded because it is not applicable when collecting cells in babies and we observed that the differences were negligible. It has also been demonstrated that cleaning the mouth prior to sampling reduces possible food residues, however this does not apply in babies with no solid food intake.

It is known that cheek cells are robust and hard to disrupt by isolated applications of detergents, ultrasound or polar solvents. Methanol and ultrasound were used in this study to disrupt cell membranes, as Klingler et al. (2011) have shown that this method has a recovery rate of >90%.

Capillary blood is another suitable matrix for assessing FA status instead of plasma, and represents a less invasive sampling technique. Collection only requires punching one finger to obtain one drop of blood. Nevertheless, capillary blood sampling for children is less recommended than cheek cells because extraction is more aggressive. Moreover, capillary blood requires the addition of BHT to prevent oxidation of the sample absorbed on the chromatographic paper, and this could lead to contamination issues.

The identification of false-positive peaks is important for accurate qualification of FAs: not only does this define the lower limit of the method, but also excludes FAs from analysis. The systematic analysis of blanks may yield information about contamination derived from chemicals or materials. In the present study, four impurities were found to co-elute with the peaks of C16:0, C18:0, C20:0 and C22:0 and their presence was attributed to SPE well plate contamination. These data were taken into account in calculations, and C16:0 and C18:0 peak areas from blanks were subtracted from samples. Meanwhile, due to their negligible contribution to the global profile, since they were minority peaks, C20:0 and C22:0 were excluded from the study. Blanks were carried out to evaluate contamination from the Rovers EndoCervex-Brushand the chromatographic paper from the blood collection kit and peaks from contaminations did not co-elute with any FA peak.

Data on the precision of FA analysis have mainly been published for plasma, and only to a lesser extent for cheek cells and capillary blood. The CV reported for FA determination in cheek cells has ranged from 1.0% to 10.5%, and in the present study, the CV for cheek cell and capillary blood samples was lower than 10%. The recovery assessment results were considered satisfactory, as described by other authors, and SPE represents a good approach in phospholipid FA analysis.

The levels of FAs found in all matrices differed considerably, but can only be compared with those found in other studies which evaluated the same FAs in order to conduct equivalent comparisons. Since the data presented by other authors are limited exclusively to PUFAs and exclude or summarize saturated and monounsaturated FAs, only the same FAs were considered in a comparison of results. Reported PUFA levels were comparable in all studies, whereas saturated acids differed, possibly because of dietary habits

or contamination. The n6:n3 ratios found in our study (11:1 for cheek cell samples, 10:1 for capillary blood samples and 7:1 for plasma samples) were considerably higher than the ratio of 1-2:1 reported by some authors. As is well known, excessive amounts of n6 and a very high n6: n3 ratio can be caused by an imbalanced diet and they promote the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases, whereas increased levels of n3 (a low n6: n3 ratio) exert suppressive effects.

In accordance with previous studies, the dominant unsaturated FAs observed in this study were the oleic, linoleic and arachidonic acids for all 3 matrices. In addition, we observed differences in AA and DHA between cheek cell, capillary blood and plasma samples. This has also been reported in previous studies, where plasma levels of these FAs were approximately three times higher.

A study of correlations between the three different matrices was useful to determine the suitability of capillary blood and cheek cells as alternatives to plasma for assessing FA status in infants. We focused on the determination of LC- PUFAs because several authors have recently found that an understanding of their metabolic synthesis routes is a key factor in the study of several diseases. As noted by other authors, we also observed good correlation coefficients for FAs in cheek cell and capillary blood samples in relation to plasma, especially for LC-PUFAs such as EPA and DHA. Therefore, in line with other studies, cheek cell and capillary blood samples reflected LC-PUFA status as well as plasma does, hence functioning as alternatives for LC-PUFA status evaluation in children.

Moreover, further studies should be carried out to improve the lipid separation step in order to avoid contamination with saturated FAs from the SPE well plate. The use of a 96-well plate for phospholipid separation represents a marked improvement over the use of cartridges since it enables a much larger quantity of samples to be processed simultaneously. The SPE 96-well plate method is simpler and less time consuming than traditional methods, such as TLC.

Impact of maternal obesity on fatty acids and cognition in early life - The PREOBE Follow-up

We analyzed the FA composition of infant cheek cells according to maternal pre-pregnancy BMI, and their relationship with the type of feeding practice and cognitive performance during the first 3 years of life. To our knowledge, there are no studies analyzing the effect of maternal weight on infant FA status during the first years of life, and this is one of the few studies that uses a biological sample that is easy to extract and reflects the dietary FA content. The PUFAs, particularly LC-PUFAs (AA, DHA), play an important and beneficial physiologic role in the offspring who especially requires these FAs for critical periods of development (first 1000 days of life). Therefore, the maternal nutritional status and FA dietary intake during pregnancy and breastfeeding are critical factors that are strongly associated with normal fetal and postnatal development. Alterations in this regard could result in modifications of fetal programming and in increased risk for developing diseases later in life. Considering the physiological importance of FAs, this study was emphasized in the analysis of essential PUFAs (LA, ALA), LC-PUFAs (AA, EPA, DHA) and the sum of individual FAs (SFAs, MUFAs, MCFA, n6 and n3 PUFAs, n6 and n3 LC-PUFAs, n6:n3 and LC-n6:n3 ratios).

When we analyzed the infant evolution of FA concentrations, we observed that, especially, the FA in the n3 series, such as DHA, n3 PUFAs and n3 LC-PUFAs decreased towards the 3 years of life, which would be explained by the infant high demand for an adequate development of the central nervous system, and these results were confirmed when we stratified by type of infant feeding practice. At 6 months of age, the infants of mothers (not considering maternal BMI) who were exclusively breastfed had higher levels of DHA than those partially breastfed; this effect disappeared when the child reached 1.5 years.

Contrary to the age related drop of n3 FAs, we found that the SFAs, n6:n3 and LC-n6:n3 ratios were increased by 3 years of life. Evidently, this could be related to the quality and increase of complementary feeding. Different studies suggest that higher n6:n3 ratios have been associated with adverse effects in the child, especially with an increased risk of obesity. High dietary intake of n6 FAs leads to increased n6 FA levels in plasma, red cells and white adipose tissue, and chronic inflammation, which are all hallmarks of obesity. On the other hand, it is known that the increased maternal BMI increases the incidence of labor induction, caesarean section, preterm labor and macrosomia. Our results show that even in postnatal life, infants born to obese woman (despite having an adequate weight gain during pregnancy) may be affected by the pre-pregnancy weight of the mother, by showing significant increases in some FA of the n6 family. The major FA of the n6 series is LA, and, at 6 months of life, infants of obese mothers had higher LA levels compared to those of normal-weight mothers, and higher PUFAs and LC-n:6:n3 ratio compared to infants of overweight mothers.

Furthermore, we analyze the effect of feeding practices on infant FAs during their first 1.5 years of life. Exclusively breastfed infants had higher levels of LA, DHA, PUFAs, n6 PUFAs and n6:n3n and lower level of ALA at 6 months compared to those who received partial breastfeeding. Since the ALA is an essential FA, the decreased level in the infant suggests a lack this FA in breast milk as a consequence of poor maternal dietary intake. At 1.5 years of life, infants fed with formula showed higher n6:n3 ratio than infants who were exclusively breastfed. These results were independent of the maternal pre-pregnancy BM and confirm that exclusive breastfeeding is the best option for the infants, since they require adequate amounts of crucial FAs to protect them from diseases later in life. When infants were separated by maternal pre-pregnancy BMI, similar results were found for infants of overweight and, especially, normal-weight mothers (these infants also showed lower SFAs after an exclusive breastfeeding). Meanwhile, infants of obese mothers showed no impact by type of feeding practice received. One possible explanation for this is that the breast milk quality of obese mothers could be compromised by their nutritional status and diet, and consequently having a similar FA content than formulas. These results are evidence that essential FAs, and consequently their products, are the influenced by diet, so exclusively breastfed infants had higher levels of PUFAs, especially in infants born to normal-weight women.

Finally, the risk of having a low cognitive score was analyzed at 6 months and 1.5 years of life in spite of the maternal BMI. At 6 months of age, higher levels of DHA, AA and n6 PUFAs were associated with higher cognitions scores. Additionally, our results showed that when the infants reached the age of 1.5 years, the protective effect of n3 FAs was continued, but the protective effect of n6 FAs was lost, and we even found a negative association between n6:n3 ratio and cognitive scores. These results are in accordance with observational, cross-sectional and longitudinal, studies in healthy children, which suggest that there might be a positive relationship between n3 FA and cognitive outcomes. Kirby et al., investigated the association between children's learning and behavior and cheek cell PUFA levels and

found that higher n3 FA levels were associated with decreased levels of inattention, hyperactivity, emotional and conduct difficulties and increased levels of prosocial behavior. When analyzing the infants according to normal-weight and overweight/obese mothers, we found that infants born to normal-weight women had a positive association between n6:n3 ratio and cognition scores at 6 months of age; and when infants turned 1.5 years old we found that n3 PUFAs were positively associated to cognition in infants of both normal-weight and obese women. Infants of obese women had a higher significance in this result, which suggests that they could benefit the most from a high n3 FA dietary intake. These results indicate the importance of a healthy diet for the infant, since it influences cognitive development and could have consequences in adolescence and adulthood.

There are some limitations in the present study. The most important is not having the information on the infant diet, however the sample used allowed us to indirectly measure the FA intake of the children since it has been demonstrated that circulating FAs reflect their dietary intake. Moreover, we were able to perform multivariate analyses to establish the relationship between the increase of maternal pre-pregnancy BMI and infant FA levels up to 3 years of age, and the association between infant cognitive performance and FA levels. It is important to emphasize that infant analyses independently of maternal pre-pregnancy BMI, showed more and higher significances compared to the analyses performed to infants classified in maternal groups of weight. One possible explanation for this could be the relatively small sample size within each group.

Limitations

Our results had some limitations: it might be limited by the relatively small sample size; however, we could identify and group participants into different weight and/or genotype categories. Information on dietary intake was obtained from validated food records and questionnaires and although each participant was guided by a nutritionist, this information could have been affected by recall bias. Likewise, supplementation data (brand, content, doses and frequency) during pregnancy were also obtained from questionnaires answered by the participants; therefore, this information might not be completely accurate. Lack of information on family lifestyle and infant dietary habits, limited our analyses as well.

7. CONCLUSIONS

CONCLUSIONS

Our results prove our hypothesis that a high maternal pre-pregnancy BMI could influence FA status of the mother and child and therefore impact growth and development of the latter. The results obtained through this thesis are novel scientific evidence and can be concluded in the following statements:

In general,

- There are many different factors that determine and individual's health status. These studies highlight the importance of a healthy pre-pregnancy weight, and identify groups of women who could benefit from a high intake of n3 FAs and controlled n6:n3 ratio to achieve an improved FA status that fulfills fetal/neonatal requirements.

Specifically,

- Maternal pre-pregnancy BMI may alter FA status in maternal plasma and colostrum, according to *FADS* and *ELOVL* genetic variants.
    - Pregnant women carrying at least 1 copy of the minor allele of *FADS1* and *FADS2* SNPs have an increased risk of obesity.
    - Compared to major homozygotes, normal-weight women carrying at least 1 minor allele of *FADS* and *ELOVL* SNPs showed reduced enzymatic activity.
    - However, in overweight/obese women, *FADS* and *ELOVL* genetic variants had a different impact, possibly improving (but not better than normal-weight women) their FA status as a physiological compensatory mechanism.
    - Alterations in maternal plasma and colostrum FAs can influence the risk of pro-inflammatory diseases and affect the nutritional supply to the fetus and neonate, which in turn would influence the child's growth and development.
    - Dietary intake of DHA in late pregnancy associated with DHA concentrations in colostrum.
    - A high intake of DHA may prevent the reduction of colostrum DHA caused by overweight or *FADS* minor alleles.

- Cheek cell and capillary blood samples are less invasive and reliable methods for FA analysis from phospholipid fraction, especially LC-PUFAs. These two methods can therefore be used as alternatives to traditional plasma sampling for analysis of these FAs.
    - Cheek cell and capillary blood sampling do not require the involvement of healthcare staff in sample collection and this markedly simplifies the analytical procedure.

- Maternal pre-pregnancy BMI altered the infant FA behavior in evolution, feeding practice and cognition.
    - A high maternal pre-pregnancy weight caused an increase of n6 FAs in infants up to 2 years of age. Infants of obese mothers had the lowest changes in FA evolution compared to infants of overweight and, especially, normal-weight mothers. In general, FA concentrations decreased towards the 3 years of life, except for the SFAs, n6:n3 and LC-n6:n3 ratios which were increased.
    - Exclusive breastfeeding seems to increase crucial FAs (e.g. DHA) in infants at 6 months of age, which is a critical period for development. With increasing maternal BMI, the influence of feeding practice on infant FA status reduced. This suggests that breast milk quality of obese mothers could be compromised by their nutritional status and diet, and consequently breast milk FA content could be similar than formulas.
    - In general, cognitive performance was improved in infants with high levels of PUFAs (e.g. DHA, AA) at 6 months of age, with the positive effect of the n3 FAs still present at 1.5 years of life.

8. FUTURE RESEARCH AND RECOMMENDATIONS

FUTURE RESEARCH AND RECOMMENDATIONS

Future Research

This thesis invites to continue research on early life programming to pursue for healthier future generations.

- Research with a larger population could be useful to confirm our results.
- Since genotypes may not have the same effects on all people, it is of interest to continue exploring gene-BMI interactions to pursue personalized health-related recommendations.
- After pregnancy follow-up of the mothers with different pregnancy conditions could assess future health risks.
- Analyzing the family lifestyle would be interesting since it is another modifiable factor for obesity and FA status alterations.
- Including data of the fathers would help to make a more accurate analysis of the children's outcomes.
- Information on children's diet and lifestyle would help to adjust the analysis of their growth and development.
- It would be interesting to follow-up these children into adolescence and adulthood to analyze their health status according to their mothers' pregnancy conditions.

Recommendations

Since maternal obesity is known to propagate obesity through generations, an interruption of the obesity cycle could help to lessen the increasing incidence of obesity and its related comorbidities. We therefore should promote a healthy weight and diet in women before, during and after pregnancy to have beneficial effects on child outcome and consequently prevent some nutrition-related issues through their life.

"Get better, look better, feel better, and you will have a chance to have a healthy pregnancy."

9. BIBLIOGRAPHY

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10. APPENDIX

10.1. CONGRESS COMMUNICATIONS

COMMUNICATION #1

MATERNAL BMI AND *FADS* POLYMORPHISMS AFFECT PUFAS IN BREAST MILK – THE PREOBE FOLLOW UP

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Presentation Method: Poster and Oral presentation
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Rationale: The aim of this study was to analyze the differences of breast milk polyunsaturated fatty acids (PUFAs) according to maternal pre-pregnancy body mass index (BMI) and fatty acid desaturase (*FADS*) genotype.

Methods: Women (n=61) from the PREOBE cohort were divided in normal-weight (BMI:18.5-24.9) and overweight/obese (BMI≥25) groups. Colostrum was collected 2-4 days postpartum and used to analyze PUFAs that were expressed as percentages of the total amount of FAs. Tag SNPs were genotyped (3 in *FADS1* and 3 in *FADS2*).

Results: Women with high BMI were associated with LC-PUFAn6 (β=0.70, P=0.003) and DHA:AA (β=-0.55, P=0.016). Among women who carried two major alleles, normal-weight group had higher concentrations of EPA (0.05 vs. 0.03%), DHA (0.46 vs. 0.33%), EPA:AA (0.08 vs. 0.05%) and DHA:AA (0.73 vs. 0.50%) and lower LC-PUFA n6:n3 (3.29 vs. 4.83%) than overweight/obese women. These differences were lost in minor allele carriers since PUFAs responded differently in BMI groups. Normal-weight women who carried minor alleles showed a tendency to decrease LC-PUFAs; they had lower levels of DHA (0.46 vs. 0.33%) and AA:DGLA (1.24 vs. 0.97%) than major homozygotes. Contrary, overweight/obese women who were minor allele carriers had higher EPA (0.03 vs. 0.05%), EPA:ALA (0.07 vs. 0.11%) and EPA:AA (0.05 vs. 0.08%) than major homozygotes (P=0.05). Dietary intake of DHA in late pregnancy correlated (R=0.32, P=0.035) with DHA level in colostrum.

Conclusion: In conclusion, women with high BMI were associated with altered FAs; however, *FADS* genotype modified these results. Contrary to normal-weight group, overweight and obese women could benefit from the *FADS* genetic variants measured in this study. A high dietary DHA intake could prevent the reduction of DHA in colostrum caused by overweight/obesity or the presence of *FADS* minor alleles in normal-weight women. Further research is needed to continue exploring gene-BMI interaction.

Keywords: Fatty acids, colostrum.
Funding: PREOBE Excellence Project Ref. P06-CTS-02341. BFU2012-40254-C03-01.

COMMUNICATION #2

MATERNAL BODY MASS INDEX ALTERS BREAST MILK FATTY ACID COMPOSITION– THE PREOBE FOLLOW UP

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Presentation Method: Poster and Oral presentation
Congress: 39th ESPEN Congress on Clinical Nutrition & Metabolism
Place and Date: The Netherlands, 2017
*Graded as a high-ranking abstract.
***Published in:**
Journal: Clinical Nutrition, Supplement.
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ISSN: 17441161
DOI: Pending

Rationale: Breast milk fatty acid (FA) composition plays an important role in children's growth and development, but there is limited information about it corresponding to maternal nutritional status. The aim of this study was to analyze the differences of mature breast milk FA composition in lactating women of different pre-pregnancy body mass index (BMI).

Methods: Women (n=65) from a population-based pregnancy cohort of the PREOBE Project were divided in 3 different groups according to pre-pregnancy BMI; normal-weight (BMI: 18.5-24.9), overweight (BMI: 25-29.9) and obese (BMI>30). Collection of mature breast milk was performed 28-32 days postpartum and samples were used to analyze FAs that were expressed as percentages of the total amount of FAs.

Results: FA levels in breast milk differed according to maternal weight. Compared to normal-weight women, the obese group displayed higher levels of total saturated FAs (27.80 vs. 25.83%), C22:2n-6 (0.06 vs. 0.04%), C22:5n-6 (0.09 vs. 0.05%) and lower C18:1n-9 (36.63 vs. 39.69%), total monounsaturated FAs (40.74 vs. 43.73%) and C18:3n-3 (ALA) (0.46 vs. 0.59%). Whereas overweight women showed higher levels of C22:5n-6 (0.07 vs. 0.05 %) and n6:n3 ratio (18.28 vs. 15.08%) and lower C22:6n-3 (DHA) (0.22 vs. 0.28 %) and EPA:AA ratio (0.09 vs. 0.12%) than normal-weight group. Significance level was established at a *P* value of 0.05.

Conclusion: In conclusion, maternal weight affects FA concentrations in mature breast milk. Our results suggest that the quality of breast milk is compromised in women with a BMI>25 which could also affect the quality of nutrients supplied to the neonate. Since diet influences breast milk FAs, overweight and obese women could benefit from dietary recommendations to optimize breast milk FA composition.

Keywords: Mature milk, BMI.
Funding: *PREOBE Excellence Project Ref. P06-CTS-02341. BFU2012-40254-C03-01.*

COMMUNICATION #3

MATERNAL WEIGHT AND GENETIC VARIANTS OF THE FADS AND ELOVL GENES ON CHILDREN'S FATTY ACIDS AND COGNITION

de la Garza A.[1,2], de Almeida L.[1], Chisaguano A.M.[3], Montes R.[4,5], Bonilla M.[1], Dinarès M.[1], Guerendiain M.E.[6], Salas I.[1], Castellote A.I.[1,2,5], Torres-Espínola F.J.[7], Arias García M.[7], Segura Moreno M.[7], Campoy C.[7,8], López-Sabater M.C.[1,2,5].

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Presentation Method: Poster presentation
Congress: INSA Workshop Annual "Cacao y Chocolate: Ciencia y Gastronomía"
Place and Date: Barcelona, 2016.

Background and objectives: Maternal polymorphisms (SNPs) in fatty acid desaturase (FADS) and elongase (ELOVL) genes alter the long chain (LC) polyunsaturated fatty acid (PUFA) availability compromising fetus supply and therefore cognitive development. We aimed to determine how maternal polymorphisms in FADS and ELOVL genes influence children's fatty acids (FAs) and cognition according to maternal weight.

Methodology: Children (n=72) from the PREOBE cohort were divided according to maternal pre-pregnancy BMI: group 1 (normoweight mothers, n=31) and group 2 (overweight/obese mothers, n=41). Maternal SNPs were genotyped (7 in FADS1, 5 in FADS2, 3 in ELOVL2 and 2 in ELOVL5). At 18 months old, children's cheek cells were analyzed to measure PUFAs in the phospholipid fraction and cognition was assessed using the Bayley III Cognitive Scale.

Results and conclusions: Major homozygotes in group 1 had higher AA:DGLA index for rs174537 (FADS1) and higher cognition for rs174545 (FADS1) than minor allele carriers. Both tendencies persisted in all SNPs in FADS1 while cognition tendency also persisted in FADS2. Group 2 showed that major homozygotes had higher cognition for rs2397142 (ELOVL5). Regarding rs2397142, group 2 showed higher cognition when mothers carrying minor alleles had high DHA intake, plasma EPA:AA and DHA:AA ratios instead of low. Maternal weight, genotype and FAs influence children's outcome. FADS1 SNPs in normoweight mothers decreased children's cognition and enzymatic activity in FA metabolism. Children's cognition was also lowered by ELOVL5 SNPs in obese, but not normoweight, mothers with low n-3 FA levels. A high n-3 FA intake should be promoted, especially in obese pregnancies, to enhance cognition in children.

Maternal Weight and Genetic Variants of the *FADS* And *ELOVL* Genes on Children's Fatty Acids & Cognition

A. de la Garza[1,2], L. de Almeida[1], A.M. Chisaguano[3], R. Montes[4,5], M. Bonilla[1], M. Dinarès[1], M.E. Guerendiain[6], I. Salas[1], A.I. Castellote[1,2,5], F.J. Torres-Espínola[7], M. Arias García[7], M. Segura Moreno[7], C. Campoy[7,8], M.C. López-Sabater[1,2,5].

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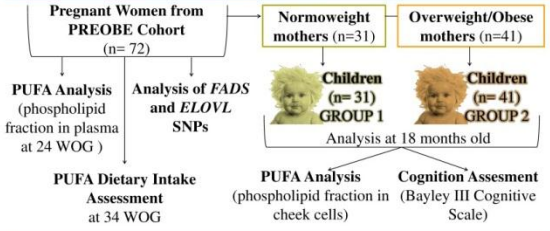
BACKGROUND

Maternal polymorphisms (**SNPs**) in fatty acid desaturase (***FADS***) and elongase (***ELOVL***) genes alter long chain (LC) polyunsaturated fatty acid (**PUFA**) availability. This compromises fetus supply of fatty acids (**FAs**) that are crucial for cognitive development.

AIM

Determine how **Maternal *FADS* and *ELOVL* SNPs influence Children's FA and Cognition** according to maternal weight.

METHODS

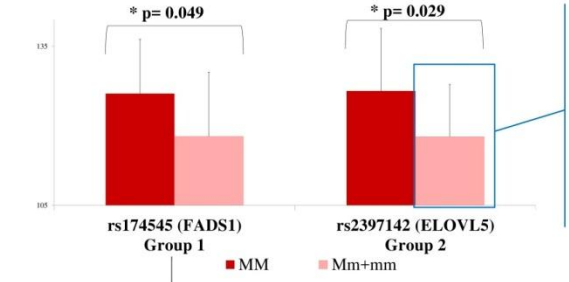


RESULTS IN CHILDREN

Group 1 had lower AA:DGLA index for rs174537 (*FADS1*) - tendency persisted in both groups and all studied SNPs in *FADS1*

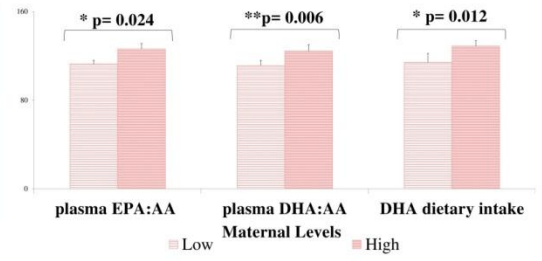
Child FA Index	Gene	SNP	Major /minor allele	GROUP 1 MM N	GROUP 1 MM Mean (sd)	GROUP 1 Mm+mm N	GROUP 1 Mm+mm Mean (sd)	P	GROUP 2 MM N	GROUP 2 MM Mean (sd)	GROUP 2 Mm+mm N	GROUP 2 Mm+mm Mean (sd)	P
AA:DGLA													
	FADS1	**rs174537**	*G/T*	12	2.80 (0.58)	14	2.32 (0.51)	**0.033***	12	2.62 (0.47)	22	2.40 (0.69)	0.325
	FADS1	**rs174545**	*C/G*	14	2.72 (0.58)	18	2.50 (0.83)	0.417	14	2.60 (0.44)	26	2.46 (0.71)	0.494
	FADS1	**rs174546**	*C/T*	14	2.72 (0.58)	18	2.50 (0.83)	0.417	14	2.60 (0.44)	27	2.47 (0.70)	0.528
	FADS1	**rs174548**	*C/G*	17	2.64 (0.52)	15	2.55 (0.92)	0.713	16	2.66 (0.43)	25	2.43 (0.71)	0.260
	FADS1	**rs174553**	*A/G*	14	2.72 (0.50)	18	2.50 (0.83)	0.417	14	2.60 (0.44)	27	2.47 (0.70)	0.528
	FADS1	**rs174547**	*T/C*	13	2.63 (0.50)	15	2.31 (0.53)	0.112	12	2.62 (0.47)	18	2.42 (0.68)	0.362

Lower cognition in children from mothers carrying minor alleles



This tendency persisted in all studied ***FADS1*** and ***FADS2*** SNPs

Cognition further impaired by maternal low levels of n-3 FAs



Maternal Genotype: Major allele (M) and minor allele (m).
Data are means from indexes of FA percentages and scores in Bayle III Cognitive Scale.
Significant differences are shown (*p< 0.05, **p<0.01) according to global ANOVA.
AA: Arachidonic acid; DGLA: Eicosatrienoic acid; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid.

CONCLUSIONS

Maternal weight, **genotype** and **FAs** influence **children's outcome**. *FADS1* SNPs in normoweight mothers decreased children's cognition and enzymatic activity in FA metabolism. Children's cognition was also lowered by *ELOVL5* SNPs in obese mothers, and it was further impaired if mothers had low n-3 FA levels. A high n-3 FA intake should be promoted, especially in obese pregnancies, to enhance cognition in children.

FUNDING

Authors thank the Spanish Ministry of Economy and Competitiveness (project BFU2012-40254-C03-02) and the CIBER-Obn for the financial support for this study. ADLG thanks the Nuevo León, México government and National Council on Science and Technology (CONACYT) for PhD grant.

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COMMUNICATION #4

IMPACT OF MATERNAL GENETIC VARIANTS OF THE FADS AND ELOVL GENE CLUSTERS ON CHILD PUFA LEVELS AND COGNITION: ALTERED BY MATERNAL PRE-PREGNANCY BMI?

A. de la Garza[1], L. de Almeida[1], A.M. Chisaguano[2], R. Montes[3,4], M. Bonilla[1], M. Dinarès[1], M.E. Guerendiain[5], I. Salas[1], A.I. Castellote[1,4], F.J. Torres-Espínola[6], M. Arias García[6], M. Segura Moreno[6], C. Campoy[6,7], M.C. López-Sabater[1,4].

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Presentation Method: Poster and Oral presentation
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***Published in** (See Appendix 10.2.3)**::**
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ISSN: 03406199, 14321076
DOI: 10.1007/s00431-016-2785-8

Background and aims: Maternal polymorphisms (SNPs) in fatty acid desaturase (FADS) and elongase (ELOVL) enzymes alter the long chain (LC) polyunsaturated fatty acid (PUFA) availability compromising fetus supply and cognitive development. We aimed to determine how maternal polymorphisms in FADS and ELOVL genes influence children's fatty acids (FAs), cognition and if maternal weight changes this.

Methods: Children (n=72) from the PREOBE cohort were divided in group 1 (maternal pre-pregnancy BMI 18.5-24.99, n=31), group 2 (BMI≥25, n=41) and ultimately in subgroups of maternal SNPs (7 in the FADS1 cluster, 5 in FADS2, 3 in ELOVL2 and 2 in ELOVL5). At 18 months old, children's cheek cells were analyzed to measure PUFAs in the phospholipid fraction and cognition was assessed using the Bayley III Cognitive Scale.

Results: Major homozygotes in group 1 had higher AA:DGLA index for rs174537 (FADS1) and higher cognition for rs174545 (FADS1) than minor allele carriers. Both tendencies persisted in all SNPs in FADS1 while cognition tendency also persisted in FADS2. Group 2 showed that major homozygotes had higher cognition for rs2397142 (ELOVL5). Regarding rs2397142, group 2 showed higher cognition when mothers carrying minor alleles had high DHA intake, plasma EPA:AA and DHA:AA ratios instead of low.

Conclusions: Decreased enzyme activity and cognition by genetic variants in FADS and ELOVL genes isn't changed by maternal pre-pregnancy BMI, but having high BMI disturbs children's cognition if the mother has genetic variants and low dietary intake of n-3 FAs. Obese pregnant women, especially minor allele carriers, should consider a n-3 FAs rich diet to enhance child cognition.

Impact of Maternal Genetic Variants of the *FADS* And *ELOVL* Genes on Child PUFA Levels & Cognition: Altered by Maternal Pre-pregnancy BMI?

A. de la Garza[1,2], L. de Almeida[1], A.M. Chisaguano[3], R. Montes[4,5], M. Bonilla[1], M. Dinarès[1], M.E. Guerendiain[6], I. Salas[1], A.I. Castellote[1,2,5], F.J. Torres-Espínola[7], M. Arias García[7], M. Segura Moreno[7], C. Campoy[7,8], M.C. López-Sabater[1,2,5].

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EAPS-0770

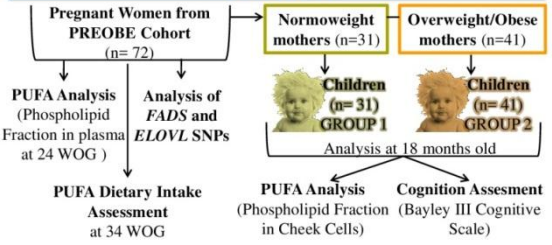
BACKGROUND

Maternal polymorphisms (**SNPs**) in fatty acid desaturase (***FADS***) and elongase (***ELOVL***) genes alter long chain (LC) polyunsaturated fatty acid (**PUFA**) availability. This compromises fetus supply of fatty acids (**FAs**) that are crucial for cognitive development.

AIM

Determine how **Maternal *FADS* and *ELOVL* SNPs influence Children's FA and Cognition** and if maternal weight changes this.

METHODS

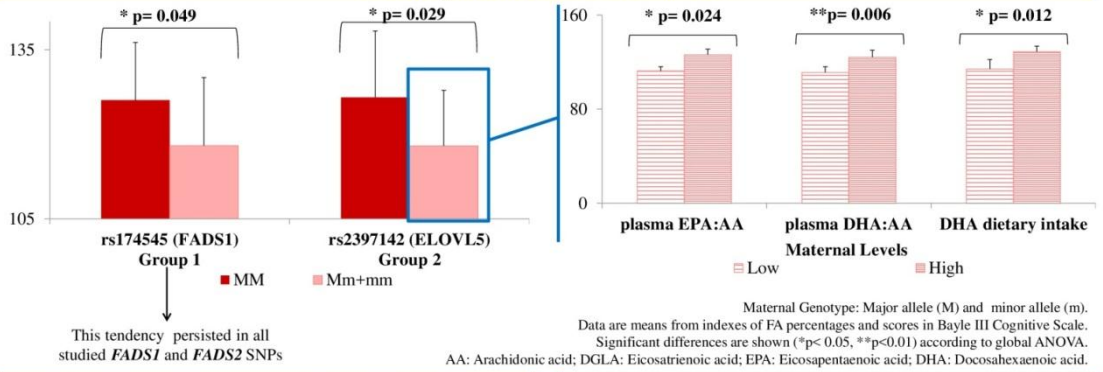


RESULTS IN CHILDREN WITH MOTHERS CARRYING MINOR ALLELES

Lower AA:DGLA index for rs174537 (***FADS1***). Tendency persisted in all studied SNPs in FADS1.

Child FA Index	Gene	SNP	Major /minor allele	GROUP 1 MM N	GROUP 1 MM Mean (sd)	GROUP 1 Mm+mm N	GROUP 1 Mm+mm Mean (sd)	GROUP 1 P	GROUP 2 MM N	GROUP 2 MM Mean (sd)	GROUP 2 Mm+mm N	GROUP 2 Mm+mm Mean (sd)	GROUP 2 P
AA:DGLA													
	FADS1	**rs174537**	*G/T*	12	2.80 (0.58)	14	↓2.32 (0.51)	**0.033***	12	2.62 (0.47)	22	2.40 (0.69)	0.325
	FADS1	**rs174545**	*C/G*	14	2.72 (0.58)	18	2.50 (0.83)	0.417	14	2.60 (0.44)	26	2.46 (0.71)	0.494
	FADS1	**rs174546**	*C/T*	14	2.72 (0.58)	18	2.50 (0.83)	0.417	14	2.60 (0.44)	27	2.47 (0.70)	0.528
	FADS1	**rs174548**	*C/G*	17	2.64 (0.52)	15	2.55 (0.92)	0.713	16	2.66 (0.43)	25	2.43 (0.71)	0.260
	FADS1	**rs174553**	*A/G*	14	2.72 (0.50)	18	2.50 (0.83)	0.417	14	2.60 (0.44)	27	2.47 (0.70)	0.528
	FADS1	**rs174547**	*T/C*	13	2.63 (0.50)	15	2.31 (0.53)	0.112	12	2.62 (0.47)	18	2.42 (0.68)	0.362

Lower cognition in children at 18 months old.



Maternal Genotype: Major allele (M) and minor allele (m).
Data are means from indexes of FA percentages and scores in Bayle III Cognitive Scale.
Significant differences are shown (*p< 0.05, **p<0.01) according to global ANOVA.
AA: Arachidonic acid; DGLA: Eicosatrienoic acid; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid.

CONCLUSIONS

Children had **lower enzyme activity** in the FA metabolism when their mothers presented genetic variants in the ***FADS*** genes. They also had **lower cognition** when their mothers presented genetic variants in either the ***FADS*** or ***ELOVL*** gene. Additionally, **if the mothers were obese, children's cognition was further impaired by maternal low levels of n-3 FAs.** Obese pregnant women, especially minor allele carriers, should consider a n-3 FAs rich diet to enhance child cognition.

FUNDING

Authors thank the Spanish Ministry of Economy and Competitiveness (project BFU2012-40254-C03-02) and the CIBER-Obn for the financial support for this study. ADLG thanks the Nuevo León, México government and National Council on Science and Technology (CONACYT) for PhD grant.

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COMMUNICATION #5

CHILDREN'S COGNITION INFLUENCED BY GESTATIONAL DIABETES AND MATERNAL LC-PUFAS IN DIET AND PLASMA

A. de la Garza[1], M. Bonilla[1], M. Dinarès[1], R. Montes[2,3], A.M. Chisaguano[4], M.E. Guerendiain[5], I. Salas[1], A.I. Castellote[1,3], F.J. Torres-Espínola[6], M. Arias García[6], L. García Valdés[6], E. Parejo Laudicina[6], C. Campoy[6,7], M.C. López-Sabater[1,3].

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Presentation Method: Poster presentation
Congress: 6th EAPS Congress of the European Academy of Paediatric Sociaties
Place and Date: Geneva, 2016
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Background and aims: Gestational diabetes (GD) alters placental transfer compromising key nutrients supply to the fetus, such as long chain polyunsaturated fatty acids (LC-PUFAs). These are crucial for cognitive development, especially arachidonic (AA) and docosahexaenoic (DHA) acids. We aimed to determine if children's cognition is influenced by GD and LC-PUFAs dietary intake and plasma levels of pregnant women.

Methods: Children (n=88) were selected from the population-based PREOBE cohort, divided in control (n=59), maternal GD (n=29) and ultimately in subgroups of maternal pre-pregnancy body mass index (BMI) (normoweight, overweight and obese). Maternal plasma was analyzed at 40 weeks of gestation (WOG) to measure LC-PUFAs in the phospholipid fraction, also nutrient intake was collected at 34–40 WOG using standardized 7 day dietary records. Children's cognition was assessed at 18 months old using the Bayley III Cognitive Scale.

Results: Children from obese mothers with GD and a high dietary intake of AA and low of EPA, DHA, EPA:AA and DHA:AA ratios, showed a lower score on cognition than children from obese mothers without GD with same dietary intake. Regarding maternal plasma, children from obese mothers with GD and low plasma levels of EPA, AA, and EPA:AA ratio showed a lower score on cognition than children from obese mothers without GD with same low plasma levels.

Conclusions: Gestational diabetes decreases score on children's cognition if the mother has a BMI≥30 and a low LC-PUFA (EPA, DHA and AA) intake. Obese pregnant women with GD should consider a high dietary intake of these FAs to enhance child cognition.

Children's Cognition Influenced by Gestational Diabetes and Maternal LC-PUFAs in Diet and Plasma

EAPS-0781

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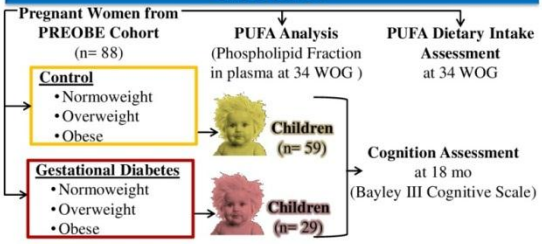
BACKGROUND

Gestational diabetes (**GD**) alters placental transfer **compromising key nutrients supply** to the fetus, such as long chain polyunsaturated fatty acids (**LC-PUFAs**). These are crucial for **cognitive development**, especially arachidonic (**AA**) and docosahexaenoic (**DHA**) acids.

AIM

The aim was to determine if **children's cognition** is **influenced** by **GD** along with **LC-PUFAs** levels in **maternal plasma** or **diet.**

METHODS



RESULTS IN CHILDREN'S COGNITION

Children´s cognition was not affected by GD if mothers had high intake/plasma levels of LC-PUFAs. But, regarding pregnant women with low levels....

Gestational diabetes affected children's cognition when mothers had low EPA status in plasma.

If mothers were normoweight or overweight, GD did not affect children's cognition even if mothers had low LC-PUFA intake/plasma levels.

But, if mothers were obese, GD did lower score on children's cognition when mothers had low LC-PUFA intake/plasma levels.

CONCLUSIONS

Maternal weight should be considered in women with gestational diabetes as it could modify the outcome. **If the mothers were obese, children's cognition was affected by gestational diabetes and LC-PUFAs levels in maternal plasma and diet.** Obese pregnant women with GD should consider a high dietary intake of these FAs to enhance child cognition.

FUNDING

Authors thank the Spanish Ministry of Economy and Competitiveness (project BFU2012-40254-C03-02) and the CIBER-Obn for the financial support for this study. ADLG thanks the Nuevo León, México government and National Council on Science and Technology (CONACYT) for PhD grant.

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COMMUNICATION #6

ASSOCIATION OF PRE-PREGNANCY BODY MASS INDEX (BMI) WITH MATERNAL GENETIC VARIANTS OF THE FADS AND ELOVL GENE CLUSTERS ON POLYUNSATURATED FATTY ACID (PUFA) LEVELS

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Presentation Method: Poster presentation
Congress: The Power of Programming, International Conference on Developmental Origins of Adiposity and Long-Term Health
Place and Date: Munich, 2016.

Background and aims: Long-chain (LC) PUFAs are key nutrients for growth and development. Fatty acid desaturase (FADS) and elongase (ELOVL) enzymes catalyze LC-PUFA synthesis, thus maternal polymorphisms (SNPs) in these enzymes may alter production during pregnancy compromising the fetus supply. We aimed to determine how these SNPs in pregnant women influence their FA response and if weight changes this.

Methods: Eighty-seven pregnant women were selected from the population-based PREOBE cohort and divided according to their pre-pregnancy BMI, group 1 (BMI<25, n=49) and group 2 (BMI≥25, n=38). Plasma samples were analyzed at 24 weeks of gestation to measure PUFAs in the phospholipid fraction. Tag SNPs were genotyped (7 in the FADS1 cluster, 5 in FADS2, 3 in ELOVL2 and 2 in ELOVL5).

Results: Minor alleles for rs174545, rs174546, rs174548 and rs174553 (FADS1) and rs1535 and rs174583 (FADS2) showed higher obesity risk. In group 1, minor allele carriers showed lower AA:DGLA and AA:LA indexes for FADS1 (rs174537, rs174545, rs174546, rs174548, rs174553 and rs174547), and AA:LA for FADS2 (rs1535 and rs174583) than major homozygotes. Similarly in group 2, minor allele carriers showed lower AA:DGLA for the same SNPs above in FADS1 and DHA:DPA for ELOVL2 (rs2236212 and rs3798713). When group 2 had no significant differences between allele carriers, they presented the same behavior than group 1.

Conclusions: Minor alleles of SNPs in FADS1 and FADS2 are associated with greater obesity risk. The drop of enzymatic activity by genetic variants in FADS genes is not necessarily changed by a high pre-pregnancy BMI but it weakens the diminution of activity.

Association of Pre-pregnancy BMI with Maternal Genetic Variants of the *FADS* and *ELOVL* Genes on Polyunsaturated Fatty Acid levels

POSTER: III-34

de la Garza A. [1], **Chisaguano A.M.** [2], **Montes R.** [3,4], **de Almeida L.** [1], **Bonilla M.** [1], **Guerendiain M.E.** [5], **Salas I.** [1], **Dinarès M.** [1], **Castellote A.I.** [1,4], **Torres-Espínola F.J.** [6], **Arias García M.** [6], **Segura Moreno M.** [6], **Campoy C.** [6,7], **López-Sabater M.C.** [1,4]

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BACKGROUND

Long-chain (LC) polyunsaturated fatty acids (PUFAs) are key nutrients for growth and development. The fatty acid desaturase (***FADS***) enzymes catalyze **LC-PUFA** synthesis.

Pregnant women presenting single nucleotide polymorphisms (**SNPs**) in the *FADS* genes may present an altered LC-PUFA production during pregnancy compromising the fetus supply.

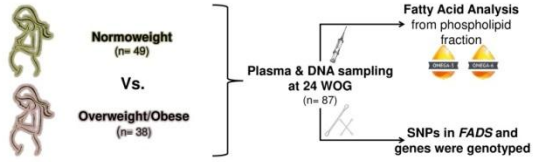
AIM

The aim was to determine if **SNPs** in ***FADS*** genes are associated with body mass index (**BMI**) in pregnant women and their impact on fatty acid (**FA**) levels.

FAs & SNPs in *FADS* BY MATERNAL WEIGHT

METHODS

Pregnant women (n= 87) were selected from the PREOBE cohort and divided according to their pre-pregnancy BMI, **group 1 (BMI<25)** and **group 2 (BMI≥25)**. Plasma samples were analyzed at 24 weeks of gestation to measure **PUFAs** in the **phospholipid fraction**. Tag **SNPs** were genotyped (**7 in *FADS1* and 5 in *FADS2***).



RESULTS

OBESITY RISK BY MINOR ALLELES FOR SNPs IN FADS GENE

Gene	SNP	Major/minor allele	N	OBESITY OR	OBESITY P	OBESITYc ORc	OBESITYc Pc
FADS1							
	rs174537	*G/T*	66	2.12	0.147	2.89	0.069
	rs174545	*C/G*	79	2.34	0.074	3.11	**0.032***
	rs174546	*C/T*	81	2.55	**0.047***	3.28	**0.025***
	rs174548	*C/G*	82	3.11	**0.015***	3.18	**0.021***
	rs174553	*A/G*	82	2.64	**0.039***	3.44	**0.019***
	rs174561	*T/C*	44	0.56	0.351	0.43	0.217
	rs174547	*T/C*	66	1.96	0.191	2.75	0.082

Gene	SNP	Major/minor allele	N	OBESITY OR	OBESITY P	OBESITYc ORc	OBESITYc Pc
FADS2							
	rs1535	*A/G*	77	2.58	**0.048***	3.42	**0.025***
	rs174575	*C/G*	70	1.57	0.351	1.77	0.273
	rs174583	*C/T*	79	2.62	**0.046***	3.38	**0.024***
	rs99780	*C/T*	71	1.75	0.261	2.37	0.122
	rs174602	*T/C*	46	1.55	0.475	1.18	0.812

SNPs were coded according to minor allele count and analyzed as numeric variable. OR= odds ratio per minor allele. Obesityc, ORc and Pc= are corrected values after adjustment for age, education, smoking and energy intake. Significant associations are denoted by stars (*p< 0.05).

LOWER ENZYME ACTIVITY INDEXES IN MINOR ALLELE CARRIERS

Gene	SNP	Major/minor allele	NORMOWEIGHT MM N	NORMOWEIGHT MM Mean (SD)	NORMOWEIGHT Mm+mm N	NORMOWEIGHT Mm+mm Mean (SD)	NORMOWEIGHT P	OVERWEIGHT/OBESE MM N	OVERWEIGHT/OBESE MM Mean (SD)	OVERWEIGHT/OBESE Mm+mm N	OVERWEIGHT/OBESE Mm+mm Mean (SD)	OVERWEIGHT/OBESE P
***FADS1* indexes**												
AA:DGLA												
FADS1	**rs174537**	*G/T*	15	3.14 (0.70)	23	↓2.20 (0.60)	**<0.001****	12	3.57 (1.73)	21	↓2.55 (0.80)	**0.032***
FADS1	**rs174545**	*C/G*	22	3.12 (0.65)	27	↓2.25 (0.57)	**<0.001****	13	3.48 (1.67)	22	↓2.51 (0.78)	**0.025***
FADS1	**rs174546**	*C/T*	22	3.12 (0.65)	27	↓2.25 (0.57)	**<0.001****	13	3.48 (1.67)	25	↓2.54 (0.74)	**0.021***
FADS1	**rs174548**	*C/G*	24	2.97 (0.65)	25	↓2.33 (0.70)	**0.002****	13	3.42 (1.69)	25	↓2.57 (0.75)	**0.037***
FADS1	**rs174553**	*A/G*	22	3.12 (0.65)	27	↓2.25 (0.57)	**<0.001****	13	3.48 (1.67)	25	↓2.54 (0.74)	**0.021***
FADS1	**rs174547**	*T/C*	15	3.20 (0.67)	23	↓2.27 (0.55)	**<0.001****	12	3.54 (1.73)	20	↓2.56 (0.82)	**0.037***
AA:LA												
FADS1	**rs174537**	*G/T*	15	0.45 (0.08)	23	↓0.39 (0.09)	**0.038***	12	0.46 (0.07)	21	0.43 (0.08)	0.390
FADS1	**rs174545**	*C/G*	22	0.46 (0.09)	27	↓0.38 (0.08)	**0.003****	13	0.47 (0.07)	22	0.42 (0.08)	0.071
FADS1	**rs174546**	*C/T*	22	0.46 (0.09)	27	↓0.38 (0.08)	**0.003****	13	0.47 (0.07)	25	0.42 (0.08)	0.098
FADS1	**rs174548**	*C/G*	24	0.45 (0.08)	25	↓0.39 (0.09)	**0.031***	13	0.45 (0.08)	25	0.43 (0.08)	0.553
FADS1	**rs174553**	*A/G*	22	0.46 (0.09)	27	↓0.38 (0.08)	**0.003****	13	0.47 (0.07)	25	0.42 (0.08)	0.098
FADS1	**rs174547**	*T/C*	15	0.45 (0.08)	23	↓0.39 (0.09)	**0.040***	12	0.46 (0.07)	20	0.43 (0.08)	0.427
***FADS2* indexes**												
AA:LA												
FADS2	**rs1535**	*A/G*	20	0.45 (0.08)	25	↓0.39 (0.09)	**0.025***	13	0.47 (0.07)	23	0.42 (0.08)	0.128
FADS2	**rs174583**	*C/T*	19	0.44 (0.08)	27	↓0.38 (0.08)	**0.019***	13	0.47 (0.07)	25	0.42 (0.08)	0.098

Indicates significant differences (*p< 0.05, **p< 0.01) according to global ANOVA within each group of weight.
Data are means from indexes of FA percentages (standard deviation).
AA: arachidonic acid; DGLA: dihomo-γ-linolenic acid; LA: linoleic acid.
Major allele: M; minor allele: m.

CONCLUSIONS

Minor alleles of SNPs in ***FADS1*** and ***FADS2*** genes are associated with greater **obesity risk**. The drop of enzymatic activity by genetic variants in *FADS* genes is not necessarily changed by a **high pre-pregnancy BMI** but it **weakens the diminution of enzymatic activity by these SNPs.**

FUNDING

Authors thank the Spanish Ministry of Economy and Competitiveness (project BFU2012-40254-C03-02) and the CIBER-Obn for the financial support for this study. ADLG thanks the Nuevo León, México government and National Council on Science and Technology (CONACYT) for PhD grant.

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COMMUNICATION #7

PRE-PREGNANCY BODY MASS INDEX AND GESTATIONAL DIABETES ON MATERNAL FATTY ACID PROFILE AND PLACENTAL TRANSFER

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Rationale: Gestational diabetes (GD) may modify maternal fatty acid (FA) availability during pregnancy and may also alter placental transfer compromising fetus supply of key nutrients such as long chain (LC) polyunsaturated fatty acids (PUFAs), especially arachidonic (AA) and docosahexaenoic (DHA) acids. A high body mass index (BMI) increases the risk for GD and is also related to an altered FA profile, hence we aimed to determine the maternal alterations in FA profile and FA placental transfer caused by GD and if they change according to maternal BMI.

Methods: Pregnant women (n=179) were selected from the population-based PREOBE cohort, divided in control (n=135) and women with GD (n= 44), these last were ultimately divided according to their pre-pregnancy BMI (normoweight, overweight and obese). Maternal plasma and umbilical cord samples were analyzed at delivery (40 weeks of gestation) to measure PUFAs in the phospholipid fraction. Placental FA transfer was analyzed calculating ratios between FAs in the umbilical cord of the offspring and FAs in maternal plasma.

Results: Women with gestational diabetes showed higher plasma levels of AA (10.46% vs. 9.32% of total detected FAs), eicosapentaenoic acid (EPA) (0.38% vs 0.30%), DHA (5.17% vs. 4.33%), PUFAs (44.25% vs 43.17%), n3 PUFAs (6.12% vs. 5.13%), LC-n3 PUFAs (6.01% vs. 5.01%), and LC-n6 PUFAs (15.12% vs. 14.18%) and lower n6:n3 (6.49% vs. 7.83%) and LCn6:LCn3 (2.61% vs. 3.00%) ($p<0.05$). A BMI$\geq$25 in these women implicated a higher ($p<0.05$) LCn6:LCn3 (2.89% vs 2.37%). Regarding FA placental transfer, women in control group showed higher transfer ratios in AA (2.02 vs. 1.73), EPA (0.70 vs 0.46), DHA (1.57 vs. 1.33), PUFAs (1.00 vs 0.95), n3 PUFAs (1.44 vs. 1.22), n6 PUFAs (0.94 vs. 0.91), LC-n3 PUFAs (1.47 vs. 1.24) and LC-n6 PUFAs (1.85 vs. 1.66) (among others) and a lower transfer in n6:n3 (0.68 vs. 0.78) ($p<0.05$). BMI didn't modify placental transfer ratios.

Conclusion: Gestational diabetes alters maternal FA profile and this may vary according to pre-pregnancy BMI. In spite of maternal weight, GD decreases placental transfer of crucial FAs (e.g. AA and DHA) involved in children development.

Disclosure of Interest: None Declared
Keywords: AA, BMI, DHA, Maternal, Nutrition, Obesity, Pregnancy

PRE-PREGNANCY BMI & GESTATIONAL DIABETES ON MATERNAL FATTY ACID PROFILE AND PLACENTAL TRANSFER

MON-LB275

A. de La Garza* [1], M. Bonilla[1], M. Dinarès[1], R. Montes[2, 3], A. M. Chisaguano[4], M. E. Guerendiain[5], I. Salas[1], A. I. Castellote[1, 3], F. J. Torres-Espínola[6], M. Arias[6], L. García[6], E. Parejo[6], C. Campoy[6, 7], M. C. López-Sabater[1, 3]

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BACKGROUND

Gestational diabetes (**GD**) may modify maternal **fatty acid** (**FA**) availability during pregnancy and may also alter placental transfer, hence compromising fetus supply of key nutrients.

Some of those nutrients are long chain (LC) polyunsaturated fatty acids (**PUFAs**), especially arachidonic (AA) and docosahexaenoic (DHA) acids.

It is known that a high body mass index (**BMI**) increases the risk for GD and is also related to an altered FA profile.

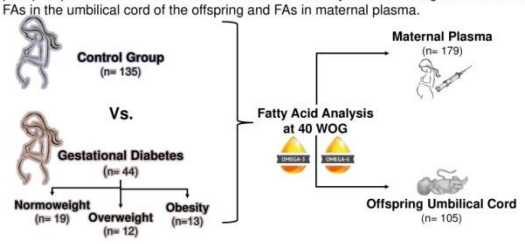
AIM

We aimed to determine the **maternal alterations** in **FA profile** and **FA placental transfer** caused by **GD** and if they change according to maternal **BMI.**

FATTY ACIDS IN GESTATIONAL DIABETES

METHODS

Pregnant women (**n=179**) from the PREOBE cohort were divided in **control** and women with **GD**, these last were ultimately divided according to their pre-pregnancy BMI (**normoweight, overweight and obese**). **Maternal plasma** and **umbilical cord** samples were analyzed at delivery (40 weeks of gestation) to measure **PUFAs** in the phospholipid fraction. **Placental FA transfer** was analyzed calculating **ratios** between FAs in the umbilical cord of the offspring and FAs in maternal plasma.



RESULTS

GD group showed **higher plasma levels** of AA, EPA, DHA, PUFAs, n3 PUFAs, LC-n3 PUFAs and LC-n6 PUFAs and **lower** n6:n3 and LCn6:LCn3. **Overweight** women in **GD** group presented **higher** LCn6:LCn3 than normoweight. Regarding FA placental transfer, women in **control** group showed **higher transfer ratios** in AA, EPA, DHA, PUFAs, n3 PUFAs, n6 PUFAs, LC-n3 PUFAs and LC-n6 PUFAs and a **lower transfer** in n6:n3. BMI didn't modify placental transfer ratios.

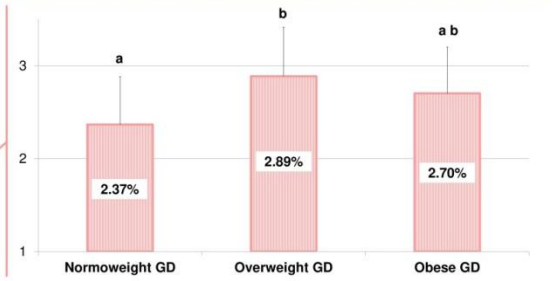
Control Group | Gestational Diabetes Group

FATTY ACID LEVELS IN MATERNAL PLASMA

Fatty Acids	CONTROL n=135 Mean % (SD)	GESTATIONAL DIABETES n=44 Mean % (SD)	P
C20:4, n-6 (AA)	9.32 (1.59)	↑ 10.46 (1.93)	<0.001**
n-6 PUFAs	37.80 (2.45)	37.89 (1.90)	0.831
LC-n-6 PUFAs	14.18 (1.96)	↑ 15.12 (1.91)	0.006**
C20:5, n-3 (EPA)	0.30 (0.19)	↑ 0.38 (0.24)	0.031*
C22:6, n-3 (DHA)	4.33 (1.04)	↑ 5.17 (0.92)	<0.001**
n-3 PUFAs	5.13 (1.22)	↑ 6.12 (1.29)	<0.001**
LC-n-3, PUFAs	5.01 (1.21)	↑ 6.01 (1.29)	<0.001**
PUFAs	43.17 (2.31)	↑ 44.25 (1.47)	0.004**
n-6:n-3	↑ 7.83 (2.09)	6.49 (1.49)	<0.001**
LCn-6:LCn-3	↑ 3.00 (0.83)	2.61 (0.55)	0.004**

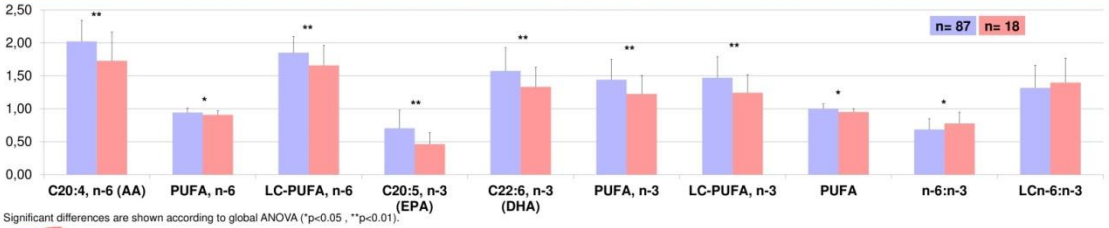
Significant differences are shown according to global ANOVA (*p<0.05 , **p<0.01).

LCn-6:LCn-3 PLASMA RATIO



Different superscript letters indicate significant differences (p<0.05) after applying Bonferroni post-hoc correction.

OFFSPRING:MATERNAL FATTY ACID RATIOS



Significant differences are shown according to global ANOVA (*p<0.05 , **p<0.01).

CONCLUSIONS

Gestational diabetes alters maternal fatty acid profile and this may vary according to pre-pregnancy BMI. In spite of maternal weight, gestational diabetes **decreases placental transfer of crucial fatty acids** (e.g. AA and DHA) involved in children development.

FUNDING

Authors thank the Spanish Ministry of Economy and Competitiveness (project BFU2012-40254-C03-02) and the CIBER-Obn for the financial support for this study. ADLG thanks the Nuevo León, México government and National Council on Science and Technology (CONACYT) for PhD grant.

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COMMUNICATION #8

IMPACTO DEL IMC PREGESTACIONAL MATERNO EN EL ESTADO NUTRICIONAL DE LA MADRE E HIJO

Andrea de la Garza Puentes

Presentation Method: Oral presentation.

Congress: Sesiones Científicas del CCNIEC 2016

Place and Date: Barcelona, 2016.

IMPACTO DEL IMC PREGESTACIONAL MATERNO EN EL ESTADO NUTRICIONAL DE MADRE E HIJO

Proyecto PREOBE (Programación Precoz de la Obesidad)

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COMMUNICATION #9

IMPACT OF MATERNAL PRE-PREGNANCY BMI IN CHILDREN'S BMI PERCENTILE AND FA PROFILE AT 6 MONTHS AND 3 YEARS OF AGE

A. de la Garza*[1, 2], L. de Almeida[1], R. Montes[1, 2], A.M. Chisaguano[1, 3], I. Salas[1], A.I. Castellote[1, 2], M.T. Segura[4], L. García-Valdés[4], F.J. Torres-Espínola[4], J. Florido[5], C. Padilla[5], C. Campoy[4, 6], M.C. López-Sabater[1, 2].

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Presentation Method: Poster presentation.
Congress: 37th ESPEN Congress, Healthy Life Through Nutrition
Place and Date: Lisbon, 2015.
***Published in** (See Appendix 10.2.6)**::**
Journal: Clinical Nutrition, Supplement.
Impact Factor: 4.487
ISSN: 17441161
DOI: 10.1016/S0261-5614(15)30782-2

Rationale: Pre-pregnancy Body Mass Index (BMI) is associated with children's health through life span, possibly leading to diseases such as obesity which is related to imbalances in n-6:n-3 ratio, n-3 and n-6 long-chain polyunsaturated fatty acids (LC-PUFAs) that are fundamental in childhood neurodevelopment and risk of diseases. The aim was to determine if maternal pre-pregnancy BMI alters fatty acid (FA) profile and/or BMI percentile of children.

Methods: Mothers and their infants from a Spanish population were included. Infant cheek cell samples and BMI percentiles were collected at 6 months and 3 years of age. Children were divided according to maternal pre-pregnancy BMI: 17 children from normoweight (group 1), 12 from overweight (group 2) and 6 from obese (group 3). All mothers had appropriate weight gain. FAs were assessed by glycerophospholipids determination and analyzed by fast gas chromatography. Statistical analyses with SPSS 20.0 using ANOVA Bonferroni's *post-hoc* and Student's T Test.

Results: At 6 months, children from group 1 (75%) and 2 (50%) tended to be at 15.1-84.9th BMI percentile and group 3 (57%) at $\leq$15th BMI percentile. Groups had few children tending to $\geq$85th BMI percentile, group 3 had the most (14%). The n6:n3 and LC-n6:n3 ratios were significantly higher ($p<0.05$) in group 3 than group 1, maintaining at 3 years of age. Without significant differences, PUFA n-6 and LC-PUFA n-6 were higher in group 2 and 3 than 1. The PUFA n-3 and LC-PUFA n-3 were higher in children from group 1 than 3.

Conclusions: Children in group 3 have a FA profile and BMI percentile altered at 6 months when compared to group 1. As this relation remains at 3 years of age, a normoweight pre-pregnancy BMI might be important on children's outcome. There is a suggestion of the importance of a complete nutritional assessment in children as they may have an altered FA profile with normal/low BMI percentile.

The authors declare no conflicts of interest.
Keywords: fatty acids, obesity, infants, pregnant women, pufa.

IMPACT OF MATERNAL PRE-PREGNANCY BMI IN CHILDREN'S BMI PERCENTILE AND FA PROFILE AT 6 MONTHS AND 3 YEARS OF AGE

MON-LB018

A. de la Garza[*1,2], L. de Almeida[1], R. Montes[1,2], A.M. Chisaguano[1,3], I. Salas[1], A.I. Castellote[1,2], M.T. Segura[4], L. García-Valdés[4], F.J. Torres-Espínola[4], J. Florido[5], C. Padilla[5], C. Campoy[4,6], M.C. López-Sabater[1,2].

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BACKGROUND

Obesity among pregnant women is becoming one important health issue. Pre-pregnancy BMI is associated with physical and analytic parameters of newborns that may predict the risk of diseases through life span.

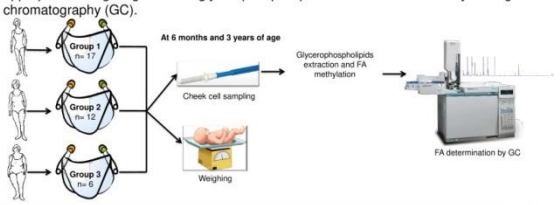
Obesity and obesity-related diseases in adulthood are mainly caused by childhood obesity. On the other hand, the n-3 and n-6 long-chain polyunsaturated fatty acids (LC-PUFAs) play important roles in childhood neurodevelopment, growth and immunity. Moreover, an imbalance in the n-6:n-3 ratio has been associated with developmental disorders in childhood.

AIM

The objective of this study was to show if maternal pre-pregnancy BMI may alter FA profile and/or BMI percentile of children at 6 months and 3 years of age.

METHODS

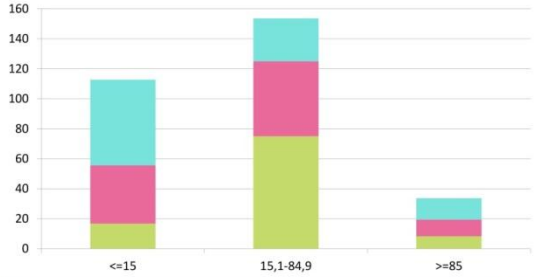
Mothers and their infants from a Spanish population were included. Infant cheek cell samples and BMI percentiles were collected at 6 months and 3 years of age. Children were divided according to maternal pre-pregnancy BMI: 17 children from normoweight (group 1), 12 from overweight (group 2) and 6 from obese (group 3). All mothers had appropriate weight gain. FA glycerophospholipids were determined by fast-gas chromatography (GC).



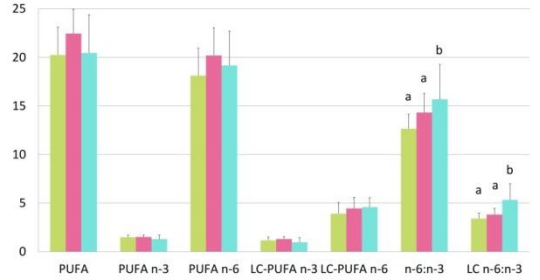
RESULTS

At 6 months, children from group 1 (75%) and 2 (50%) tended to be at 15.1-84.9th BMI percentile and group 3 (57%) at ≤15th BMI percentile. Groups had few children tending to ≥85th BMI percentile, group 3 had the most (14%). The n6:n3 and LC-n6:n3 ratios were significantly higher ($p<0.05$) in group 3 than group 1, maintaining at 3 years of age. Without significant differences, PUFA n-6 and LC-PUFA n-6 were higher in group 2 and 3 than 1. The PUFA n-3 and LC-PUFA n-3 were higher in children from group 1 than 3.

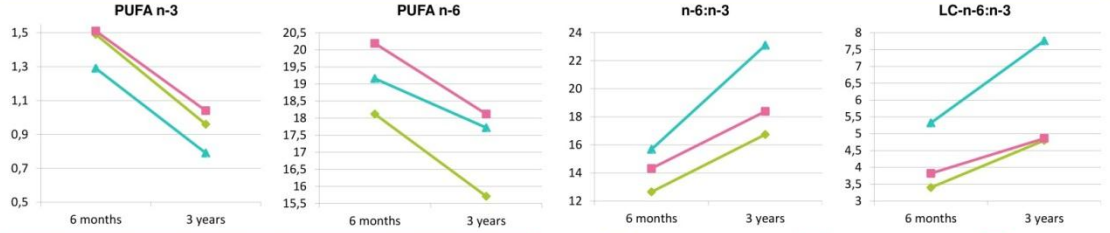
CHILDREN'S WEIGHT CATEGORY AT 6 MONTHS OF AGE ACCORDING TO THEIR BMI PERCENTILE



CHILDREN'S FAs AT 6 MONTHS OF AGE



EVOLUTION



Group 1 (Normoweight*) | Group 2 (Overweight*) | Group 3 (Obesity*)

*Children's groups according to maternal pre-pregnancy BMI

CONCLUSIONS

Children in group 3 had a FA profile and BMI percentile altered at 6 months when compared to group 1 and this relation remained at 3 years of age. **In spite of an appropriate pregnancy weight gain, a normoweight pre-pregnancy BMI might be crucial on children's outcome.** There is a suggestion of the importance of a **complete nutritional assessment in children** as they may have an altered FA profile with normal/low BMI percentile.

FUNDING

The authors thank the Spanish Ministry of Economy and Competitiveness (project BFU2012-40254-C03-02) and the CIBER-Obn for the financial support of this work. ADLG thanks the Nuevo León, México government and National Council on Science and Technology (CONACYT) for the PhD grant.

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COMMUNICATION #10

BREAST MILK FATTY ACID COMPOSITION IN NORMOWEIGHT, OVERWEIGHT AND OBESE WOMEN: ASSOCIATION WITH INFANT OUTCOMES

Maribel Chisaguano, Andrea de la Garza, Rosa Montes, Ana Isabel Castellote, Laura Martín-Dinares, María Teresa Segura, Luz García Valdés, Francisco Jose Torres-Espínola, Jesús Florido, Carmen Padilla, Cristina Campoy, M. Carmen López-Sabater.

Presentation Method: Poster presentation.
Congress: Lipids, Molecular & Cellular Biology of Gordon Research Conference
Place and Date: NH, EUA, 2015.

Background and aim: Few studies have explored the associations between the breast milk fatty acid (FA) compositions according to maternal nutritional status, with especial interest in long chain polyunsaturated fatty acids (LC-PUFAs) and infant outcomes. The aim of this study was to analyze the relationships between the concentration of n-3 and n-6 LC-PUFAs in breast milk and the anthropometric measurements of the newborns over the first 18 months of life.

Methods: A subgroup from a population-based pregnancy cohort of the PREOBE Project was analyzed. FA composition, particularly n-3 and n-6 LC-PUFAs, was analyzed in both colostrum and mature milk. The infant anthropometric measurements were transformed to weight-for-age (WAZ), length-for-age (LAZ), head circumference-for-age (HCAZ) and BMI-for-age (BMIZ) Z-scores (SD scores), according to the WHO child growth standards.

Results and conclusion: We found that the n-3 LC-PUFAs, especially docosahexaenoic (DHA), in colostrum had a strong negative association with both WAZ and LAZ until to 18 months of age. Linoleic acid (LA) and total n-6 FA content in breast milk were positively associated with BMI at 3 months of age. We conclude that the higher levels of n-6 FAs in breast milk may influence the anthropometric measures of infants during the first months of life.

Breast milk fatty acid composition in normoweight, overweight and obese women: association with infant outcomes

Aida Maribel Chisaguano*[1,2], Andrea de la Garza[2,3], Rosa Montes[2,3], Ana Isabel Castellote[2,3], Laura Martín-Dinares[2], María Teresa Segura[4], Luz García-Valdés[4], Francisco José Torres-Espínola[4], Jesús Florido[5], Carmen Padilla[5], Cristina Campoy[4,6] and M. Carmen López-Sabater[2,3]

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Universitat de Barcelona

INTRODUCTION

The diet consumed by pregnant and breastfeeding women and the presence or development of obesity during pregnancy could be key determinants of the metabolic health of future generations.

Since breast milk is usually the only source of nutrients for newborn infants, its composition greatly influences growth and health during the period of rapid growth and development in the first months of life.

Many studies suggest that the profile of the LC-PUFAs in breast milk is related with the development of obesity in offspring. While a protective role against obesity has been attributed to n-3 LC-PUFAs, an adverse effect of n-6 LC-PUFAs has been reported; thus, the results appear to be inconsistent.

In Western countries such as Spain, the increasing incidence of obesity has coincided with a marked increase in the intake of n-6 PUFAs, which has led to the suggestions that the two may be causally related.

AIM

The aim of this study was to investigate the association between breast milk PUFA content and infant growth until 18 months of age.

EXPERIMENTAL DESIGN

PARTICIPANTS

The PREOBE study (*Role of Nutrition and Maternal Genetics on the Programming of Development of Fetal Adipose Tissue*) was an observational cohort study of a total of 331 pregnant women, classified as normoweight (pre-conception body mass index (BMI) 18.5 ≤ BMI < 25), overweight (25 ≤ BMI < 30) and obese (BMI ≥30), and women who developed gestational diabetes[1].

For this study, analyses were performed only to data pertaining to pregnant women (classified as normoweight, overweight and obese) and their offspring who were exclusively breastfed.

SAMPLES

Colostrum was collected 2–4 days postpartum and mature milk at 28–32 days, by an experienced nurse at the hospital.

STATISTICAL ANALYSIS

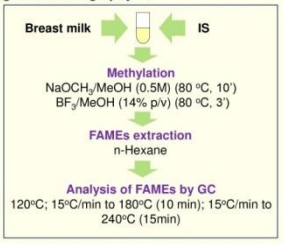
The association between the FA composition of breast milk and anthropometric measurements was analyzed by partial correlations after adjusting for potential confounders.

The confounders included gestational age, maternal BMI, maternal education, maternal smoking, pregnancy weight gain and infant gender.

METHODS

FATTY ACIDS DETERMINATION

Breast milk fatty acids were analyzed by **fast-gas chromatography** [2].



INFANT OUTCOMES

Weight, length, head circumference and BMI measurements were converted to weight-for-age **(WAZ)**, length-for-age **(LAZ)**, head circumference-for-age **(HCAZ)** and BMI-for-age **(BMIZ)** Z-scores (SD scores), according to the WHO child growth standards.

RESULTS

Population characteristics

Characteristics	
Maternal characteristics	
Maternal age, years (mean±SD)	32.1 ± 3.50
Weight gain during pregnancy, kg (mean±SD)	
T1	5.1 ± 4.09
T2	11.4 ± 1.24
T3	17.7 ± 3.22
Maternal BMI, Kg/m² (%)	
Normoweight (18.5 ≤ BMI < 25)	58.0
Overweight (25 ≤ BMI < 30)	26.0
Obese (BMI ≥ 30)	16.0
Maternal education (%)	
Low (Primary or less)	9.2
Medium (Secondary)	27.6
High (University or more)	63.2
Parity (%)	
0	44.1
1	42.9
≥2	13.0
Smoking in pregnancy, yes (%)	6.9
Children characteristics	
Sex, male (%)	52.6
Birth weight, g (mean±SD)	3386.4 ± 485.0
Birth length, cm (mean±SD)	50.7 ± 1.87
Birth head Circumference, cm (mean±SD)	34.6 ± 1.33
Birth BMI, kg/m² (mean±SD)	13.2 ± 1.23
Newborn according Lubchenco curves# (%)	
SGA	1.4
AGA	75.4
LGA	23.2

#The newborns were divided into three groups according to the Lubchenco curves as: small for gestational age (SGA), appropriate for gestational age (AGA) and large for gestational age (LGA).

Correlations between breast milk PUFA composition and anthropometric measurements in infants at 3 and 18 months of age

Fatty acid	HCAZ		WAZ		LAZ		BMIZ	
	Colostrum	Mature milk	Colostrum	Mature milk	Colostrum	Mature milk	Colostrum	Mature milk
C18:2 n-6 (LA)								
3 mo.	0.311	0.155	0.535	0.572*	0.188	0.280	0.650*	0.620*
18 mo.	0.128	0.156	0.280	0.452	0.031	0.317	0.321	0.334
C18:3 n-3 (ALA)								
3 mo.	0.181	0.383	0.087	0.424	-0.030	0.376	0.159	0.307
18 mo.	0.097	0.281	-0.043	0.033	-0.178	0.224	0.074	-0.110
C20:4 n-6 (AA)								
3 mo.	0.334	0.318	-0.071	0.311	0.124	0.282	-0.222	0.221
18 mo.	0.000	0.164	-0.347	-0.052	-0.154	0.238	-0.321	-0.206
C20:5 n-3 (EPA)								
3 mo.	-0.271	-0.033	-0.204	-0.310	-0.126	-0.570*	-0.202	0.043
18 mo.	-0.240	-0.347	-0.124	-0.268	-0.120	-0.764**	-0.057	0.193
C22:6 n-3 (DHA)								
3 mo.	-0.380	-0.033	-0.811**	-0.082	-0.788**	-0.116	-0.528	-0.010
18 mo.	-0.507	0.122	-0.676*	-0.207	-0.814**	-0.118	-0.269	-0.161
LC-PUFA n-3								
3 mo.	-0.385	0.033	-0.842**	-0.324	-0.758**	-0.438	-0.603*	-0.089
18 mo.	-0.492	-0.130	-0.710**	-0.555*	-0.777**	-0.586*	-0.330	-0.259
LC-PUFA n-6								
3 mo.	-0.030	0.157	-0.394	0.413	-0.146	0.487	-0.477	0.183
18 mo.	-0.248	0.032	-0.270	0.076	-0.362	0.425	-0.095	-0.168
Σn-3								
3 mo.	-0.237	0.232	-0.680*	0.225	-0.668*	0.134	-0.436	0.232
18 mo.	-0.373	0.136	-0.633*	-0.299	*-0.762***	0.062	-0.246	-0.382
Σn-6								
3 mo.	0.315	0.164	0.464	0.583*	0.162	0.301	0.565*	0.617*
18 mo.	0.082	0.158	0.225	0.444	-0.046	0.331	0.304	0.316
n-6:n-3 ratio								
3 mo.	0.305	-0.104	*0.645**	0.317	0.456	0.158	0.575*	0.334
18 mo.	0.243	-0.017	0.471	0.631*	0.385	0.249	0.309	0.580*

All values were adjusted for maternal age, maternal BMI (normoweight, overweight and obese women), maternal education, maternal smoking, pregnancy weight gain and sex of child. HCAZ, head circumference-for-age; WAZ, weight-for-age; LAZ, length-for-age; BMIZ, body mass index-for-age. (*p<0.05, **p<0.01).

Our findings showed that n-3 LCPUFAs, especially DHA, in colostrum had a **strong negative association with both WAZ and LAZ**. Meanwhile, n-6 FAs present effects opposed to those of n-3 FAs. Thus, some n-6 FAs in both colostrum and mature milk had a **positive association with WAZ and BMIZ**; specifically LA and total n-6 FAs, as well as the n-6:n-3 ratio. Our data support that n-6 levels may contribute to increase the fat mass in children.

CONCLUSIONS

The n-6 FA content of breast milk was positively correlated with BMIZ. Overall, our results suggest that maternal nutritional status may affect the FA composition of breast milk and that a higher intake of n-6 FAs, due to different breast milk composition, influences the anthropometric measurements of infants over the first 18 months of life.

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FINANCIAL SUPPORT

The authors thank the Spanish Ministry of Economy and Competitiveness (project BFU2012-40254-C03-02) for financial support for the research.

COMMUNICATION #11

EVALUATION OF NONINVASIVE ALTERNATIVES TO ASSESS FATTY ACIDS STATUS: BUCCAL MUCOSAL CELL AND CAPILLARY BLOOD SAMPLING

A. De la Garza[1], R. Montes[1], A.I. Castellote-Bargalló[1], R. Moreno-Torres[2], C. Campoy[2], M.C. López Sabater[1].

[1]Department of Nutrition and Food Science, Faculty of Pharmacy, University of Barcelona, Barcelona, Spain. [2]EURISTIKOS Excellence Centre for Pediatric Research, University of Granada. Granada, Spain.

Presentation Method: Poster presentation.
Congress: 2nd International Conference on Nutrition and Growth
Place and Date: Barcelona, 2014.

Background and aim: Plasma is the most common matrix to analyze fatty acid (FA) status, but its extraction is invasive and not well accepted, particularly in infants. Previous studies have suggested buccal mucosa cell and capillary blood samples as alternative markers, but the sampling and analytical methods differ widely limiting the comparison of results. The objective of this work was to evaluate buccal mucosal cells and capillary blood as noninvasive methods for phospholipids FA, especially long chain polyunsaturated fatty acids (LC-PUFA), analysis as alternatives of traditional plasma sampling and to contribute to the standardization of the procedure to analyze FA.

Methods: Buccal mucosal cell, capillary blood and plasma samples were obtained from 20 children. Lipid fraction was extracted with dichloromethane/methanol (DCM:MeOH) 2:1 and then, phospholipids were isolated by Solid Phase Extraction (SPE) in a 96 well plate. Methyl esters of FA were obtained and finally analysed bygas cromatography (GC).

Results: The precision of quantitative data was confirmed by the intra-assay and inter-assay for the three different matrices. Values obtained for the ratio LC-PUFA n-6: LC-PUFA n-3 were equivalent in buccal mucosal cell samples with the two other matrices. Correlation coefficients of the main LC-PUFA, docosahexaenoic acid (DHA), in buccal mucosal cell and capillary blood samples in relation to plasma samples, ranged between r= 0.78-0.87.

Conclusions: Buccal mucosal cells and capillary blood can be used as an alternative of traditional plasma sampling for FA analysis. The applied methodology resulted in a highly accurate determination of LC-PUFAs.

EVALUATION OF NONINVASIVE ALTERNATIVES TO ASSESS FATTY ACIDS STATUS: BUCCAL MUCOSAL CELLS AND CAPILLARY BLOOD SAMPLING

Universitat de Barcelona

A. De la Garza[1], R. Montes[1], A.I. Castellote-Bargalló[1], R. Moreno-Torres[2], C. Campoy[2], M.C. López-Sabater*[1].
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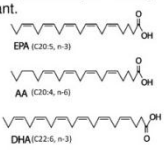
BACKGROUND

In many clinical and epidemiological studies has been observed that the n-3 and n-6 long-chain polyunsaturated fatty acids (LC-PUFA) play important roles in human health and common diseases. For this reason, the analysis of fatty acid (FA) status has become important.



Plasma is the traditional matrix to analyze FA status, but its extraction is invasive and not well accepted, particularly in infants. Previous studies have suggested buccal mucosa cell and capillary blood samples as alternative markers, but the sampling and analytical methods differ widely limiting the comparison of results.

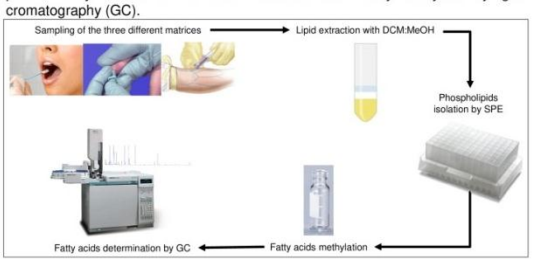
AIM

The aim of this study was to evaluate buccal mucosal cells and capillary blood sampling as noninvasive methods for phospholipids FA, especially long chain polyunsaturated fatty acids (LC-PUFA), analysis as alternatives of traditional plasma sampling and to contribute to the standardization of the procedure to analyze FA.

METHODS

Buccal mucosal cells, capillary blood and plasma samples were obtained from 20 fasted children volunteers at the Granada Hospital, Spain.

Lipid fraction was extracted with dichloromethane/methanol (DCM:MeOH) 2:1 and then, phospholipids were isolated by Solid Phase Extraction (SPE) in a 96 well plate. Methyl esters of FA were obtained and finally analysed by gas cromatography (GC).



RESULTS

The precision of quantitative data was confirmed by the intra-assay and inter-assay for the three different matrices. Values obtained for the ratio LC-PUFA n-6: LC-PUFA n-3 were equivalent in buccal mucosal cell samples with the two other matrices. Correlation coefficients (r) of the main LC-PUFA, docosahexaenoic acid (DHA), in buccal mucosal cell and capillary blood samples in relation to plasma samples, ranged between $r = 0.78$-0.87.

PRECISION OF METHODS

Fatty Acids	Buccal Mucosa Cells		Capillary Blood	
	Intra day (n=10)	Inter day (n=10)	Intra day (n=10)	Inter day (n=10)
	CV (%)	CV (%)	CV (%)	CV (%)
C20:2, n-6	5,01	4,56	4,85	3,91
C20:3, n-9	5,29	9,23	4,07	5,22
C20:3, n-6	1,99	1,88	0,69	0,89
C20:4, n-6 (AA)	0,64	0,72	0,30	0,22
C20:5, n-3 (EPA)	4,53	5,58	2,43	3,46
C22:4, n-6	3,84	2,69	0,80	1,58
C22:5, n-6	4,35	9,29	2,98	3,8
C22:5, n-3	4,55	6,42	1,41	1,85
C22:6, n-3 (DHA)	0,55	2,17	0,31	0,55

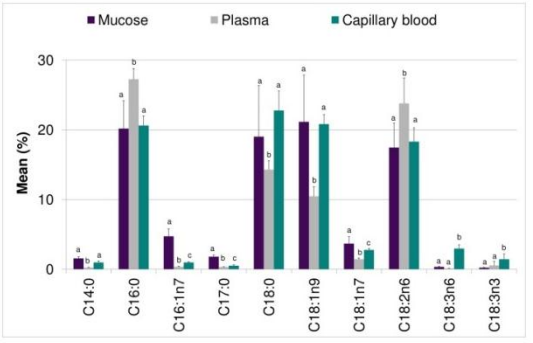
Coefficients of variation (CV) for different determinations of pool samples (n=20 individuals).

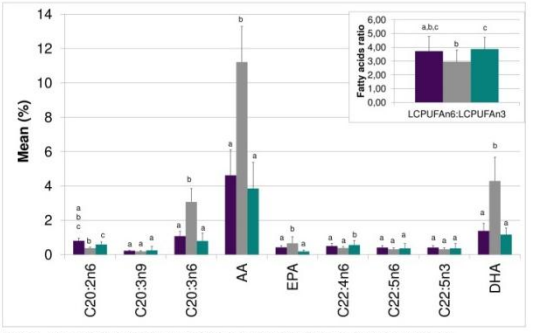
CORRELATIONS OF ALTERNATIVE MATRICES

Fatty Acids	Mucose vs. Plasma		Capillary blood vs. Plasma	
	r	p	r	p
C20:2n6	-0,08	0,315	0,02	0,454
C20:3n9	0,57	0,121	0,59	0,320
C20:3n6	0,64**	0,000	0,94**	0,000
C20:4, n-6 (AA)	0,50**	0,000	0,70**	0,000
C20:5, n-3 (EPA)	0,36*	0,020	0,90**	0,000
C22:4n6	0,57**	0,001	0,56**	0,000
C22:5n6	0,15	0,208	0,17	0,159
C22:5n3	0,57**	0,002	0,76**	0,000
C22:6, n-3 (DHA)	0,78**	0,000	0,87**	0,000

Coefficients for different individuals (n=20) (*P < 0.05, **P < 0.01).
r= correlation coefficient.

FATTY ACIDS PROFILE





Means standard deviation of FA with different superscript letter are statistically different.

CONCLUSIONS

Buccal mucosal cells and capillary blood can be used as an alternative of traditional plasma sampling for FA analysis. The applied methodology resulted in a highly accurate determination of LC-PUFAs.

FUNDING

The authors thank the Spanish Ministry of Economy and Competitiveness (project BFU2012-40254-C03-02) and the CIBER Obn for the financial support of this work. ADLG thanks the Nuevo León, México government and National Council on Science and Technology (CONACYT) for the PhD grant.

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COMMUNICATION #12

DIFFERENCES IN BREAST MILK FATTY ACID COMPOSITION BETWEEN OBESE, OVERWEIGHT AND NORMOWEIGHT MOTHER

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Presentation Method: Poster presentation.
Congress: 14th International Nutrition & Diagnostics Conference
Place and Date: Prague, 2014.

Background and aim: The main sources of breast milk fatty acids (FA) are the maternal diet, the mobilization of endogenous stores of FA and the novo synthesis of FA by breast tissue or liver. The study of FA composition of breast milk in obese or overweight populations is still limited. It has been hypothesized that the content of the long-chain polyunsaturated fatty acids (LC-PUFA) in breast milk is related with obesity.

Methods: We studied the differences of the FA composition of colostrum and mature milk between obese and normoweight mothers from a Spanish population. Maternal nutritional status (normoweight, overweight or obesity) was estimated on the Body Mass Index (BMI). Thirteen obese (BMI $\geq$ 30 kg/m^2), 17 overweight (25 $\geq$ BMI < 30 kg/m^2) and 16 normoweight (18 $\geq$ BMI $\leq$ 25 kg/m^2) mothers were selected for this study. Participants provided samples of colostrum (2-4 days after delivery) and mature milk (28-32 days after delivery). Breast milk fatty acid methyl esters (FAMEs) were separated and quantified by fast-gas chromatography. Differences between groups were assessed.

Results and conclusion: The FA composition of breast milk showed a considerable variation with regards to factors such as the stage of lactation. Thus, we found that colostrum of the three groups have higher levels of n-6 and n-3 LC-PUFA, especially AA and DHA, in relation to mature milk. On the other hand, we observed higher concentrations of total n-6 LC-PUFA and the ratio n-6/n-3 FA in colostrum of obese compared to normoweight mothers. There were no significant differences between the levels of the main saturated and monounsaturated fatty acids in both, colostrum and mature milk.

Keywords: BMI, LC-PUFAs, breast milk.

DIFFERENCES IN BREAST MILK FATTY ACID COMPOSITION BETWEEN OBESE, OVERWEIGHT AND NORMOWEIGHT MOTHER

A. Chisaguano*[1], L. Martín-Dinares[1], S. Caballero[1], A.I. Castellote[1], R. Montes[1], A. de la Garza[1], P. Brandi[2], M. Segura[2], R. Moreno-Torres[2], C. Campoy[2], M.C. López-Sabater[2]
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ciberesp

INTRODUCTION

Overweight and obesity in childhood and adolescence, are a major health problem which is increasing disproportionately worldwide.

To date, there are 110 million children in the world with this disease and the increase forecasts for the coming years are very alarming.

The main sources of breast milk fatty acids (FA) are the maternal diet, the mobilization of endogenous stores of FA and the de novo synthesis of FA by breast tissue or liver. The study of the breast milk FA composition in obese or overweight populations is still limited.

It has been hypothesized that the content of the long-chain polyunsaturated fatty acids (LC-PUFA) in breast milk is related with obesity.

AIM

The aim of this study was to investigate the differences of the FA composition of colostrum and mature breast milk between obese, overweight and normoweight mothers from a Spanish population.

METHODS

PARTICIPANTS

Subgroup of mothers from PREOBE study (Clinical Trials Identifier: NCT01634464)[1] was included in this study. Maternal nutritional status (normoweight, overweight or obesity) was assessed based on the Body Mass Index (BMI).

We selected 13 obese (BMI ≥ 30 kg/m^2), 17 overweight (25 ≥ BMI < 30 kg/m^2) and 16 normoweight (18 ≥ BMI < 25 kg/m^2) mothers.

SAMPLES

Participants provided samples of colostrum (2–4 days after delivery) and mature breast milk (28–32 days after delivery).

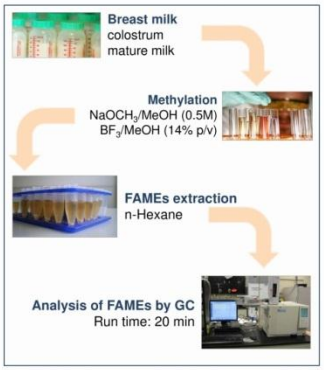
STATISTICAL ANALYSIS

To compare gestational age groups at each stage of lactation, an ANOVA (one-way) analysis was used. Differences between gestational age groups were subsequently identified by using the Bonferroni post-hoc test.

Differences associated with *p* values lower than 0.05 were considered to be statistically significant.

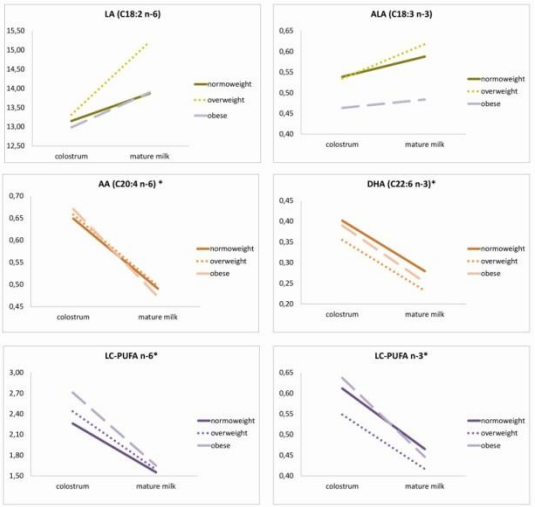
FATTY ACIDS DETERMINATION

Total fatty acids were analyzed by **fast-gas chromatography**[2].



RESULTS

Evolution of fatty acids in breast milk during lactation



Colostrum of the three groups, **normoweight, overweight and obese mother**, have higher levels of n-6 and n-3 LC-PUFA, especially **AA** and **DHA** in relation to mature breast milk (*p<0.05, colostrum *vs* mature breast milk).

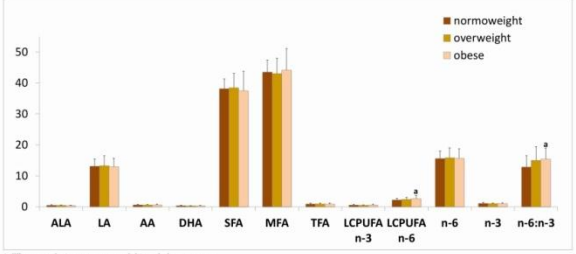
Groups of fatty acids: colostrum *vs* mature breast milk

	Normoweight			Overweight			Obese		
Σ FA	Colostrum	Mature milk		Colostrum	Mature milk		Colostrum	Mature milk	
	Mean ± SD	Mean ± SD	p	Mean ± SD	Mean ± SD	p	Mean ± SD	Mean ± SD	p
SFA	38,19 ± 3,13	37,14 ± 4,48	0,32	38,50 ± 4,61	37,24 ± 4,27	0,35	37,53 ± 6,29	40,35 ± 3,23	0,14
MFA	43,57 ± 3,85	44,92 ± 4,59	0,22	43,07 ± 4,94	43,53 ± 5,74	0,78	44,19 ± 6,93	41,77 ± 2,86	0,23
TFA	0,96 ± 0,28	0,92 ± 0,23	0,50	0,98 ± 0,23	0,85 ± 0,26	0,10	0,94 ± 0,33	0,84 ± 0,20	0,32
n-6:n-3	12,89 ± 3,63	15,48 ± 4,39	0,77	15,06 ± 4,41	17,34 ± 5,67	0,14	15,46 ± 3,56	17,46 ± 5,20	0,14

SFA (Saturated fatty acids). **MFA** (Monounsaturated fatty acid). **TFA** (Trans fatty acids). Mean (% of total fatty acids)

There were no significant differences for **SFA**, **MFA** and **TFA** between colostrum and mature breast milk within each group .

Fatty acids between groups in colostrum samples



[a] differences between normoweight and obese women

Total **n-6 LC-PUFA** and ratio **n-6:n-3** were significantly different between **obese and normoweight women** in colostrum samples. There were no differences in **mature breast milk** between the three groups.

CONCLUSIONS

Obese woman have higher levels of total n-6 LC-PUFA compared to normoweight mothers. The high consumption of foods sources of n-6 fatty acids may contribute to increment the risk of obesity.

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FINANCIAL SUPPORT

The authors thank the Spanish Ministry of Economy and Competitiveness for the predoctoral grant awarded to Aida Maribel Chisaguano, for a postdoctoral *Juan de la Cierva* fellowship awarded to Rosa Montes, and financial support for the research project BFU2012-40254-C03-02. We also thank the CIBER-Obn for the financial support of this work.

COMMUNICATION #13

PLASMA VITAMIN C IN NORMAL WEIGHT, OVERWEIGHT AND OBESE PREGNANT WOMEN AND OFFSPRING AT BIRTH

A. De la Garza[1], C. Marysael[1], M. E. Guerendiain[1], A.I. Castellote-Bargalló[1], R. Montes[1], M. Chisaguano[1], R. Moreno-Torres[2], L. García-Valdés[2], C. Campoy[2], M.C. López Sabater[1].

[1]Department of Nutrition and Food Science, Faculty of Pharmacy, University of Barcelona, Barcelona, Spain. [2]EURISTIKOS Excellence Centre for Pediatric Research, University of Granada. Granada, Spain.

Presentation Method: Poster presentation.
Congress: 14th International Nutrition & Diagnostics Conference
Place and Date: Prague, 2014.
*Chosen among the best 10 posters.

Background and aim: Vitamin C is an essential water-soluble vitamin of great interest due to its role in the prevention of oxidative stress, which is a key feature in the development of complications during pregnancy. The objective of our study was to evaluate plasma ascorbic acid (AA) concentration in pregnant women belonging to different body mass index (BMI) groups and to search for an association between plasma AA levels in both mother and child.

Methods: Plasma samples were obtained from 302 pregnant women divided in 3 different groups according to their BMI; normal weight, overweight and obesity. Samples were collected at 24, 34 and 40 weeks of gestation and a cord plasma sample from the fetus was collected at offspring along with anthropometric measures such as weight, length and head circumference. Plasma AA concentration was determined by a validated Ultra High Performance Liquid Chromatography (UHPLC) method.

Results: Plasma AA levels were significantly different between overweight and obese women at 24 weeks of gestation. No differences were observed for other periods even when compared with control group. Overweight women presented significant differences in plasma AA levels at all weeks of sample collection, and there was a tendency of AA values to decrease towards delivery. A positive correlation was found between the newborns and the AA plasma level of the mothers in all groups at 40 weeks of gestation. AA concentration of the newborns was always significantly higher than the mothers'.

Conclusions: AA plasma concentration of the newborns is related the mothers' at 40 weeks of gestation, independently of the maternal BMI.

Keywords: ascorbic acid, BMI, antioxidant, newborns.

PLASMA VITAMIN C IN NORMAL WEIGHT, OVERWEIGHT AND OBESE PREGNANT WOMEN AND OFFSPRING AT BIRTH

Universitat de Barcelona

A. de la Garza[1], C. Marysael[1], M. E. Guerendiain[1], A.I. Castellote-Bargalló[1], R. Montes[1], M. Chisaguano[1], R. Moreno-Torres[2], L. García-Valdés[2], C. Campoy[2], M.C. López-Sabater*[1].

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BACKGROUND

Vitamin C is an essential water-soluble vitamin of great interest in many scientific research topics because it has several important functions in the human organism. As humans have lost the ability to synthesize it, intake through nutrition has become mandatory.

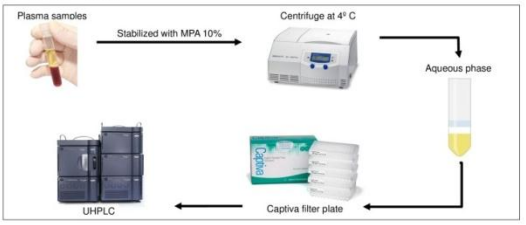
One of its main properties is as an antioxidant having an important role in the prevention of oxidative stress, which is a key feature in the development of complications during pregnancy. For instance, an increased body mass index (BMI) is associated with a higher amount of oxidative stress in the body. During pregnancy, this oxidative stress may lead to an increased risk of pre-eclampsia.

AIM

The objective of our study was to evaluate plasma ascorbic acid (AA) concentration in pregnant women belonging to different BMI groups and to search for an association between plasma AA levels in both mother and child.

METHODS

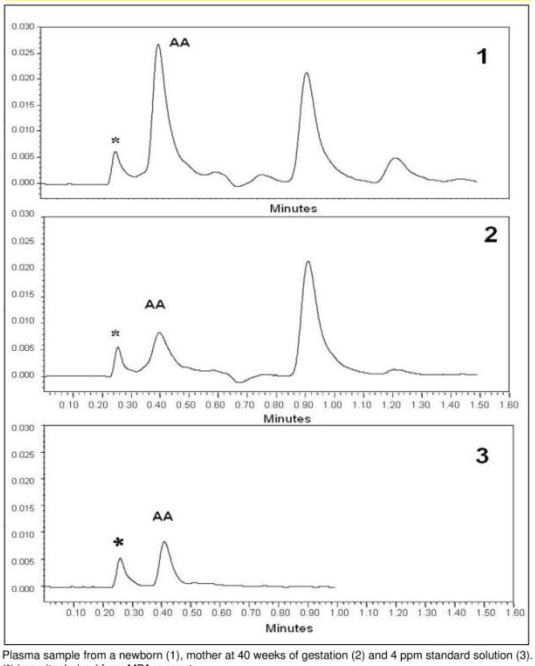
Plasma samples were obtained from 302 pregnant women divided in 3 different groups according to their BMI; normal weight, overweight and obesity. Samples were collected at 24, 34 and 40 weeks of gestation and a cord plasma sample from the fetus was collected at offspring along with anthropometric measures such as weight, length and head circumference. Plasma AA concentration was determined by a validated Ultra High Performance Liquid Chromatography (UHPLC) method.



RESULTS

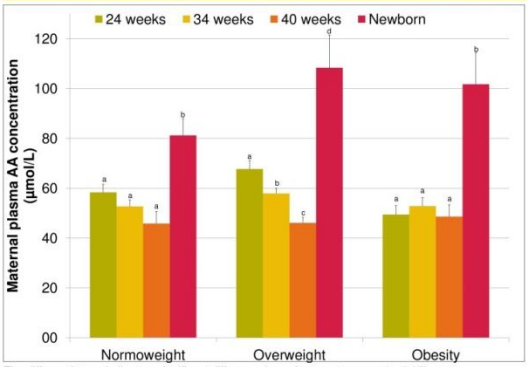
AA concentration of the newborns was always significantly higher than the mothers'. Overweight women presented significant differences in plasma AA levels at all weeks of sample collection, and there was a tendency of AA values to decrease towards delivery. Plasma AA levels were significantly different between overweight and obese women at 24 weeks of gestation. No differences were observed for other periods even when compared with control group. A positive correlation was found between the newborns and the AA plasma level of the mothers in all groups at 40 weeks of gestation.

AA DETERMINATION BY UHPLC



Plasma sample from a newborn (1), mother at 40 weeks of gestation (2) and 4 ppm standard solution (3).
(*) impurity derived from MPA reagent

PLASMA AA CONCENTRATION WITHIN EACH GROUP



The different letters indicate a significant difference in each separate group ($p<0.05$)

AA IN WEEK 24 OF GESTATION BETWEEN GROUPS

Dependent variables			Diff. Means	SD	p
AA 24 weeks of gestation	Normal weight	Overweight	-878.41	499.39	0.25
		Obesity	949.70	516.00	0.21
	Overweight	Normal weight	878.41	499.39	0.25
		Obesity	18.28*	519.49	**0.00***
	Obesity	Normal weight	-949.70	516.00	0.21
		Overweight	-18.28*	519.49	**0.00***

(*) $p < 0.05$ with confidence interval 95%

CORRELATION ACCORDING TO WEEKS OF GESTATION

Weeks	Newborn
24	0.53
34	0.087
40+	**0.248***

(*) p< 0.05
+ Naturally transformed log of 40 weeks of gestation

CONCLUSIONS

AA plasma concentration of the newborns is related the mothers' at 40 weeks of gestation, independently of the maternal BMI.

FUNDING

The authors thank the Spanish Ministry of Economy and Competitiveness (project BFU2012-40254-C03-02) and the CIBER-Obn for the financial support of this work. ADLG thanks the Nuevo León, México government and National Council on Science and Technology (CONACYT) for the PhD grant.

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10.2. PUBLICATIONS

10.2.1. PUBLICATION #1

PLOS ONE

RESEARCH ARTICLE

Association of maternal weight with *FADS* and *ELOVL* genetic variants and fatty acid levels- The PREOBE follow-up

Andrea de la Garza Puentes[1], Rosa Montes Goyanes[2,3], Aida Maribel Chisaguano Tonato[4], Francisco José Torres-Espínola[5,6], Miriam Arias García[5,6], Leonor de Almeida[1], María Bonilla Aguirre[1], Marcela Guerendiain[7], Ana Isabel Castellote Bargalló[1,3], Maite Segura Moreno[5,6], Luz García-Valdés[5,6], Cristina Campoy[5,6,8], M. Carmen Lopez-Sabater[1,3]*, PREOBE team[¶]

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¶ PREOBE team: Membership of the PREOBE group is provided in the Acknowledgments.
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OPEN ACCESS

Citation: de la Garza Puentes A, Montes Goyanes R, Chisaguano Tonato AM, Torres-Espínola FJ, Arias García M, de Almeida L, et al. (2017) Association of maternal weight with *FADS* and *ELOVL* genetic variants and fatty acid levels- The PREOBE follow-up. PLoS ONE 12(6): e0179135. https://doi.org/10.1371/journal.pone.0179135

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

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Abstract

Single nucleotide polymorphisms (SNPs) in the genes encoding the fatty acid desaturase (*FADS*) and elongase (*ELOVL*) enzymes affect long-chain polyunsaturated fatty acid (LC-PUFA) production. We aimed to determine if these SNPs are associated with body mass index (BMI) or affect fatty acids (FAs) in pregnant women. Participants (n = 180) from the PREOBE cohort were grouped according to pre-pregnancy BMI: normal-weight (BMI = 18.5–24.9, n = 88) and overweight/obese (BMI≥25, n = 92). Plasma samples were analyzed at 24 weeks of gestation to measure FA levels in the phospholipid fraction. Selected SNPs were genotyped (7 in *FADS1*, 5 in *FADS2*, 3 in *ELOVL2* and 2 in *ELOVL5*). Minor allele carriers of rs174545, rs174546, rs174548 and rs174553 (*FADS1*), and rs1535 and rs174583 (*FADS2*) were nominally associated with an increased risk of having a BMI≥25. Only for the normal-weight group, minor allele carriers of rs174537, rs174545, rs174546, and rs174553 (*FADS1*) were negatively associated with AA:DGLA index. Normal-weight women who were minor allele carriers of *FADS* SNPs had lower levels of AA, AA:DGLA and AA:LA indexes, and higher levels of DGLA, compared to major homozygotes. Among minor allele carriers of *FADS2* and *ELOVL2* SNPs, overweight/obese women showed higher DHA:EPA index than the normal-weight group; however, they did not present higher DHA concentrations than the normal-weight women. In conclusion, minor allele carriers of *FADS* SNPs have an increased risk of obesity. Maternal weight changes the effect of genotype on FA levels. Only in the normal-weight group, minor allele carriers of *FADS* SNPs displayed reduced enzymatic activity and FA levels. This suggests that women with a BMI≥25 are less affected by *FADS* genetic variants in this regard. In the presence of *FADS2* and *ELOVL2* SNPs,

data collection, data analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

overweight/obese women showed higher n-3 LC-PUFA production indexes than women with normal weight, but this was not enough to obtain a higher n-3 LC-PUFA concentration.

Introduction

During pregnancy, the mother is the sole source of key nutrients for the fetus, such as n-6 and n-3 long-chain (LC) polyunsaturated fatty acids (PUFAs) [1–3]. Studies have shown that arachidonic acid (AA; C20:4n−6) and particularly docosahexaenoic acid (DHA; C22:6n−3), play an important role in many physiological conditions [4], such as neural and visual development [1, 3, 5]. Hyperlipidemia develops in normal pregnancy; accordingly, it has been observed that levels of fatty acid (FA), such as DHA, increase in maternal plasma during pregnancy. Nevertheless, a higher activity of the enzymes encoded by the *FADS* and *ELOVL* genes could also be related to this hyperlipidemia [6]. Due to the beneficial effects of n-3 LC-PUFAs, it is recommended to increase their intake during pregnancy [4]. This is in contrast with the effect of excessive n-6 FA intake, which may lead to maternal obesity and obesity-related complications such as an increased risk of cardiovascular disease [7, 8]. Moreover, obese pregnancies lead to a higher risk of obesity in newborn babies, thereby increasing the likelihood of lifelong obesity and obesity-related complications [9, 10].

Apart from diet, LC-PUFAs can be obtained via endogenous synthesis from their essential n-6 and n-3 PUFA precursors: linoleic acid (LA) and α-linolenic acid (ALA), respectively [1] (S1 Fig). This process requires desaturation and elongation reactions, which are catalyzed by delta-5 and delta-6 fatty acid desaturases (encoded by *FADS*1 and *FADS*2 genes, respectively) and elongases (encoded by *ELOVL* gene (elongation of very long chain fatty acids)) [1, 11, 12]. The n-3 and n-6 series compete for these enzymes since they participate in both pathways. Single nucleotide polymorphisms (SNPs) in these genes may affect LC-PUFA production, and consequently alter FA levels [12]. For instance, minor allele carriers of the *FADS1*, *FADS2* and *ELOVL2* genes, have been linked to lower LC-PUFA production, and consequently they show increased concentrations of substrates and decreased levels of products in the LC-PUFA metabolic pathway [1, 12–15].

Certain SNPs in the *FADS* gene have been linked to diseases such as coronary artery disease [16] and type 2 diabetes [17]. Obesity is related to these conditions and is already known to affect lipid metabolism during pregnancy [18]; however, to the best of our knowledge, maternal obesity has not yet been directly linked to SNPs in the *FADS* and *ELOVL* genes.

Current studies analyzing the impact of *FADS* and *ELOVL* polymorphisms on FA levels are not properly comparable because their analyses include different tissue samples, FAs and SNPs. Given the impact of both obesity and SNPs on LC-PUFA production, the association between obesity and SNPs in *FADS* and *ELOVL* genes is of interest. These nutritional studies involving pregnant women are of great importance since the synthesis of LC-PUFA is induced in pregnancy and therefore the SNPs might have a different effect on pregnant women than on the general population. Thus, the aim of this study was to determine if *FADS* and *ELOVL* genetic variants are associated with body mass index (BMI) or affect PUFA levels in pregnant women.

Materials and methods

Study design and participants

The study complies with the Declaration of Helsinki. The protocol was approved by the medical ethics committees of the Clinical University Hospital San Cecilio and the Mother–Infant

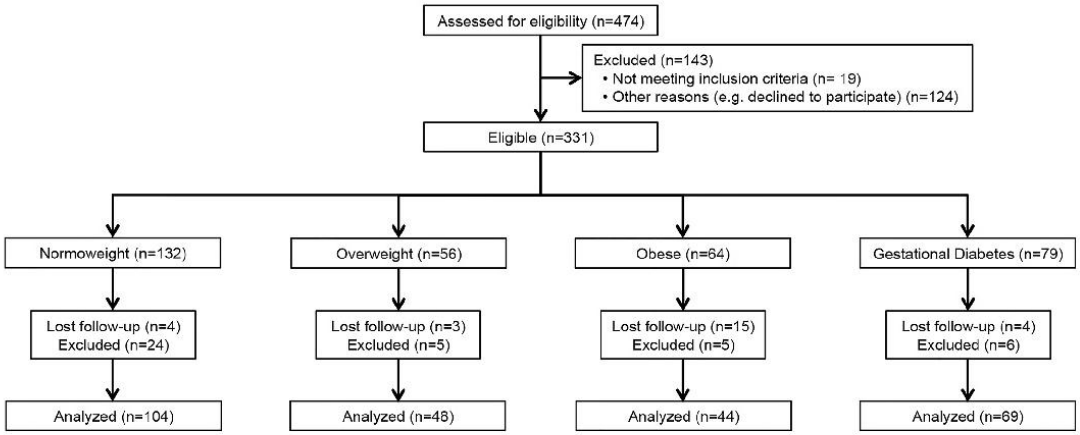

Fig 1. Participants in the PREOBE study and classification following BMI and gestational diabetes criteria.

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Hospital in the city of Granada, Spain. Written informed consent was obtained from all the participants at the beginning of the study.

Pregnant women (n = 180) were selected from the 331 individuals participating in the observational PREOBE cohort study (study of maternal nutrition and genetics on fetal adiposity programming) [19]. Participants were recruited at the Clinical University Hospital San Cecilio and the Mother–Infant Hospital in the city of Granada, Spain, where samples and information were also collected. Study design and information on PREOBE participants are exhibited in Fig 1.

The inclusion criteria were singleton pregnancy, gestation between 12 and 20 weeks at enrollment, and intention to deliver at the centers involved. Women were excluded if they were participating in other research studies, if they had been receiving drug treatment or supplements of either DHA or folate for more than the first 3 months of pregnancy, if they were suffering from disorders such as hypertension, pre-eclampsia, fetal intrauterine growth retardation, infections, hypo- or hyperthyroidism or hepatic renal diseases, or if they were following an extravagant or vegan diet. Using questionnaires and medical records, baseline and background characteristics were recorded such as maternal age, pre-pregnancy BMI, parity, smoking status, diet, alcohol drinking habits, socio-demographic information, educational level, and weight gain during pregnancy.

For this study, 180 pregnant women were divided into two groups according to their pre-pregnancy BMI, normal-weight (BMI = 18.5–24.9, n = 88) and overweight/obese (BMI≥25, n = 92). Plasma samples were collected at 24 weeks of gestation for FA analysis. Seventeen SNPs (7 in the *FADS1* gene, 5 in *FADS2*, 3 in *ELOVL2* and 2 in *ELOVL5*) out of the 32 initially selected were successfully genotyped and included in the analysis (S2 Table).

DNA analysis

Maternal material for DNA analysis was collected by scraping the inside of the cheek of the pregnant participants with a buccal swab. Thirty-two SNPs in genes involved in lipid metabolism (*FADS1*, *FADS2*, *FADS3*, *ELOVL2*, *ELOVL5*, *ELOVL6* and *FASN*) were initially genotyped from 5 μl of maternal DNA mixed with 5 μl of 2X TaqMan® OpenArray® Genotyping Master

Mix. The analysis was then performed with 3 μl of the mixture in a microplate using Taqman® Open Array® genotyping technology. The OpenArray® instrument (which consists of a Dual Flat Block GeneAmp® PCR System 9700, Bio-Rad® thermal cycler with a Slide Chambers Dual-Block Alpha unit and Thermo Electron PX2 thermal cycler) and the corresponding OpenArray® SNP Genotyping Analysis software, located at the *Autonomous University of Barcelona* (UAB), were used for the analysis. The genotyping required two phases: a thermal cycle (PCR amplification) and detection of the final fluorescence signal. The reagents used were supplied by Applied Biosystem (Foster City, CA, USA).

Fatty acid analysis

At 24 weeks of gestation, blood was obtained by arm venipuncture. Plasma was separated by centrifugation and immediately frozen and stored at -80˚C until analysis. FAs were determined in the phospholipid fraction using the method developed by Chisaguano et al. [20]. Plasma lipids were extracted using 2:1 dichloromethane:methanol and phospholipids were isolated using solid-phase extraction (SPE). FA methyl esters from plasma phospholipids were analyzed using fast gas chromatography with a flame ionization detector. The results were expressed as percentages of the total FAs detected.

We analyzed the FAs involved in enzymatic reactions encoded by the *FADS* and *ELOVL* genes (substrates, products or indexes (product/substrates)). Furthermore, we studied the n3:n6 ratios (eicosapentaenoic acid (EPA):AA and DHA:AA), associated with obesity risk [7, 21–23].

Statistical analysis

Statistical analysis was performed using the SPSS software (version 20.0; SPSS Inc., Chicago, IL, USA). The Kolmogorov–Smirnov test was used to study the normal distribution of data; non-normal data were natural-log transformed. The agreement of genotype frequencies with Hardy-Weinberg equilibrium expectations was tested by the chi-square test. Due to the limited sample size, heterozygotes and homozygous for minor alleles, were analyzed as one group. SNPs were coded according to minor allele count (0 for major homozygotes and 1 for the carriers of at least one minor allele) and analyzed as a numerical variable. However, this codification implies an additive and dominant model. The associations between SNPs and FAs were analyzed using linear regression; while the associations between SNPs and obesity were analyzed by logistic regression. All associations were corrected for potential confounders such as age, education, smoking status and energy intake. FA levels were compared using univariate ANOVA. Since DHA supplementation was largely absent and it did not affect DHA levels in plasma, supplementation data were omitted for parsimony. The Bonferroni correction was applied to take multiple testing into account and p-value thresholds were set at 0.004, which was applied within each trait.

Results

Population characteristics

The characteristics of the groups are shown in Table 1. Normal-weight women were more likely to have a higher level of education and take DHA supplementation; while the overweight/obese group had a lower energy intake and weight gain during pregnancy. No differences were observed in dietary FA levels. S1 Table shows a comparison of plasma DHA concentrations at 24 weeks of gestation between pregnant women who took DHA supplementation and those who did not. After finding that the participants who reported taking DHA supplement (n = 17) did not have higher serum DHA concentrations (p = 0.636) than the

Table 1. Characteristics of the population.

	NORMAL-WEIGHT (n = 88)	OVERWEIGHT/OBESE (n = 92)	P
	Mean (SD)	Mean (SD)	
Age (years)	30.91 (4.09)	30.64 (4.20)	0.665
Pre-pregnancy BMI (kg/m2)	22.00 (1.64)	30.33 (4.20)	**<0.001***
Weight Gain (kg)	12.64 (5.47)	9.19 (6.29)	**0.001***
Education (%)			
<Highschool	10.23%	15.38%	0.401
Highschool	15.91%	19.78%	
>Highschool	73.86%	64.84%	
Smoking during pregnancy (%)	21.92%	27.27%	0.447
DHA supplementation during pregnancy (%)	25.00%	7.61%	**0.002***
Dietary intakes			
Energy intake (kcal/d)	2177.54 (335.41)	1938.07 (591.28)	**0.017**
EPA intake (g/d)	0.13 (0.13)	0.12 (0.10)	0.713
DHA intake (g/d)	0.25 (0.20)	0.27 (0.18)	0.646
AA intakes (g/d)	0.13 (0.07)	0.13 (0.06)	0.98

P-value derived from global ANOVA and significance level ($p \leq 0.004$) was adjusted for multiple testing by Bonferroni correction. P-values <0.05 are highlighted in bold and significant associations that persisted after Bonferroni correction are additionally denoted by stars (*$p < 0.004$). BMI: body mass index; AA: arachidonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

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participants who did not take supplements (n = 93), we decided to include all the subjects in the analysis regardless of supplement use.

S2 Table presents the characteristics of the studied SNPs, including the distribution of participants in each allele group (major homozygotes, heterozygotes and minor homozygotes). Normal-weight women tended to be major homozygotes for all the *FADS1* and *FADS2* SNPs studied, while women in the overweight/obese group were mostly heterozygotes. Regarding *ELOVL2* SNPs, both groups presented mostly heterozygous alleles, and no pattern was observed for *ELOVL5* SNPs.

Association between SNPs and fatty acids

Table 2 shows nominal and significant associations between PUFA levels and *FADS* and *ELOVL* SNPs after adjusting for age, education, smoking status and energy intake (the complete analysis can be found in S3 Table). The most significant associations ($p \leq 0.004$) were only found in the normal-weight group, where minor allele carriers of rs174537, rs174545, rs174546 and rs174553 (*FADS1*) were negatively associated with the AA:dihomo-gamma-linolenic acid (DGLA) index.

Association of *FADS* SNPs with obesity risk

After adjusting for age, education, smoking status and energy intake, participants who were minor allele carriers of rs174545, rs174546, rs174548 and rs174553 (*FADS1*), and rs1535 and rs174583 (*FADS2*) were nominally associated ($p < 0.05$) with an increased risk of having a BMI$\geq$25, compared with two major allele carriers (Table 3).

Fatty acid levels according to genotype and weight

Plasma LC-PUFA levels according to genotypes are presented in Tables 4 and 5 (the complete analysis can be found in S4 Table). Significant differences were found for normal-weight

Table 2. Associations between plasma proportions of PUFAs and *FADS* and *ELOVL* polymorphisms.

Fatty acid	Gene	SNP *Major/minor allele*		NORMAL-WEIGHT			OVERWEIGHT/OBESE		
				N	β	P	N	β	P
***FADS1* indexes**									
AA:DGLA									
	FADS1	rs174537	*G/T*	22	-0.81	**0.004***	18	-0.48	0.155
	FADS1	rs174545	*C/G*	28	-0.70	**0.003***	17	-0.48	0.174
	FADS1	rs174546	*C/T*	28	-0.70	**0.003***	19	-0.49	0.139
	FADS1	rs174553	*A/G*	28	-0.70	**0.003***	19	-0.49	0.139
	FADS1	rs174547	*T/C*	23	-0.67	**0.013**	18	-0.48	0.155
***FADS2* indexes**									
DGLA:LA									
	FADS2	rs1535	*A/G*	26	0.56	**0.029**	18	0.42	0.226
	FADS2	rs174583	*C/T*	27	0.53	**0.040**	19	0.41	0.226
	FADS2	rs99780	*C/T*	25	0.54	**0.042**	18	0.42	0.226
***ELOVL2* indexes**									
DHA:DPAn3									
	ELOVL2	rs2236212	*G/C*	25	-0.05	0.806	19	-0.58	**0.016**
	ELOVL2	rs3798713	*G/C*	25	0.00	0.995	19	-0.58	**0.016**
Fatty acids involved in *FADS1* indexes									
C20:3n6 (DGLA)									
	FADS1	rs174537	*G/T*	22	0.75	**0.012**	18	0.53	0.119
	FADS1	rs174545	*C/G*	28	0.57	**0.028**	17	0.52	0.137
	FADS1	rs174546	*C/T*	28	0.57	**0.028**	19	0.52	0.118
	FADS1	rs174553	*A/G*	28	0.57	**0.028**	19	0.52	0.118
	FADS1	rs174547	*T/C*	23	0.60	**0.034**	18	0.53	0.119
C20:4n6 (AA)									
	FADS1	rs174545	*C/G*	28	-0.55	**0.042**	17	-0.01	0.977
	FADS1	rs174546	*C/T*	28	-0.55	**0.042**	19	-0.01	0.971
	FADS1	rs174553	*A/G*	28	-0.55	**0.042**	19	-0.01	0.971
C18:3n3 (ALA)									
	FADS1	rs174537	*G/T*	22	0.71	**0.027**	18	-0.09	0.790
	FADS1	rs174547	*T/C*	23	0.66	**0.026**	18	-0.09	0.790
Fatty acids involved in *FADS2* indexes									
C18:3n6 (GLA)									
	FADS2	rs174575	*C/G*	22	0.12	0.672	19	0.64	**0.031**
C20:3n6 (DGLA)									
	FADS2	rs1535	*A/G*	26	0.65	**0.010**	18	0.53	0.119
	FADS2	rs174583	*C/G*	27	0.62	**0.015**	19	0.52	0.118
C18:3n3 (ALA)									
	FADS2	rs174575	*C/G*	23	0.50	**0.043**	19	0.22	0.494
C20:5n3 (EPA)									
	FADS2	rs99780	*C/T*	25	0.52	**0.035**	17	0.11	0.749
C22:5n3 (DPAn3)									
	FADS2	rs99780	*C/T*	25	0.62	**0.011**	18	-0.41	0.173

Associations between SNPs and fatty acids were analyzed using linear regression. SNPs were coded according to minor allele count and analyzed as a numeric variable. "β" = beta per minor allele standardized per the major allele. All associations were adjusted for potential confounders such as age, education, smoking and energy intake. P-values <0.05 are highlighted in bold and significant associations that persisted after Bonferroni correction are additionally denoted by stars (*$p \leq 0.004$). LA: Linoleic Acid; GLA: γ-Linolenic Acid; DGLA: Dihomo-γ-Linolenic Acid; AA: Arachidonic Acid; ALA: α-linolenic Acid; EPA: Eicosapentaenoic acid; DPAn3: Docosapentaenoic acid n3; DHA: Docosahexaenoic acid.

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Table 3. Associations between *FADS* and *ELOVL* genes and maternal obesity.

Gene	SNP *Major/Minor alleles*		N	BMI≥25		BMI≥25c	
				OR	P	ORc	Pc
FADS1							
	rs174537	*G/T*	66	2.12	0.147	2.89	0.069
	rs174545	*C/G*	79	2.34	0.074	3.11	**0.032**
	rs174546	*C/T*	81	2.55	**0.047**	3.28	**0.025**
	rs174548	*C/G*	82	3.11	**0.015**	3.18	**0.021**
	rs174553	*A/G*	82	2.64	**0.039**	3.44	**0.019**
	rs174561	*T/C*	44	0.56	0.351	0.43	0.217
	rs174547	*T/C*	66	1.96	0.191	2.75	0.082
FADS2							
	rs1535	*A/G*	77	2.58	**0.048**	3.42	**0.025**
	rs174575	*C/G*	70	1.57	0.351	1.77	0.273
	rs174583	*C/T*	79	2.62	**0.046**	3.38	**0.024**
	rs99780	*C/T*	71	1.75	0.261	2.37	0.122
	rs174602	*T/C*	46	1.55	0.475	1.18	0.812
ELOVL2							
	rs2236212	*G/C*	76	0.76	0.568	0.88	0.805
	rs3798713	*G/C*	75	0.67	0.430	0.81	0.708
	rs953413	*A/G*	58	1.00	1.000	0.58	0.425
ELOVL5							
	rs2397142	*C/G*	79	0.78	0.573	0.75	0.556
	rs9395855	*T/G*	54	0.50	0.316	0.50	0.345

The association between SNPs and obesity risk was analyzed with logistic regression. SNPs were coded according to minor allele count and analyzed as numeric variable. BMI = Body Mass Index. OR = odds ratio per minor allele with the major allele as reference. ORc and Pc = are corrected values after adjustment for age, education, smoking and energy intake. Nominal associations are highlighted in bold ($p<0.05$).

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women: minor allele carriers of *FADS1* SNPs showed lower AA level and both AA:DGLA and AA:LA indexes than major homozygotes; while minor allele carriers of *FADS2* SNPs showed lower AA and higher DGLA levels ($p\leq0.004$). Overweight/obese women presented the same tendencies, but without the results reaching statistical significance. No differences were found between genotypes of *ELOVL* SNPs.

Significant differences were also shown when comparing groups of weight ($p\leq0.004$). Among the minor allele carriers of rs1535, rs174583 and rs99780 (*FADS2*), and rs2236212, rs3798713 and rs953413 (*ELOVL2*), overweight/obese women showed a higher DHA:EPA index than those in the normal-weight group (Table 4). Meanwhile, among minor alleles carriers of rs174537, rs174545, rs174546, rs174553 and rs174547 (*FADS1*), and rs1535, rs174575, rs174583 and rs99780 (*FADS2*), normal-weight women had higher levels of the substrate ALA than those in the overweight/obese group. In addition, among the major homozygotes of rs2397142 (*ELOVL5*), normal-weight women presented higher levels of EPA than women who were overweight/obese (Table 5).

Maternal plasma n3:n6 ratios, according to genotype, are presented in S5 Table. Among the minor allele carriers of rs3798713 (*ELOVL2*), normal-weight women had significantly higher EPA:AA ratio than those who were overweight/obese. This trend was also found in all gene clusters studied, even when comparing major homozygotes.

Table 4. *FADS1*, *FADS2 and ELOVL2* enzymatic indexes according to maternal SNPs and LC-PUFA levels in plasma.

Gene	SNP *Major/minor allele*		NORMAL-WEIGHT					OVERWEIGHT/OBESITY					P (*MM*)	P (*Mm+mm*)
			MM		*Mm+mm*		P	*MM*		*Mm+mm*		P		
			N	Mean (SD)	N	Mean (SD)		N	Mean (SD)	N	Mean (SD)			
***FADS1* indexes**														
AA:LA														
FADS1	rs174537	*G/T*	15	0.45 (0.08)	23	0.39 (0.09)	**0.038**	12	0.46 (0.07)	21	0.43 (0.08)	0.390	0.811	0.085
FADS1	rs174545	*C/G*	22	0.46 (0.09)	27	0.38 (0.08)	**0.003***	13	0.47 (0.07)	22	0.42 (0.08)	0.071	0.877	0.171
FADS1	rs174546	*C/T*	22	0.46 (0.09)	27	0.38 (0.08)	**0.003***	13	0.47 (0.07)	25	0.42 (0.08)	0.098	0.877	0.109
FADS1	rs174548	*C/G*	24	0.45 (0.08)	25	0.39 (0.09)	**0.031**	13	0.45 (0.08)	25	0.43 (0.08)	0.553	0.969	0.115
FADS1	rs174553	*A/G*	22	0.46 (0.09)	27	0.38 (0.08)	**0.003***	13	0.47 (0.07)	25	0.42 (0.08)	0.098	0.877	0.109
FADS1	rs174547	*T/C*	15	0.45 (0.08)	23	0.39 (0.09)	**0.040**	12	0.46 (0.07)	20	0.43 (0.08)	0.427	0.950	0.124
AA:DGLA														
FADS1	rs174537	*G/T*	15	3.14 (0.70)	23	2.20 (0.60)	**<0.001***	12	3.57 (1.73)	21	2.55 (0.80)	**0.032**	0.414	0.107
FADS1	rs174545	*C/G*	22	3.12 (0.65)	27	2.25 (0.57)	**<0.001***	13	3.48 (1.67)	22	2.51 (0.78)	**0.025**	0.362	0.183
FADS1	rs174546	*C/T*	22	3.12 (0.65)	27	2.25 (0.57)	**<0.001***	13	3.48 (1.67)	25	2.54 (0.74)	**0.021**	0.362	0.120
FADS1	rs174548	*C/G*	24	2.97 (0.65)	25	2.33 (0.70)	**0.002***	13	3.42 (1.69)	25	2.57 (0.75)	**0.037**	0.244	0.246
FADS1	rs174553	*A/G*	22	3.12 (0.65)	27	2.25 (0.57)	**<0.001***	13	3.48 (1.67)	25	2.54 (0.74)	**0.021**	0.362	0.120
FADS1	rs174547	*T/C*	15	3.20 (0.67)	23	2.27 (0.55)	**<0.001***	12	3.54 (1.73)	20	2.56 (0.82)	**0.037**	0.291	0.194
***FADS2* indexes**														
DGLA:LA														
FADS2	rs1535	*A/G*	20	0.15 (0.04)	25	0.18 (0.05)	**0.011**	13	0.15 (0.04)	23	0.18 (0.05)	0.115	0.877	0.798
FADS2	rs174575	*C/G*	24	0.15 (0.04)	16	0.19 (0.06)	**0.017**	21	0.17 (0.06)	14	0.17 (0.05)	0.943	0.276	0.315
FADS2	rs174583	*C/T*	19	0.15 (0.04)	27	0.18 (0.05)	**0.036**	13	0.15 (0.04)	25	0.18 (0.05)	0.130	0.967	0.907
FADS2	rs99780	*C/T*	15	0.15 (0.04)	26	0.18 (0.05)	**0.037**	12	0.15 (0.05)	22	0.18 (0.05)	0.076	0.953	0.961
AA:LA														
FADS2	rs1535	*A/G*	20	0.45 (0.08)	25	0.39 (0.09)	**0.025**	13	0.47 (0.07)	23	0.42 (0.08)	0.128	0.504	0.170
FADS2	rs174583	*C/T*	19	0.44 (0.08)	27	0.38 (0.08)	**0.019**	13	0.47 (0.07)	25	0.42 (0.08)	0.098	0.447	0.109
DHA:EPA														
FADS2	rs1535	*A/G*	20	15.02 (5.64)	25	14.66 (5.92)	0.840	13	19.69 (6.63)	23	20.89 (7.87)	0.655	**0.045**	**0.004†**
FADS2	rs174575	*C/G*	24	16.41 (5.99)	16	13.41 (5.21)	0.119	21	20.99 (6.36)	14	20.27 (8.32)	0.778	**0.018**	**0.012**
FADS2	rs174583	*C/T*	19	15.02 (5.64)	27	14.48 (5.71)	0.753	13	19.69 (6.63)	25	20.58 (7.89)	0.737	**0.045**	**0.003†**
FADS2	rs99780	*C/T*	15	16.22 (5.52)	26	13.71 (4.76)	0.138	12	20.50 (6.29)	22	20.19 (8.14)	0.911	0.078	**0.002†**
FADS2	rs174602	*T/C*	19	16.06 (5.17)	13	14.76 (6.12)	0.531	23	19.97 (6.82)	14	18.02 (6.37)	0.393	**0.046**	0.199
DHA:DPAn3														
FADS2	rs1535	*A/G*	20	10.84 (1.96)	25	10.03 (2.40)	0.232	13	11.14 (2.26)	23	11.58 (2.26)	0.577	0.687	**0.027**
FADS2	rs174575	*C/G*	24	10.58 (2.21)	16	10.15 (2.39)	0.574	21	11.23 (2.29)	14	12.20 (2.28)	0.230	0.334	**0.026**
FADS2	rs174583	*C/T*	19	10.76 (1.98)	27	10.13 (2.33)	0.350	13	11.14 (2.26)	25	11.53 (2.50)	0.642	0.613	**0.044**
FADS2	rs99780	*C/T*	15	11.04 (2.07)	26	9.83 (2.17)	0.090	12	11.42 (2.11)	22	11.41 (2.45)	0.990	0.640	**0.024**
***ELOVL2* indexes**														
DPAn3:EPA														
ELOVL2	rs2236212	*G/C*	14	1.51 (0.51)	29	1.48 (0.67)	0.876	13	1.66 (0.71)	22	1.88 (0.71)	0.388	0.530	**0.047**
ELOVL2	rs3798713	*G/C*	11	1.48 (0.52)	31	1.44 (0.67)	0.863	12	1.67 (0.74)	25	1.88 (0.70)	0.410	0.476	**0.021**
DHA:EPA														
ELOVL2	rs2236212	*G/C*	14	16.39 (6.29)	28	14.45 (5.43)	0.307	13	20.58 (8.33)	22	20.05 (7.02)	0.841	0.151	**0.003†**
ELOVL2	rs3798713	*G/C*	11	15.48 (5.10)	30	13.96 (5.33)	0.418	12	20.76 (8.67)	24	20.13 (7.07)	0.819	0.094	**<0.001†**
ELOVL2	rs953413	*A/G*	10	15.33 (5.93)	25	14.78 (5.23)	0.789	7	21.14 (7.60)	20	20.88 (7.57)	0.939	0.096	**0.003†**
DHA:DPAn3														
ELOVL2	rs2236212	*G/C*	14	10.87 (2.20)	29	10.14 (2.32)	0.337	13	12.57 (1.53)	22	10.77 (2.67)	**0.032**	**0.029**	0.375

(*Continued*)

Table 4. (*Continued*)

Gene	SNP Major/minor allele		NORMAL-WEIGHT					OVERWEIGHT/OBESITY					P (*MM*)	P (*Mm+mm*)
			MM		*Mm+mm*		P	*MM*		*Mm+mm*		P		
			N	Mean (SD)	N	Mean (SD)		N	Mean (SD)	N	Mean (SD)			
ELOVL2	rs3798713	*G/C*	11	10.68 (2.24)	31	10.10 (2.20)	0.460	12	12.64 (1.58)	25	10.78 (2.55)	**0.028**	**0.024**	0.291
ELOVL2	rs953413	*A/G*	10	9.83 (2.22)	26	10.38 (1.99)	0.478	7	10.68 (2.80)	20	12.04 (2.09)	0.183	0.493	**0.008**

P-value derived from global ANOVA and significance level ($p \leq 0.004$) was adjusted for multiple testing by Bonferroni correction. Data are means of FAs expressed as percentages of the total phospholipid profile (standard error). P-values <0.05 are highlighted in bold and significant associations that persisted after Bonferroni correction are additionally denoted by stars or daggers ($p \leq 0.004$). *Indicates significant differences within each group of weight and † Indicates significant differences between groups of weight. Major allele: M; minor allele: m; LA: Linoleic Acid; GLA: γ-Linolenic Acid; DGLA: Dihomo-γ-Linolenic Acid; AA: Arachidonic Acid; AdA: Adrenic Acid; DPAn6: Docosapentaenoic acid n6; ALA: α-linolenic Acid; EPA: Eicosapentaenoic acid; DPAn3: Docosapentaenoic acid n3; DHA: Docosahexaenoic acid.

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Discussion

The present study analyzed the effect of *FADS* and *ELOVL* genetic variants on a broad FA profile. To the best of our knowledge, this is the first study to explore associations between *FADS* and *ELOVL* SNPs, FA levels and maternal pre-pregnancy weight. Despite a lack of studies analyzing the association between obesity risk and *FADS* or *ELOVL* polymorphisms, some authors have observed that minor alleles of rs174547 (*FADS1*) confer a higher risk of obesity-related conditions, such as increased triglyceride levels and decreased high-density lipoprotein cholesterol concentrations [24–26], as well as an increased risk of coronary disease [26, 27]. In the present study, we found that women who carried at least one minor allele of the *FADS1* and *FADS2* SNPs, were associated with a higher risk of having a BMI≥25 than homozygotes for the major allele. This association could explain why most of the overweight/obese women carried one minor allele copy and normal-weight women were mostly homozygous for the major alleles. Both weight groups showed very similar distributions within the allele groups of *ELOVL* SNPs; thus, we did not expect to find any associations between *ELOVL* genotypes and weight.

In line with other studies [5, 12], we found that the *FADS1* and *FADS2* SNPs were associated with FAs, mainly from the n-6 series, and less with those from the n-3 series. The only significant association (p-value ≤0.004) was found in the normal-weight group, where minor alleles of *FADS1* were associated with a lower AA:DGLA index. Several nominal associations were also found; nevertheless, they were mainly in normal-weight women and the n-6 series. Regarding the overweight/obese group, minor allele carriers of rs2236212 and rs3798713 (*ELOVL2)* were nominally associated with a lower DHA:n-3 docosapentaenoic acid (DPAn3) index. Barman et al. observed results similar to this last association, but their significance did not persist after correction either [12].

We also observed that *FADS* genetic variants affected FA concentrations. Normal-weight women who were minor allele carriers of *FADS1* SNPs had significantly lower levels of product (AA) and indexes (AA:LA and AA:DGLA) than major homozygotes. Consistently with this, nominal differences were also observed; minor allele carriers of *FADS1* SNPs showed nominally lower substrates (DGLA and ALA). This was previously reported by other authors [1, 5, 12, 13, 28, 29], who observed that minor allele carriers in *FADS* displayed lower FA indexes and products, and increased amounts of substrates. Overweight/obese women with *FADS1* SNPs showed the same trends and some nominal differences in DGLA and AA:DGLA, but none of them with a p-value ≤0.004. Other studies [1, 28, 29] have also found that *FADS2* SNPs were related to lower levels of AA and lower AA:LA index. This supports our findings

Table 5. Substrates and products of enzymatic indexes according to maternal SNPs and LC-PUFA levels in plasma.

Gene	SNP *Major/minor allele*		NORMAL-WEIGHT					OVERWEIGHT/OBESITY					P (*MM*)	P (*Mm+mm*)
			MM		*Mm+mm*		P	*MM*		*Mm+mm*		P		
			N	Mean (SD)	N	Mean (SD)		N	Mean (SD)	N	Mean (SD)			
Fatty acids involved in *FADS1* indexes														
C20:3n-6 (DGLA)														
FADS1	rs174537	*G/T*	15	3.51 (0.81)	23	4.27 (0.85)	**0.010**	12	3.31 (0.80)	21	4.18 (0.96)	**0.012**	0.529	0.754
FADS1	rs174545	*C/G*	22	3.50 (0.79)	27	4.14 (0.86)	**0.009**	13	3.39 (0.81)	22	4.15 (0.94)	**0.021**	0.691	0.976
FADS1	rs174546	*C/T*	22	3.50 (0.79)	27	4.14 (0.86)	**0.009**	13	3.39 (0.81)	25	4.11 (0.90)	**0.021**	0.691	0.893
FADS1	rs174548	*C/G*	24	3.59 (0.79)	25	4.10 (0.93)	**0.041**	13	3.38 (0.81)	25	4.11 (0.90)	**0.019**	0.431	0.969
FADS1	rs174553	*A/G*	22	3.50 (0.79)	27	4.14 (0.86)	**0.009**	13	3.39 (0.81)	25	4.11 (0.90)	**0.021**	0.691	0.893
FADS1	rs174547	*T/C*	15	3.61 (0.78)	23	4.17 (0.76)	**0.036**	12	3.31 (0.80)	20	4.17 (0.98)	**0.016**	0.335	0.980
C20:4n-6 (AA)														
FADS1	rs174537	*G/T*	15	10.55 (1.33)	23	9.02 (1.54)	**0.003***	12	10.53 (0.89)	21	10.04 (1.36)	0.273	0.973	**0.026**
FADS1	rs174545	*C/G*	22	10.50 (1.39)	27	8.96 (1.49)	**0.001***	13	10.64 (0.93)	22	9.83 (1.31)	0.061	0.759	**0.037**
FADS1	rs174546	*C/T*	22	10.50 (1.39)	27	8.96 (1.49)	**0.001***	13	10.64 (0.93)	25	9.90 (1.31)	0.080	0.759	**0.019**
FADS1	rs174548	*C/G*	24	10.28 (1.39)	25	9.05 (1.64)	**0.007**	13	10.40 (1.02)	25	10.03 (1.33)	0.389	0.790	**0.025**
FADS1	rs174553	*A/G*	22	10.50 (1.39)	27	8.96 (1.49)	**0.001***	13	10.64 (0.93)	25	9.90 (1.31)	0.080	0.759	**0.019**
FADS1	rs174561	*T/C*	15	9.88 (1.32)	14	9.30 (1.12)	0.212	20	10.27 (1.48)	17	10.33 (1.54)	0.910	0.423	**0.046**
FADS1	rs174547	*T/C*	15	10.49 (1.33)	23	9.18 (1.47)	**0.009**	12	10.53 (0.89)	20	10.00 (1.38)	0.243	0.917	0.067
C18:3n-3 (ALA)														
FADS1	rs174537	*G/T*	15	0.12 (0.04)	23	0.16 (0.04)	**0.021**	12	0.11 (0.03)	21	0.11 (0.04)	0.632	0.496	**<0.001†**
FADS1	rs174545	*C/G*	22	0.12 (0.04)	27	0.15 (0.04)	**0.018**	13	0.12 (0.03)	22	0.11 (0.04)	0.532	0.536	**0.001†**
FADS1	rs174546	*C/T*	22	0.12 (0.04)	27	0.15 (0.04)	**0.018**	13	0.12 (0.03)	25	0.11 (0.04)	0.580	0.536	**0.001†**
FADS1	rs174548	*C/G*	24	0.13 (0.04)	22	0.15 (0.04)	0.146	12	0.12 (0.03)	25	0.11 (0.04)	0.454	0.371	**0.001**
FADS1	rs174553	*A/G*	22	0.12 (0.04)	27	0.15 (0.04)	**0.018**	13	0.12 (0.03)	25	0.11 (0.04)	0.580	0.536	**0.001†**
FADS1	rs174561	*T/C*	15	0.13 (0.04)	14	0.13 (0.03)	0.695	20	0.12 (0.05)	17	0.11 (0.03)	0.497	0.397	**0.024**
FADS1	rs174547	*T/C*	15	0.12 (0.04)	23	0.15 (0.04)	**0.027**	12	0.11 (0.03)	20	0.11 (0.04)	0.734	0.444	**<0.001†**
C20:5n3 (EPA)														
FADS1	rs174561	*T/C*	15	0.33 (0.14)	13	0.31 (0.11)	0.683	20	0.26 (0.11)	17	0.23 (0.10)	0.491	0.092	**0.044**
Fatty acids involved in *FADS2* indexes														
C20:3n-6 (DGLA)														
FADS2	rs1535	*A/G*	20	3.41 (0.77)	25	4.23 (0.82)	**0.001***	13	3.39 (0.81)	23	4.15 (0.92)	**0.019**	0.927	0.736
FADS2	rs174575	*C/G*	24	3.57 (0.71)	16	4.28 (0.97)	**0.012**	21	3.82 (1.30)	14	3.90 (0.90)	0.805	0.358	0.283
FADS2	rs174583	*C/T*	19	3.47 (0.75)	27	4.14 (0.86)	**0.009**	13	3.39 (0.81)	25	4.11 (0.90)	**0.021**	0.762	0.893
FADS2	rs99780	*C/T*	15	3.51 (0.78)	26	4.16 (0.89)	**0.025**	12	3.35 (0.84)	22	4.14 (0.94)	**0.022**	0.613	0.941
FADS2	rs174602	*T/C*	19	3.77 (0.82)	13	3.50 (0.85)	0.387	23	3.95 (0.76)	14	4.19 (0.54)	0.297	0.463	**0.018**
C20:4n-6 (AA)														
FADS2	rs1535	*A/G*	20	10.31 (1.30)	25	9.07 (1.50)	**0.005**	13	10.64 (0.93)	23	9.92 (1.35)	0.102	0.439	**0.044**
FADS2	rs174583	*C/T*	19	10.33 (1.34)	27	8.96 (1.49)	**0.003***	13	10.64 (0.93)	25	9.90 (1.31)	0.080	0.482	**0.019**
FADS2	rs99780	*C/T*	15	10.39 (1.48)	26	9.15 (1.51)	**0.015**	12	10.54 (0.91)	22	10.02 (1.40)	0.254	0.757	**0.046**
C18:3n-3 (ALA)														
FADS2	rs1535	*A/G*	20	0.13 (0.04)	25	0.15 (0.04)	**0.025**	13	0.12 (0.03)	23	0.11 (0.04)	0.448	0.500	**<0.001†**
FADS2	rs174575	*C/G*	24	0.13 (0.04)	16	0.16 (0.04)	**0.013**	21	0.11 (0.03)	14	0.11 (0.03)	0.972	**0.035**	**<0.001†**
FADS2	rs174583	*C/T*	19	0.13 (0.04)	27	0.15 (0.04)	**0.033**	13	0.12 (0.03)	25	0.11 (0.04)	0.580	0.498	**0.001†**
FADS2	rs99780	*C/T*	15	0.13 (0.04)	26	0.15 (0.04)	0.060	12	0.12 (0.03)	22	0.11 (0.04)	0.584	0.576	**0.001†**
C20:5n-3 (EPA)														
FADS2	rs174602	*T/C*	19	0.31 (0.14)	12	0.32 (0.10)	0.780	23	0.23 (0.09)	14	0.27 (0.13)	0.347	**0.040**	0.241
C22:5n-3 (DPAn3)														

(*Continued*)

Table 5. (*Continued*)

Gene	SNP *Major/minor allele*		NORMAL-WEIGHT					OVERWEIGHT/OBESITY					P (*MM*)	P (*Mm+mm*)
			MM		*Mm+mm*		P	*MM*		*Mm+mm*		P		
			N	Mean (SD)	N	Mean (SD)		N	Mean (SD)	N	Mean (SD)			
FADS2	rs1535	*A/G*	20	0.41 (0.10)	25	0.42 (0.06)	0.730	13	0.42 (0.11)	23	0.37 (0.10)	0.190	0.887	**0.034**
FADS2	rs174575	*C/G*	24	0.40 (0.09)	16	0.44 (0.05)	0.056	21	0.39 (0.11)	14	0.37 (0.10)	0.632	0.872	**0.033**
FADS2	rs174583	*C/T*	19	0.40 (0.10)	27	0.42 (0.06)	0.388	13	0.42 (0.11)	25	0.37 (0.10)	0.158	0.674	**0.018**
FADS2	rs99780	*C/T*	15	0.38 (0.07)	26	0.44 (0.08)	**0.017**	12	0.42 (0.12)	22	0.39 (0.10)	0.429	0.297	**0.043**
C22:6n-3 (DHA)														
FADS2	rs1535	*A/G*	20	4.48 (1.35)	25	4.23 (1.04)	0.496	13	4.56 (0.98)	23	4.18 (0.93)	0.255	0.841	0.869
FADS2	rs174575	*C/G*	24	4.19 (1.22)	16	4.50 (1.02)	0.400	21	4.25 (0.92)	14	4.44 (1.01)	0.573	0.856	0.859
FADS2	rs174583	*C/T*	19	4.31 (1.16)	27	4.29 (1.06)	0.955	13	4.56 (0.98)	25	4.11 (0.93)	0.169	0.528	0.511
FADS2	rs99780	*C/T*	15	4.14 (0.95)	26	4.33 (1.19)	0.599	12	4.64 (0.98)	22	4.26 (0.88)	0.260	0.188	0.835
FADS2	rs174602	*T/C*	19	4.32 (1.09)	13	4.52 (1.22)	0.635	23	4.10 (0.91)	14	4.19 (1.02)	0.794	0.480	0.450
Fatty acids involved in *ELOVL2* indexes														
C20:5n-3 (EPA)														
ELOVL2	rs2236212	*G/C*	14	0.31 (0.12)	29	0.33 (0.15)	0.776	13	0.27 (0.23)	22	0.24 (0.11)	0.612	0.571	**0.032**
ELOVL2	rs3798713	*G/C*	11	0.32 (0.13)	31	0.34 (0.15)	0.765	12	0.28 (0.24)	25	0.24 (0.11)	0.490	0.626	**0.011**
C22:5n-3 (DPAn3)														
ELOVL2	rs2236212	*G/C*	14	0.43 (0.10)	29	0.41 (0.07)	0.424	13	0.34 (0.06)	22	0.41 (0.12)	**0.047**	**0.011**	0.787
ELOVL2	rs3798713	*G/C*	11	0.43 (0.11)	31	0.41 (0.07)	0.465	12	0.35 (0.06)	25	0.41 (0.12)	0.099	**0.033**	0.992
C22:6n-3 (DHA)														
ELOVL2	rs2236212	*G/C*	14	4.64 (1.32)	29	4.10 (0.98)	0.139	13	4.30 (0.99)	22	4.28 (0.97)	0.942	0.466	0.514
ELOVL2	rs3798713	*G/C*	11	4.60 (1.49)	31	4.10 (0.91)	0.200	12	4.41 (0.94)	25	4.24 (0.96)	0.617	0.728	0.565
ELOVL2	rs953413	*A/G*	10	3.82 (0.83)	26	4.31 (1.22)	0.250	7	3.85 (0.73)	20	4.61 (0.92)	0.059	0.929	0.357
Fatty acids involved in *ELOVL5* indexes														
C18:3n-3 (ALA)														
ELOVL5	rs2397142	*C/G*	23	0.13 (0.04)	20	0.15 (0.04)	0.168	19	0.11 (0.03)	16	0.11 (0.04)	0.605	**0.022**	**0.016**
ELOVL5	rs9395855	*T/G*	6	0.16 (0.05)	25	0.14 (0.04)	0.405	8	0.10 (0.02)	20	0.11 (0.04)	0.519	**0.010**	**0.047**
C20:5n-3 (EPA)														
ELOVL5	rs2397142	*C/G*	24	0.33 (0.14)	22	0.34 (0.14)	0.855	18	0.21 (0.09)	17	0.29 (0.22)	0.168	**0.004†**	0.411

P-value derived from global ANOVA and significance level ($p \leq 0.004$) was adjusted for multiple testing by Bonferroni correction. Data are means of FAs expressed as percentages of the total phospholipid profile (standard error). P-values <0.05 are highlighted in bold and significant associations that persisted after Bonferroni correction are additionally denoted by stars or daggers ($p \leq 0.004$). *Indicates significant differences within each group of weight and † Indicates significant differences between groups of weight. Major allele: M; minor allele: m; LA: Linoleic Acid; GLA: γ-Linolenic Acid; DGLA: Dihomo-γ-Linolenic Acid; AA: Arachidonic Acid; AdA: Adrenic Acid; DPAn6: Docosapentaenoic acid n6; ALA: α-linolenic Acid; EPA: Eicosapentaenoic acid; DPAn3: Docosapentaenoic acid n3; DHA: Docosahexaenoic acid.

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for the normal-weight group, where minor allele carriers of *FADS2* SNPs had significantly lower levels of AA and higher DGLA. We also found nominal differences consistent with the previous results (lower AA:LA index and higher ALA and DPAn3). Similarly to other studies [30], the DGLA:LA index was nominally higher in minor allele carriers. This could be because the DGLA:LA index precedes the AA:LA index and, therefore, a lower AA:LA index would cause an accumulation of DGLA, thereby "increasing" the DGLA:LA index. Overweight/obese women who were minor allele carriers of *FADS2* SNPs only showed nominally higher levels of DGLA than major homozygotes.

As shown previously, the *FADS1* and *FADS2* genetic variants were found to affect mainly the n-6 FAs, even though desaturases and elongases work on both the n-6 and n-3 series.

Several studies have observed that the DHA status, or that of the n-3 series, is less influenced by genetic variants in the *FADS* genes [1, 5, 12, 13, 28, 31]. One possible explanation for this is that the final conversion step from DPA to DHA requires translocation to the peroxisomes (which is not performed in the endoplasmic reticulum where the other reactions occur) [1], making DHA the least efficiently synthesized n-3 LC-PUFA. Therefore, the influence of SNPs might not ultimately affect DHA levels [1, 32]. It has also been postulated that DHA supplementation during pregnancy could reduce dependence on endogenous DHA synthesis [1]. However, previous studies have reported that increased dietary intake of EPA and DHA is linked to higher *FADS1* and lower *FADS2* activities, suggesting that endogenous LC-PUFA production remains active and is possibly enhanced, despite additional dietary intake of LC-PUFAs [1, 33]. Moreover, in our study, we observed no differences in dietary intake of EPA, DHA or AA between the two BMI groups; and although normal-weight pregnant women were more likely to take DHA supplementation, it was very unusual and this supplementation did not affect DHA level in plasma.

Our results suggest that BMI modifies genotype responses. According to *FADS* SNPs, n-6 FAs showed the same effects in the 2 groups studied, but significant effects were only found in the group of normal-weight women. This suggests that overweight/obese women are less affected. This could explain why women in the normal-weight group had significantly higher n-3 substrate (ALA) levels and nominally lower n-6 product (AA) levels than overweight/obese women, only when we compared minor allele carriers of SNPs in *FADS1* and *FADS2*. Additionally, among the minor allele carriers of *FADS2*, overweight/obese women showed significantly higher DHA:EPA index than the normal-weight group (the DHA:DPAn3 index showed the same trend). This suggests that minor alleles of *FADS2* SNPS in overweight/obese women could have a positive impact on n-3 LC-PUFA production. Furthermore, among major homozygotes, overweight/obese women also showed nominally higher DHA:EPA index than those in the normal-weight group. Perhaps, regardless of genetic variants in the *FADS* gene, a high BMI could be linked to increased activity of enzymes involved in n-3 FA synthesis. Nevertheless, there were no differences in amounts of DHA between the weight groups, suggesting that even if enzymatic activity in n-3 FA production is increased in overweight/obese individuals, it is not enough to elicit greater n-3 LC-PUFA levels than in normal-weight subjects.

We analyzed 3 *ELOVL2* SNPs (rs2236212, rs3798713 and rs953413) and found that their genetic variants only affected n-3 FA levels in overweight/obese women. Overweight/obese women who were minor allele carriers of rs2236212 [12] and rs3798713 had nominally lower DHA:DPAn3 index compared with major homozygotes. This led to nominally higher amounts of the substrate DPAn3. Lemaitre et al. found decreased DHA levels in the presence of at least one minor allele of rs2236212 [15], which is consistent with the tendency observed in our results. The normal-weight group generally showed the same trends. Moreover, among minor allele carriers of *ELOVL2* SNPs, overweight/obese women showed significantly higher DHA:EPA index than normal-weight women (DPA:EPA and DHA:DPA indexes showed the same tendency). This is in accordance with *FADS2* results. Therefore, minor alleles of *FADS2* and *ELOVL2* SNPs in overweight/obese women, could increase n-3 LC-PUFA production indexes, but without surpassing levels in normal-weight women.

Estrogen facilitates LC-PUFA synthesis [34, 35], probably by regulating delta-6 desaturase. During pregnancy, estrogen levels are higher, leading to increased amounts of DHA and AA until delivery, when the release of prolactin inhibits estrogen activity [35]. Since estrogen is produced in adipocytes, obesity is linked to high estrogen levels which increase proportionally to total body adiposity [36]. This could be an alternative explanation of the increase in n-3 LC-PUFA production observed in overweight/obese individuals. In this case, more studies introducing measurements of estrogen in both populations are needed.

We also studied n3:n6 ratios (EPA:AA and DHA:AA), which are associated with obesity risk. Among minor allele carriers of the *FADS* and *ELOVL* genes, normal-weight women showed nominally higher plasma ratios of EPA:AA and DHA:AA than women who were overweight/obese. The same trend was observed when comparing major homozygotes, but with a weaker (or no) association. This suggests that a high BMI leads to increased levels of AA and/or lower levels of DHA and EPA, more importantly in women who carry at least one copy of the minor allele of *FADS* and *ELOVL* SNPs.

To the best of our knowledge, this is the first report to directly associate *FADS* SNPs with obesity risk and to analyze how weight affects the impact of variations in genes involved in FA metabolism. Our results further justify the need for personalized nutrition by showing that metabolism is affected by nutritional status and genes. Our present study had some limitations: it might be limited by the relatively small sample size; however, we could identify and group participants into different weight and genotype categories. Information on dietary intake was obtained from validated food records and questionnaires and although each participant was guided by a nutritionist, this information could have been affected by recall bias. Likewise, supplementation data (brand, content, doses and frequency) during pregnancy were also obtained from questionnaires answered by the participants; therefore, this information might not be completely accurate.

Conclusions

In conclusion, minor allele carriers of *FADS1* and *FADS2* SNPs have an increased risk of obesity ($p \leq 0.05$). The effects of genotype on FA concentrations differed by maternal pre-pregnancy weight status. Enzymatic activity and FA levels were reduced in normal-weight women who were minor allele carriers of *FADS* SNPs; these reductions were not significant in overweight/obese participants. This suggests that women with a BMI ≥ 25 are less affected by *FADS* genetic variants in this regard. In the presence of *FADS2* and *ELOVL2* SNPs, overweight/obese women showed higher n-3 LC-PUFA production indexes than those women in the normal-weight group, but this was not enough to obtain a higher n-3 LC-PUFA concentration ($p \leq 0.004$). Since genotypes may not have the same effects on all people, it is of interest to continue exploring gene-BMI interactions to pursue personalized health-related recommendations. Alterations in maternal FAs modify the risk of pro-inflammatory diseases and affect FA delivery to the fetus/neonate, which has an impact on child growth and development. Therefore, this study also supports the importance of a healthy pre-pregnancy weight, and identifies groups of women who could benefit from a high intake of n-3 FAs in order to achieve an improved FA status that fulfills fetal/neonatal requirements.

Supporting information

S1 Fig. Metabolism pathways of omega-6 and omega-3 PUFAs.
(TIF)

S1 Table. DHA in plasma according to DHA supplementation.
(DOCX)

S2 Table. Characteristics of the studied SNPs within the *FADS* and *ELOVL* genes.
(DOCX)

S3 Table. Associations between plasma proportions of PUFAs and *FADS* and *ELOVL* polymorphisms.
(DOCX)

S4 Table. PUFA levels in plasma according to maternal *FADS* and *ELOVL* SNPs.
(DOCX)

S5 Table. Maternal EPA:AA and DHA:AA ratios in plasma according to their genotypes.
(DOCX)

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RESEARCH ARTICLE

Evaluation of less invasive methods to assess fatty acids from phospholipid fraction: cheek cell and capillary blood sampling

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Abstract

Plasma is the most commonly employed matrix for analyzing fatty acids (FAs), but its extraction is not well accepted in the infant population. The objectives of this study were to evaluate cheek cells and capillary blood as alternatives to plasma sampling for FA analysis and to standardize the methodology. Samples were obtained from 20 children who underwent lipid extraction, phospholipid isolation by Solid Phase Extraction (SPE) in a 96-well plate, methylation, and analysis by fast gas chromatography (GC). A positive correlation was found for most of the FAs, especially long-chain polyunsaturated fatty acids (LC-PUFAs), in cheek cells and capillary blood versus plasma samples ($r = 0.32–0.99$). No differences were found in the levels of n-6: n-3 PUFA and n-6: n-3 LC-PUFA ratios between cheek cells and capillary blood. These two proposed samples can therefore be used as alternatives to plasma sampling for phospholipid FA analysis, especially LC-PUFAs.

Keywords

Buccal mucosal cells, fingertip blood, infants, phospholipids, plasma

History

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Introduction

Many epidemiological and interventional studies have reported that n-3 and n-6 long-chain polyunsaturated fatty acids (LC-PUFAs) play important roles in human health and common diseases (Calder & Yaqoob, 2009; Lopez-Huertas, 2010; Riediger et al., 2009). LC-PUFAs have a critical influence on neurodevelopment in childhood since they are implicated in the normal growth and functioning of the brain and central nervous system (Calder & Yaqoob, 2009; Lopez-Huertas, 2010; Riediger et al., 2009; Ryan et al., 2010). It is well known that imbalances in these nutrients, from either dietary intake or metabolism, may have significant negative effects on brain function in both the developmental phase and throughout the entire life span (Schuchardt et al., 2010) and there is increasing evidence regarding the positive effects of supplementation with LC-PUFAs, especially with those involved in neuronal processes such as the n-3 fatty acids (FAs) docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Lopez-Huertas, 2010, Ryan et al., 2010, Schuchardt et al., 2010). Some of the positive effects of supplementation shown by controlled trials are improvement in visual, cognitive and motor functions (Calder & Yaqoob, 2009; Lopez-Huertas, 2010). Moreover, a link has been shown between developmental disorders in childhood and imbalances in the n-6:n-3 ratio (Schuchardt et al., 2010), and several authors agree on the need to lower the n-6:n-3 ratio to 1–2:1 (Gómez et al., 2011; Granados et al., 2006). Therefore, further research on newborns and infants is of special interest to evaluate how FAs influence health disorders.

Current studies use different biological compartments for FA analysis, such as whole blood, red blood cells, plasma, and adipose tissue. Of these, plasma is most commonly used to determine an individual's fatty acid status (Klingler & Koletzko, 2012). Separation of the phospholipid fraction is the best way to assess FAs since other lipid fractions, such as triglycerides or total FAs, are more influenced by recent diet. However, these approaches are invasive and not well accepted in population studies, particularly in infants and young children. For this reason, studies on FAs often rely on food intake questionnaires which do not allow accurate estimation of FA status, since they merely provide data about the type of diet consumed and therefore dietary fat intake (Klingler et al., 2011; Klingler & Koletzko, 2012).

Since the 1980s several studies have proposed the cheek cell sampling as an alternative matrix for evaluating FA status. However, the sampling and analytical methods applied in these studies have differed widely, thus hindering the comparison of results. A wide variety of materials has been used for sample collection, such as distilled water (Browning et al., 2012; Hodson et al., 2014), plastic spoons (Sampugna et al., 1988), sterile cotton swabs (Koletzko et al., 1999), sterile wooden spatulas (Hoffman et al., 1999), sterile gauzes (Connor et al., 2000) and endocervical brushes (Grindel et al., 2013; Kirby et al., 2010; Klingler et al., 2011; Laitinen et al., 2006). Furthermore, the lipid extraction methods proposed by Bligh & Dyer (1959), Folch et al. (1957) and Hoffman & Uauy (1992), have been previously used as references (Browning et al., 2012; Connor et al., 2000; Grindel et al., 2013; Hodson et al., 2014; Hoffman et al., 1999; Kirby et al., 2010; Klingler et al., 2011; Koletzko et al., 1999; Laitinen

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et al., 2006; McMurchie et al., 1984; Sampugna et al., 1988). Regarding the separation of phospholipids, two methods have commonly been used: thin-layer chromatography (TLC) (Connor et al., 2000; Grindel et al., 2013; Hodson et al., 2014; Hoffman et al., 1999; Koletzko et al., 1999; McMurchie et al., 1984; Sampugna et al., 1988) or solid phase extraction (SPE) with cartridges (Browning et al., 2012; Laitinen et al., 2006), the latter of which limits the quantity of samples that can be processed simultaneously. Another important obstacle to overcome is small sample size, which may hinder proper analysis of FAs, as well as loss of lipid fractions during sample preparation (Klingler & Koletzko, 2012).

Meanwhile, capillary blood, commonly called drop blood spots (DBS), has also been proposed as alternative to plasma analysis (Armstrong et al., 2008; Bailey-Hall et al., 2008; Bicalho et al., 2008; Marangoni et al., 2004; Metherel et al., 2012; Min et al., 2011; Ryan & Nelson, 2008). Normally, the process consists of direct methylation of whole capillary blood from fingertips absorbed on a chromatographic paper (Armstrong et al., 2008; Bailey-Hall et al., 2008; Bicalho et al., 2008; Metherel et al., 2012; Ryan & Nelson, 2008). This method is much simpler and reduces the amount of analytical time required when compared with plasma samples (Min et al., 2011).

To date, plasma remains the most common matrix for FA analysis and yet there is no agreed standard procedure. However, plasma sampling is not recommended in studies involving infants and young children, or subjects with inaccessible veins. This presents an issue when recruiting volunteers for studies, especially if multiple blood sampling is necessary. Although attempts have been made to compare plasma sampling with other less invasive techniques, such as mucosal sampling (Klingler & Koletzko, 2012), to the best of our knowledge, no previous studies have compared the results of these three matrices: cheek cells, capillary blood and plasma.

The aim of this study was (1) to evaluate cheek cells and capillary blood as less invasive alternatives to traditional plasma sampling for FA analysis and (2) to contribute to standardization of sample preparation independently of the matrix. In addition, an assessment was conducted of correlations between FA status in these two biological matrices, cheek cells and capillary blood, versus plasma (as the standard matrix).

Methods

Subjects

Cheek cell, capillary blood and plasma samples were obtained from child volunteers at the ''San Cecilio'' Clinical University Hospital in Granada, Spain, who had fasted prior to the collection procedure. Complete sets of data were available from 20 subjects. Written informed consent was obtained from the parents of all study participants prior to sample collection. For method validation, 12 samples of each matrix from different adult volunteers belonging to our research group at the time were collected and mixed as explained below to create working pools.

Reagents and materials

Methanol (HPLC grade) and anhydrous sodium sulfate were purchased from Scharlab (Sentmenat, Spain). Sodium chloride, diethyl ether, 2-propanol, acetic acid, ethanol 96%, dichloromethane and chloroform were purchased from Panreac (Barcelona, Spain). The *n*-hexane was purchased from Merck (Darmstadt, Germany). A 14% boron trifluoride methanol solution (BF_3/MeOH), 50 mg/mL butylated hydroxytoluene (BHT) in ethanol and sodium methylate in 0.5 M methanol ($NaOCH_3$/MeOH) were purchased from Sigma Aldrich (St. Louis, MO). Discovery DSC-NH_2 SPE 96-well plates (100 mg/well) were purchased from Supelco (Bellefonte, PA). Cheek cells were collected using a Rovers EndoCervex-Brush® supplied by Heinz Herenz (Hamburg, Germany) and a Fluka blood collection kit purchased from Sigma Aldrich was used to collect capillary blood samples (St. Louis, MO). Other mucosal cell extraction devices were also tested, such as sterile cotton swabs with a wooden handle, supplied by Deltalab (Barcelona, Spain), sterile cotton swabs with a plastic handle from COPAN Innovation (Brescia, Italy), sterile gauzes from the INDAS Laboratory (Portillo, Spain) and a circular-shaped Gynobrush from Heinz Herenz (Hamburg, Germany).

A supelco 37-component fatty acid methyl ester (FAME) mix and polyunsaturated fatty acid (PUFA) methyl ester mix (animal source) were used for peak identification, and 1,2-dipentadecanoyl-*sn*-glycero-3-phosphocholine (PC-C15:0) was used as the internal standard solution (IS), all purchased from Supelco (Bellefonte, PA). Stock standard solutions were prepared by dissolving the commercial standards in *n*-hexane in appropriate concentrations (200–1000 μg/mL) and stored at −20 °C until use.

Sampling and lipid extraction

Plasma samples

Blood was obtained by arm venipuncture and plasma was separated by centrifugation and immediately frozen and stored at −80 °C until analysis. Lipid extraction and phospholipid isolation were performed according to a modified version of the method described by Chisaguano et al. (2013). Prior to lipid extraction, 40 μL of IS (PC-C15:0) at a concentration of 0.4 μg/μL was added to 300 μL of plasma, and lipids were subsequently extracted with 3 mL of dichloromethane/methanol (DCM:MeOH) (2:1). After sonication for 5 min, 500 μL of a saturated sodium chloride solution in water was added to the tube, shaken in vortex and centrifuged for 7 min at 3000 rpm and 25 °C. After centrifugation, the organic phase was collected by aspiration, transferred into a conical-shaped tube and dried under a stream of N_2 at 40 °C in a multiple position evaporator.

Cheek cell samples

Prior to cheek cell collection, the mouth was rinsed 3 times with tap water to eliminate possible food residues. Then, each inner cheek was gently scraped back and forth 25 times with a Rovers EndoCervex-Brush® using one different side of the brush for each cheek. The tip of the brush was transferred into a 4 mL cryotube and stored at −80 °C until analysis.

For analysis, the brush was inserted into an assay glass tube. The remains in the cryotube were swept with 3 mL of DCM:MeOH (2:1) and transferred to the assay glass tube along with the brush. The tube was vortexed for 1 min and sonicated for 5 min. The brush was removed and 750 μL of sodium chloride (NaCl) (0.9%) was added. The tube was shaken and centrifuged at 3000 rpm for 5 min. The supernatant was discarded and the organic phase was collected by aspiration and transferred into a conical-shaped tube. 20 μL of 0.4 μg/μL IS solution (PC-C15:0) was added and then vortexed for 30 s. The organic extract was then dried under a stream of N_2 at 40 °C.

Capillary blood samples

One drop of blood was obtained by punching the index finger with a lancet and, avoiding skin contact, the drop was absorbed on a chromatographic paper from the blood collection kit. 10 μL of BHT (50 mg/mL in ethanol) was added to the paper to prevent oxidation. The sample was stored at −80 °C in a cellophane envelope with airtight closure until analysis.

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For analysis, the filter paper was transferred to an assay tube with 3 mL of DCM:MeOH (2:1). The tube was sonicated for 5 min and then 750 µL of NaCl (0.9%) was added. The tube was vortexed for 1 min and centrifuged at 3000 rpm for 5 min. The supernatant was discarded and the organic phase was collected by aspiration and transferred into a conical-shaped tube to which 20 µL of 0.4 µg/µL IS solution (PC-C15:0) was added and then vortexed for 30 s. The extract was then dried under a stream of N_2 at 40 °C.

Phospholipid isolation using solid phase extraction

The dry lipid extracts were dissolved in 400 µL of chloroform and loaded into a previously activated solid phase extraction (SPE) 96-well plate. Each well was activated with 2 × 1 mL of hexane and 1 mL of chloroform/2-propanol (2:1). After sample load, wells were washed with another 1 mL of chloroform/2-propanol (2:1) to remove neutral lipids, followed by 1 mL of 2% acetic acid in diethyl ether to remove the free FA and finally eluted with 1.5 mL of methanol to collect the phospholipid fraction in the 96-well collection plate.

Fatty acids methylation

Methylation was performed according to a modified version of the method described by Chisaguano et al. (2013). Briefly, 2 mL of sodium methylate in 0.5 M methanol was added to the phospholipid fraction obtained from SPE. The mixture was vortexed for 30 s and heated (Thermoblock P-Selecta, Barcelona, Spain) at 80 °C for 10 min. After subsequent cooling in an ice bath, 2 mL of boron trifluoride methanolic solution (14% v/v) was added, vortexed, and the mixture was heated again at 80 °C for 3 min. After that, the tubes were cooled down. Fatty acid methyl esters (FAMEs) were then extracted by adding 500 µL of *n*-hexane and 2 mL of saturated sodium chloride solution in water was used to enhance phase separation. The mixture was centrifuged for 10 min at 3000 rpm. After this, the clear organic top layer was transferred into another tube and dried over anhydrous sodium sulfate. Finally, 300 µL of the extract was transferred into an automatic injector vial equipped with a volume adapter. The aliquot was evaporated to dryness under a stream of N_2 and re-diluted with 50 µL of *n*-hexane.

Fatty acid determination by gas chromatography

Separation of FAMEs was performed by fast gas chromatography following a modified version of the method developed by Chisaguano et al. (2013). The chromatographic system consisted of an HP-6890 Series GC System (Hewlett-Packard, Waldbronn, Germany) equipped with a split/splitless injector, an HP-7683B Series autoinjector, an electronic pressure control unit, a flame ionization detector (FID), and a fused-silica capillary column RTX-2330 (40 m × 0.18 mm internal diameter, 0.10 µm film thickness) coated with a 10% cyanopropyl phenyl-90% biscyanopropyl polysiloxane non-bonded stationary phase from Restek (Souderton, UK). The chromatographic conditions used were: helium was used as a carrier gas at a constant pressure of 37.97 psi (equivalent to linear velocity of 26 cm/s at 110 °C). The detector and injector temperatures were set at 300 °C and 250 °C, respectively; the split ratio was at 1:15 for capillary blood and mucosa, and 1:50 for plasma and standard analyzes. The injection volume was 1 µL. Oven temperatures were programed as follows: initial temperature was set at 110 °C, which was held for 0.5 min, then the temperature was increased at a rate of 52 °C/min to 195 °C. This temperature was held for 6 min and finally increased to 230 °C at a rate of 25 °C/min, and held for 6.5 min.

Identification of FAMEs from both biological matrices was performed by comparing peak retention times of FAME samples and standard FAME mix solutions. FAMEs were quantified by standard normalization (% total FA).

Method validation

Cheek cells and capillary blood from 12 different individuals were used to prepare a pool from each matrix after lipid extraction. The pools were aliquoted in different samples which were then processed in groups to evaluate repeatability ($n = 3$) and reproducibility ($n = 9$ on 3 different days). Two blank samples from each process were prepared in order to identify possible contaminations.

The recovery of the method was assessed according to Chisaguano et al. (2013), adding known amounts of 1,2-dipentadecanoyl-sn-glycero-3-phosphocholine (10, 50 and 100 µg) to samples before and after the SPE stage. Although PC-C15:0 was used as the IS solution in the final methodology, in this specific evaluation of recovery, PC-C15:0 was selected to assess the yield of the method, and C13:0 was also added as IS. Triplicate determinations were performed for each level. All samples were subjected to the complete proposed procedure.

Statistical analysis

All statistical analyzes were performed using SPSS 20.0 for Windows (SPSS Inc., Chicago, IL). The Kolmogorov–Smirnov test was used to study the normal distribution of data and the non-normal distributed were naturally log transformed.

FA levels within the different matrices were compared by means of a univariate ANOVA applying Bonferroni's *post-hoc* correction. Correlations between different variables were determined by Pearson's correlation coefficient. The confidence level was established at 95% for all analyses. Thus, results obtaining a p value of below 0.05 were considered statistically significant.

Results

The same 19 FAs were analyzed from the phospholipid fraction and identified in the chromatograms of each different matrix, as shown in Figure 1.

The FA composition of cheek cell, plasma and capillary blood samples is presented in Table 1. Statistical differences were found in the mean of FAs from each matrix. Within the unsaturated FAs, the quantitatively dominant FAs in all matrices were oleic (C18:1, n-9), linoleic (C18:2, n-6) and arachidonic (C20:4, n-6) acids. As noted in the table, arachidonic acid (AA) and DHA contents were approximately 2–4 times higher in plasma than in cheek cells and capillary blood. The n-6 series PUFAs were more abundant than the n-3 series. The mean of the n-6: n-3 and n-6: n-3 LC-PUFA ratios was found to be similar between cheek cell and capillary blood samples, but both of these groups were statistically different from the plasma samples.

The precision of the method was calculated through the coefficients of variation (CV) which are shown in Table 2. The CV for cheek cell samples ranged from 1.3 to 5.6% (intra-day) and 3.7 to 9.3% (inter-day) while the CV from capillary blood samples ranged from 0.5 to 7.7% (intra-day) and 1.7 to 9.8% (inter-day). Relative recovery of the SPE process was 100.5%, expressed as an average (3 different addition levels) ratio of calculated and nominal spiked amount × 100.

Fatty acids of cheek cell and capillary blood samples were correlated with those of plasma samples. Most of the FAs showed a statistically significant association with values from $r = 0.32$ to $r = 0.99$, especially LC-PUFAs, where FA such as AA, EPA and DHA are included (Table 3).

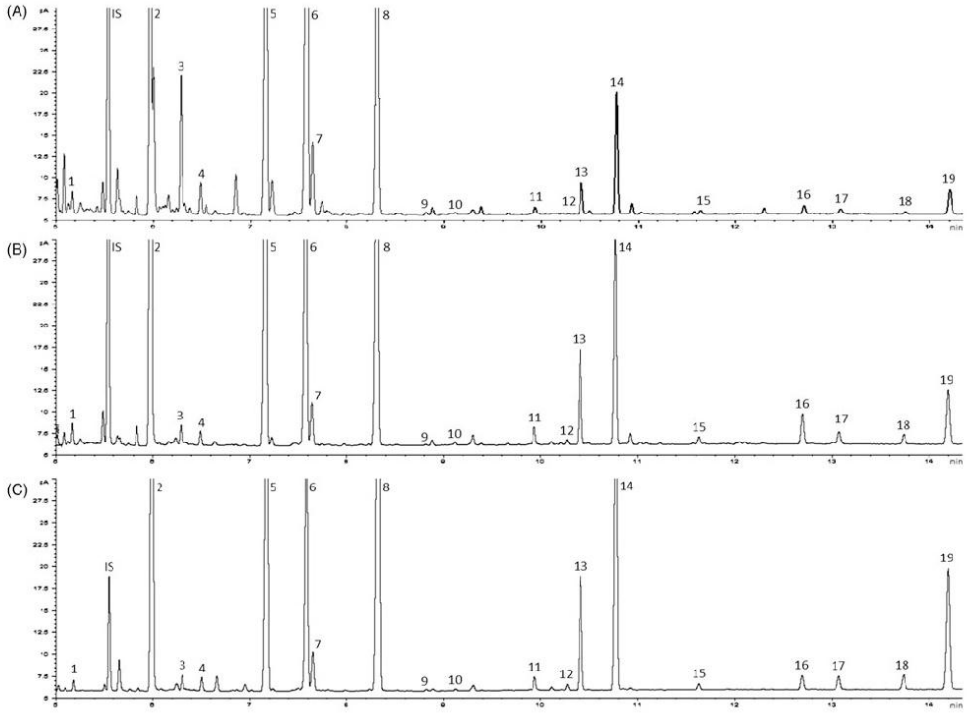

Figure 1. Chromatograms of the FAs profile from each matrix. (A) Chromatogram from cheek cell sample. (B) Chromatogram from capillary blood sample. (C) Chromatogram from plasma sample. 1. C14:0, 2. C16:0, 3. C16:1, n-7, 4. C17:0, 5. C18:0, 6. C18:1, n-9, 7. C18:1, n-7, 8. C18:2, n-6, 9. C18:3, n-6, 10. C18:3, n-3, 11. C20:2, n-6, 12. C20:3, n-9, 13. C20:3, n-6, 14. AA, 15. EPA, 16. C22:4, n-6, 17. C22:5, n-6, 18. C22:5, n-3, 19. DHA, IS. C15:0.

Discussion

As plasma sampling status is not well accepted to assess FA, especially when dealing with newborn or child populations (Klingler et al., 2011; Klingler & Koletzko, 2012), it has become increasingly important to study alternative matrices. Cheek cells and capillary blood are the most commonly considered alternatives, but to our knowledge there are still no studies that have evaluated and compared the results obtained for these two different approaches with those of traditional plasma sampling.

In preliminary studies on cheek cell samples, we tested different devices commonly used in other studies for their extraction (Connor et al., 2000; Grindel et al., 2013; Hoffman et al., 1999; Kirby et al., 2010; Klingler et al., 2011; Koletzko et al., 1999; Laitinen et al., 2006), including sterile cotton swabs a wooden handle, sterile cotton swabs a plastic handle, sterile gauzes, a circular-shaped Gynobrush and a Rovers *EndoCervex-Brush*. First, the wooden spatula was rejected because of the unpleasant sensation it caused in comparison with the other options, and the sterile cotton swabs with a wooden handle and the sterile gauzes were ruled out because of high levels of contamination that interfered with the analyzes (data not shown). The sterile cotton swabs with a plastic handle were also found to be inadequate because the plastic was not resistant to the solvent used for lipid extraction. The circular-shaped Gynobrush was also dismissed because it had a wire and nylon bristle combination that could potentially hurt the patient if not used carefully. However, the Rovers EndoCervex-Brush was found to be a perfect sampling device for several reasons: it was made entirely of flexible, soft polypropylene, and it consisted of a brush head that could be detached from the handle after collection, making it easy to store in a small tube. Additionally, the use of a brush for scraping was selected in preference to the cotton swab with a wooden handle because it has been shown that the average cell yield obtained with a cotton swab is approximately two-thirds of that collected with an endocervical brush. Consequently, the use of a brush might be crucial to collect an adequate amount of cells in young children for a reliable analysis of FAs with this method (Klingler et al., 2011).

Proper collection of cheek cells requires a strict sampling procedure. It has been proved that rinsing the mouth after scraping the inner cheeks and collecting this residue, leads to significantly higher cell yields (Devereux-Graminski & Sampugna, 1993). However, the rinsing residue may be discarded because it is not applicable when collecting cells in babies and we observed that the differences were negligible. It has also been demonstrated that cleaning the mouth prior to sampling reduces possible food residues (Devereux-Graminski & Sampugna, 1993), however this does not apply in babies with no solid food intake.

It is known that cheek cells are robust and hard to disrupt by isolated applications of detergents, ultrasound or polar solvents. Methanol and ultrasound were used in this study to disrupt cell membranes, as Klingler et al. (2011) have shown that this method has a recovery rate of >90%.

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Table 1. Fatty acids profile according to each different matrix.

Fatty Acid	Cheek cells Mean (%) ± SD	Plasma Mean (%) ± SD	Capillary blood Mean (%) ± SD	*p*
C14:0	1.12 ± 0.28[a]	0.24 ± 0.06[b]	0.66 ± 0.17[c]	<0.0001
C16:0	24.96 ± 2.11[a]	27.25 ± 1.54[a]	34.61 ± 1.43[b]	<0.0001
C16:1, n-7	3.94 ± 1.34[a]	0.35 ± 0.07[b]	0.19 ± 0.07[b]	<0.0001
C17:0	1.39 ± 0.22[a]	0.33 ± 0.05[b]	0.40 ± 0.04[b]	<0.0001
C18:0	25.82 ± 10.60[a]	14.22 ± 1.23[b]	40.13 ± 4.05[c]	<0.0001
C18:1, n-9	21.29 ± 5.60[a]	10.52 ± 1.33[b]	7.45 ± 1.51[c]	<0.0001
C18:1, n-7	3.00 ± 0.89[a]	1.44 ± 0.19[b]	0.70 ± 0.13[c]	<0.0001
C18:2, n-6	10.22 ± 3.51[a]	23.93 ± 3.53[b]	8.37 ± 2.54[a]	<0.0001
C18:3, n-6	0.27 ± 0.10[a]	0.12 ± 0.05[b]	0.05 ± 0.01[c]	<0.0001
C18:3, n-3	0.18 ± 0.06[a]	0.58 ± 0.56[b]	0.06 ± 0.01[a]	<0.0001
C20:2, n-6	0.57 ± 0.15[a]	0.37 ± 0.04[b]	0.13 ± 0.04[c]	<0.0001
C20:3, n-9	0.15 ± 0.03[a]	0.19 ± 0.04[b]	0.08 ± 0.02[c]	<0.0001
C20:3, n-6	0.98 ± 0.32[a]	3.07 ± 0.77[b]	0.84 ± 0.34[a]	<0.0001
C20:4, n-6 (AA)	4.11 ± 1.66[a]	11.14 ± 2.03[b]	3.93 ± 1.13[a]	<0.0001
C20:5, n-3 (EPA)	0.31 ± 0.12[a]	0.66 ± 0.36[b]	0.19 ± 0.07[a]	<0.0001
C22:4, n-6	0.38 ± 0.19[a]	0.39 ± 0.08[a]	0.78 ± 0.36[b]	<0.0001
C22:5, n-6	0.31 ± 0.15[a]	0.31 ± 0.08[b]	0.12 ± 0.04[c]	<0.0001
C22:5, n-3	0.22 ± 0.03[a]	0.66 ± 0.14[a]	0.29 ± 0.11[a]	<0.0001
C22:6, n-3 (DHA)	1.13 ± 0.58[a]	4.24 ± 1.36[b]	1.05 ± 0.38[a]	<0.0001
SFAs	53.29 ± 11.37[a]	42.03 ± 1.16[b]	75.81 ± 5.09[c]	<0.0001
MUFAs	28.22 ± 6.87[a]	12.31 ± 1.37[b]	8.34 ± 1.62[c]	<0.0001
PUFAs	18.50 ± 5.54[a]	45.66 ± 1.42[b]	15.83 ± 3.81[c]	<0.0001
n-3 PUFAs	1.70 ± 0.73[a]	6.15 ± 1.86[b]	1.58 ± 0.51[a]	<0.0001
n-6 PUFAs	16.77 ± 5.05[a]	39.33 ± 2.53[b]	14.18 ± 3.56[a]	<0.0001
n-3 LC-PUFAs	1.61 ± 0.71[a]	5.57 ± 1.65[b]	1.52 ± 0.52[a]	<0.0001
n-6 LC-PUFAs	6.30 ± 2.05[a]	15.29 ± 2.15[b]	5.80 ± 1.56[c]	<0.0001
n-6:n-3 PUFAs	11.25 ± 5.03[a]	6.97 ± 2.48[b]	9.97 ± 4.52[a]	<0.0001
n-6: n-3 LC-PUFAs	4.48 ± 2.17[a]	2.94 ± 0.84[b]	4.08 ± 1.22[a]	<0.0001

$p < 0.05$ indicates statistical significant differences according to global ANOVA. Natural Log was used for non-normal variables. Means of FAs with different superscript letters indicate significant differences after applying the Bonferroni *post-hoc* correction.

Table 2. Precision of the method for cheek cell and capillary blood samples.

	Cheek Cells		Capillary Blood	
Fatty Acid	Intra day ($n = 3$) CV (%)	Inter day ($n = 9$) CV (%)	Intra day ($n = 3$) CV (%)	Inter day ($n = 9$) CV (%)
C14:0	6.2	9.3	4.0	7.8
C16:0	3.0	3.7	0.5	1.7
C16:1, n-7	4.4	6.0	5.3	5.3
C17:0	3.1	6.9	3.4	9.8
C18:0	6.3	6.8	2.4	8.1
C18:1, n-9	3.0	6.0	5.3	9.3
C18:1, n-7	4.0	5.3	5.7	9.5
C18:2, n-6	3.5	7.1	6.3	5.5
C18:3, n-6	3.7	4.1	3.8	3.8
C20:0	1.3	6.6	5.0	9.2
C18:3, n-3	6.0	5.9	3.9	4.8
C20:2, n-6	2.5	5.8	6.3	6.7
C20:3, n-9	4.4	8.5	7.7	9.0
C20:3, n-6	3.1	5.7	6.3	6.6
C22:0	5.1	7.8	6.8	8.5
C20:4, n-6 (AA)	3.4	6.2	4.5	5.1
C20:5, n-3 (EPA)	5.0	9.3	5.3	9.6
C22:4, n-6	4.0	8.8	2.9	2.8
C22:5, n-6	4.3	5.7	3.5	5.3
C22:5, n-3	6.5	6.1	3.8	5.2
C22:6, n-3 (DHA)	1.5	5.6	3.1	5.0

Table 3. Correlation coefficients of each matrix referred to plasma.

	Cheek cells vs. Plasma		Capillary Blood vs. Plasma	
Fatty Acid	*r*	*p*	*r*	*p*
C14:0	0.09	0.610	0.49**	0.001
C16:0	0.37*	0.038	0.64**	<0.001
C16:1, n-7	0.20	0.265	0.40*	0.011
C17:0	0.18	0.338	0.45**	0.004
C18:0	0.07	0.719	0.05	0.777
C18:1, n-9	0.38*	0.032	0.34*	0.030
C18:1, n-7	0.36*	0.041	0.32*	0.045
C18:2, n-6	0.36*	0.045	0.63**	<0.001
C18:3, n-6	0.59**	0.001	0.58*	0.029
C18:3, n-3	0.76**	0.001	0.42**	0.007
C20:2, n-6	0.50**	0.004	0.32*	0.047
C20:3, n-9	0.99**	<0.001	0.39*	0.013
C20:3, n-6	0.46**	0.008	0.73**	<0.001
C20:4, n-6 (AA)	0.43*	0.015	0.32*	0.044
C20:5, n-3 (EPA)	0.68**	<0.001	0.81**	<0.001
C22:4, n-6	0.36*	0.049	0.32*	0.044
C22:5, n-6	0.62**	<0.001	0.47**	0.002
C22:5, n-3	0.42*	0.040	0.36*	0.023
C22:6, n-3 (DHA)	0.59**	<0.001	0.57**	<0.001

*$p < 0.05$, **$p < 0.01$.

Capillary blood is another suitable matrix for assessing FA status instead of plasma, and represents a less invasive sampling technique. Collection only requires punching one finger to obtain one drop of blood. Nevertheless, capillary blood sampling for children is less recommended than cheek cells because extraction is more aggressive. Moreover, capillary blood requires the addition of BHT to prevent oxidation of the sample absorbed on the chromatographic paper, and this could lead to contamination issues.

The identification of false-positive peaks is important for accurate qualification of FAs: not only does this define the lower limit of the method, but also excludes FAs from analysis (Klingler

et al., 2011). The systematic analysis of blanks may yield information about contamination derived from chemicals or materials. In the present study, four impurities were found to co-elute with the peaks of C16:0, C18:0, C20:0 and C22:0 and their presence was attributed to SPE well plate contamination. These data were taken into account in calculations, and C16:0 and C18:0 peak areas from blanks were subtracted from samples. Meanwhile, due to their negligible contribution to the global profile, since they were minority peaks, C20:0 and C22:0 were excluded from the study. Blanks were carried out to evaluate contamination from the Rovers EndoCervex-Brush® and the chromatographic paper from the blood collection kit and peaks from contaminations did not co-elute with any FA peak.

Data on the precision of FA analysis have mainly been published for plasma, and only to a lesser extent for cheek cells and capillary blood. The CV reported for FA determination in cheek cells has ranged from 1.0% to 10.5% (Glaser et al., 2009; Klingler et al., 2011), and in the present study, the CV for cheek cell and capillary blood samples was lower than 10%. The recovery assessment results were considered satisfactory, as described by other authors (Browning et al., 2012; Chisaguano et al., 2013; Laitinen et al., 2006), and SPE represents a good approach in phospholipid FA analysis.

The levels of FAs found in all matrices differed considerably, but can only be compared with those found in other studies which evaluated the same FAs in order to conduct equivalent comparisons. Since the data presented by other authors are limited exclusively to PUFAs and exclude or summarize saturated and monounsaturated FAs (Browning et al., 2012; Grindel et al., 2013; Hodson et al., 2014; Hoffman et al., 1999; Klingler et al., 2011; Laitinen et al., 2006), only the same FAs were considered in a comparison of results. Reported PUFA levels were comparable in all studies, whereas saturated acids differed, possibly because of dietary habits or contamination (Klingler et al., 2011). The n-6:n-3 ratios found in our study (11:1 for cheek cell samples, 10:1 for capillary blood samples and 7:1 for plasma samples) were considerably higher than the ratio of 1-2:1 reported by some authors (Gómez et al., 2011; Granados et al., 2006). As is well known, excessive amounts of n-6 and a very high n-6: n-3 ratio can be caused by an imbalanced diet and they promote the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases, whereas increased levels of n-3 (a low n-6: n-3 ratio) exert suppressive effects (Simopoulos, 2006).

In accordance with previous studies (Grindel et al., 2013; Klingler et al., 2011; Marangoni et al., 2004), the dominant unsaturated FAs observed in this study were the oleic, linoleic and arachidonic acids for all 3 matrices. In addition, we observed differences in AA and DHA between cheek cell, capillary blood and plasma samples. This has also been reported in previous studies, where plasma levels of these FAs were approximately three times higher (Klingler et al., 2011).

A study of correlations between the three different matrices was useful to determine the suitability of capillary blood and cheek cells as alternatives to plasma for assessing FA status in infants. We focused on the determination of LC- PUFAs because several authors have recently found that an understanding of their metabolic synthesis routes is a key factor in the study of several diseases (Barman et al., 2013; Gottrand, 2008). As noted by other authors, we also observed good correlation coefficients for FAs in cheek cell (Grindel et al., 2013; Klingler et al., 2011) and capillary blood samples in relation to plasma, especially for LC-PUFAs such as EPA and DHA.

Therefore, in line with other studies, cheek cell (Connor et al., 2000; Grindel et al., 2013; Hoffman et al., 1999; Kirby et al., 2010; Klingler et al., 2011) and capillary blood samples (Marangoni et al., 2004; Metherel et al., 2012) reflected LC-PUFA status as well as plasma does, hence functioning as alternatives for LC-PUFA status evaluation in children. Moreover, further studies should be carried out to improve the lipid separation step in order to avoid contamination with saturated FAs from the SPE well plate. The use of a 96-well plate for phospholipid separation represents a marked improvement over the use of cartridges since it enables a much larger quantity of samples to be processed simultaneously. The SPE 96-well plate method is simpler and less time consuming than traditional methods, such as TLC.

Conclusions

Cheek cell and capillary blood samples are less invasive and reliable methods for FA analysis from phospholipid fraction. The modifications applied to sampling and sample preparation methodology of both matrices, resulted in a positive correlation for most of the FAs, especially LC-PUFAs, when compared with plasma samples. These two methods can therefore be used as alternatives to traditional plasma sampling for analysis of these FAs. In particular, their use does not necessitate the involvement of healthcare staff in sample collection and this markedly simplifies the analytical procedure.

Declaration of interest

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10.2.3. PUBLICATION #3

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ABSTRACT

EAPS Congress 2016

October 21-25, 2016

Invited Speaker Abstracts

1
EAPS-1570
Educational Symposium 1: Preventive health care

Is hip screening by ultrasound worthwhile

R. Schmid[1]
[1], *Baar, Switzerland*

Developmental dysplasia of the hip (DDH) is the most frequent "inborn" malformation on the musculoscelettal system. Its prevalence varies significantly amongst different ehnies and is estimated to be 1-2% in Switzerland.
The ultrasound method of Prof. R. Graf (Austria) is gold standard for earliest detection of DDH in babies. It not only detects dislocation of the hip, but also quantifies the degree of dysplasia, using a geometric measurement system.
DDH can be healed quite simply, if detected early - which has an important impact on individual health and abilities.
Experiences from screening-like programs in Switzerland and from a help project in Mongolia (www.smopp.ch) including the data from a large screening study are presented and shall be the basis for analysis and discussion.

2
EAPS-1559
Educational Symposium 2: Child with asthma

Education of the asthmatic patient or his/her parent

M. Schöni[1]
[1], *Switzerland*

EDUCATION OF THE ASTHMATIC PATIENT OR HIS/HER PARENTS
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Education for asthmatic children and/or parents is essential for the understanding of the disease and the therapy. Whether it is done in educational classes (i.e. lessons for parents or children) or individually depends on the availability of sources, professionals and caregivers. Years of practicing teach us, that individual education seems to be far more efficient than learning classes. In individual counselling the health professional can teach and demonstrate on the individual patient the topics which must be covered: questions to the diagnosis itself, speculations or facts of the reason for asthma, preventive and clear actions to be taken in the individual case, testing for therapeutic interventions and modalities (i.e. choice of medication, device for inhalation, technique of inhalation etc.), and control of success of failure for the therapy.
In most cases the failure to get success in the leading and treating asthmatic children is based on wrong application of medication, bad or wrong technique of handling inhalation devices, compliance and adherence to and with medication, wrong medicaments and finally wrong diagnosis.
The personal and individual approach however is time consuming, needs personal engagement and at least an interpersonal contact that must be based on understanding each other (from language to empathy). It also needs control, repeated contacts and good follow-up. When being aware, that compliance with therapy in asthma ranges between 20-60% the personal approach is demanding.

3
EAPS-1578
Educational Symposium 3: Update in pulmonary hypertension

New treatment strategies in pediatric pulmonary hypertension

M. Beghetti[1]
[1], *Switzerland*

Pulmonary hypertension is a hemodynamic condition occurring rarely in newborn, infants and children. Nevertheless, it is associated with significant morbidity and mortality. When characterized by progressive pulmonary vascular structural changes, the disease is called pulmonary arterial hypertension (PAH). It results in increased pulmonary vascular resistance and eventual right ventricular failure and death. In the vast majority of cases, pediatric PAH is idiopathic or associated with congenital heart disease, and contrary to adult PAH, is rarely is associated with connective tissue, portal hypertension, HIV infection or thromboembolic disease. Although there is still no cure for PAH, quality of life and survival have been improved significantly with specific drug therapies. These treatments target the recognized pathophysiological pathways of PAH with Endothelin-1 receptor antagonists, Prostacyclin analogs, and Phosphodiesterase type 5 inhibitors whereas new pathways are currently explored. However beside sildenafil in Europe no medical therapies have been formally approved for pediatric PAH. Non medical therapies such as atrioseptostomy and Potts shunt have shown beneficial effects. Lung transplantation remain the final cure. Nevertheless, the management of pediatric PAH remains challenging, therapeutic strategy and treatment goals depending mainly on results from adult clinical trials and pediatric experts. We will discuss the current drug therapies available for the management of pediatric PAH.

4
EAPS-1566
Educational Symposium 4: Update in neurocritical care

Post-cardiac arrest management

H. Krishnan Kanthimathinathan[1]
[1], *United Kingdom*

Paediatric cardiac arrest remains a devastating event. Good neurological survival is still unfortunately rare after out-of-hospital (1-4%) and in-hospital cardiac arrest (25-35%). Paediatric cardiac arrests differ from those in adults. They are predominately secondary to hypoxia rather than

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Conclusions
Our developed computerized balance assessment programs combined with Wii Balance Board has a high potential for balance assessment system.

638
EAPS-0093
E-Poster Discussion Session 16: Gastroenterology

Association of anterior displacement of anus and constipation in infancy

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Background and aims
Anterior displacement of the anus (ADA), a common congenital abnormality of anorectal region, is recognized as a common cause of constipation. However, the recent studies have shown inconsistent results of the association. This study aims to investigate the association of ADA and constipation in infancy.

Methods
403 neonates have been examined and their anal position index (API) has been determined. API less than 0.46 and 0.34 in males and females, respectively, were defined as ADA. A 6-month prospective cohort study was conducted to evaluate the association between ADA and constipation.

Results
Overall incidence of ADA was 24.8%, while the incidences were significantly higher in females than males (32.0% and 17.7%, respectively; P<0.01). Of the 316 children, completed a 6-month cohort, 10.1% reported the constipated events. The overall incidences of constipation in ADA group and control were comparable (12.0% and 9.5%, respectively). Nevertheless, at 6 months incidence of constipation is higher in ADA than in control (6.7% and 0.8%, respectively; P< 0.01). Finally, the incidences of ADA in infants with constipated events at 2, 4 and 6 months of age are 10.0%, 33.3% and 71.4%, respectively.

Conclusions
ADA is a common finding with reported incidence of one-quarter of neonates. Children with ADA have increasingly a tendency toward constipation corresponding with increasing age. Including API measurement during pediatric examination is recommended in order to achieve a complete evaluation of infancy constipation.
Acknowledgements: The study was supported by grants from Faculty of Medicine, Srinakharinwirot University.

639
EAPS-0682
E-Poster Discussion Session 16: Gastroenterology

ANALYSIS OF FEEDING PATTERNS IN EARLY CHILDHOOD

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Background and aims
Observational studies suggest that longer duration of breastfeeding plays an important role on development and health in early childhood. The aim of this position paper is to investigate to what extent the feeding practice in infancy matches the recommended guidelines from ESPHGAN.

Methods
The research was conducted via questionnaire, in nurseries in the city of Zagreb. The study included 157 toddlers between 12 and 36 months of age. Parameters such as duration of breastfeeding, exclusive breastfeeding, time when formula and cow's milk were introduced, mother's age and education were considered. Descriptive and nonparametric statistics, χ^2 test and Mann–Whitney U test were used in data analysis. The study groups did not differ in regard to gender and age at the time of investigation.
Results
75% of infants were still breastfed after the age of 6 months, whereas 40% were exclusively breastfed longer than 6 months. Only 3.3% of mothers introduced cow's milk to their infants before the age of 6 months. No statistically significant result were found between the mothers' education or age and the duration of exclusive breastfeeding (χ^2= 5,998; p=0,112) or time when formula feeding was introduced. However, mothers over 35 years have breastfed longer than 12 months (45%).
Conclusions
The feeding practices of the surveyed population matched the recommended guideline which encourages breastfeeding longer than 6 months. Most mothers introduced cow's milk for the first time after the age of 1 year (67%) which indicates that the negative influence of hyperprotein nutrition in early childhood was recognised.

640
EAPS-0770
E-Poster Discussion Sessions
E-Poster Discussion Session 16: Gastroenterology

IMPACT OF MATERNAL GENETIC VARIANTS OF THE FADS AND ELOVL GENE CLUSTERS ON CHILD PUFA LEVELS AND COGNITION: ALTERED BY MATERNAL PRE-PREGNANCY BMI?

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Background and aims
Maternal polymorphisms (SNPs) in fatty acid desaturase (FADS) and elongase (ELOVL) enzymes alter the long chain (LC) polyunsaturated fatty acid (PUFA) availability compromising fetus supply and cognitive development. We aimed to determine how maternal polymorphisms in FADS and ELOVL genes influence children's fatty acids (FAs), cognition and if maternal weight changes this.
Methods
Children (n=72) from the PREOBE cohort were divided in group 1 (maternal pre-pregnancy BMI 18.5-24.99, n=31), group 2 (BMI>25, n=41) and ultimately in subgroups of maternal SNPs (7 in the FADS1 cluster, 5 in FADS2, 3 in ELOVL2 and 2 in ELOVL5). At 18 months old, children's cheek cells were analyzed to measure PUFAs in the phospholipid fraction and cognition was assessed using the Bayley III Cognitive Scale.
Results
Major homozygotes in group 1 had higher AA:DGLA index for rs174537 (FADS1) and higher cognition for rs174545 (FADS1) than minor allele carriers. Both tendencies persisted in all SNPs in FADS1 while cognition

tendency also persisted in FADS2. Group 2 showed that major homozygotes had higher cognition for rs2397142 (ELOVL5). Regarding rs2397142, group 2 showed higher cognition when mothers carrying minor alleles had high DHA intake, plasma EPA:AA and DHA:AA ratios instead of low.

Conclusions
Decreased enzyme activity and cognition by genetic variants in FADS and ELOVL genes isn't changed by maternal pre-pregnancy BMI, but having high BMI disturbs children's cognition if the mother has genetic variants and low dietary intake of n-3 FAs. Obese pregnant women, especially minor allele carriers, should consider a n-3 FAs rich diet to enhance child cognition.

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EAPS-0866
E-Poster Discussion Session 16: Gastroenterology

BONE METABOLISM METABOLITES LEVEL IN CELIAC CHILDREN

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[2]Russian Medical Academy of Postgraduate Study, Pediatrica, Moscow, Russia
[3]North-Caucasus Federal University, Center of bioengeenir, Stavropol, Russia

Background and aims
Study of bone remodelling markers in celiac patients, analysis of influence of gluten-free diet (GFD) on these parameters.

Methods
76 celiac children between 1 and 17 years of age have been examined. 29 of them were at the acute stage of the disease (AC), and 47 children were on GFD. Control group (CG) consisted of 14 healthy children.

Results
In children at the AC osteocalcin level was significantly lower – 40.7 ± 2.93 ng/ml ($p<0.05$), and parameters of C- telopeptides (C-tp) were higher 58.2 ± 12.9 pg/ml ($p<0,05$), than those of the CG – 64.7 ± 7.4 ng/ml and 24.3 ± 6.2 pg/ml, respectively. These data confirm that bone tissue resorption process significantly prevails over osteosynthesis in children at the AC. Parathyroid hormone (PH) level during the AC 28.5 ± 3.7 pg/ml, which is 1.7 fold higher than that for the CG17.2 ± 1.0 pg/ml ($p<0.01$). For 10.3% patients during coeliacia verification period a secondary hyperparathyroidism was detected (PH > 70 pg/ml). In patients on GFD increase of volumetric blood flow – 49.5 ± 5.8 ng/ml ($p>0.05$), decrease of PH – 20.7 ± 1.2 pg/ml ($p<0,05$) and C-tp – 51.9 ± 9.3 pg/ml ($p>0.05$) C-tp were observed versus those parameters during the AC. This supports a positive effect of GFD on bone tissue metabolism processes – activation of osteosynthesis and decrease of bone resorption.

Conclusions
Monitoring of biochemical bone formation markers in celiac patients allows to diagnose and prevent osteopenia at early development stages, before any severe complications, such as pathologic fractures, occur.

642
EAPS-0835
E-Poster Discussion Session 16: Gastroenterology

The strong association between a Congenital Diaphragmatic Hernia and the undescended testis

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[1]Radboud University Medical Center, Pediatric Surgery, Nijmegen, Netherlands
[2]Radboud University Medical Center, Health Evidence, Nijmegen, Netherlands

Background and aims
The incidence of an undescended testis (UDT) is 1-3%, with a known increase in lower birth weight and premature infants. However, this study shows the incidence of infants surviving a congenital diaphragmatic hernia repair (CDH).

Methods
A retrospective cohort of CDH patients from an expertise center was evaluated from 2000–2013. A minimum follow-up of two years was included because a UDT is generally diagnosed before that age.

Results
There were 75 males included, with a median follow-up of 7,4 years (range 2,0-13,2), of which 31% (N=23) were diagnosed with an UDT. Twenty-one underwent orchiopexy, with a median age of 24 months (range 1,5-106). Although the majority had the UDT ipsilateral to the side of the CDH (n=18), five of these were bilateral UDT with a unilateral CDH. Only nine of the patients were reported as a UDT at birth. There was no significant difference in birth weight and gestational age in patients with or without an UDT (38,3 versus 38,6 weeks, p= 0,63 and 3119gr versus 3236gr, p=0,14) . Additionally, type of repair had a comparable distribution between patients with and without a descended testis (p=0,39).

Conclusions
This study shows a strong correlation between a Congenital Diaphragmatic Hernia and an undescended testis, of one in three boys. However, this could be an underestimation because testis evaluation is currently not standard in the follow up of CDH patients. Therefore, testes of all patients with a CDH should be evaluated thoroughly during the first years of life.

643
EAPS-0446
E-Poster Discussion Session 16: Gastroenterology

The efficiency of different treatment of autoimmune liver diseases in children

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Background and aims
Autoimmune liver diseases (AILD) is a life-threatening disease, especially when diagnosed in young and still growing children. The aim of this study was to evaluate the efficiency of different treatment for the induction and maintenance of remission in children with AILD

Methods
The analysis included 113 patients (40 boys, 73 girls) with AILD who had been treated in the clinic from 1999 to 2015. Initially, all patients were treated with prednisolone 2 mg/kg/day, followed by a gradual taper according to each patient individual response over 4–8 weeks. Azathioprine was added (maximum 2 mg/kg/day) in patients not responding to high dose prednisolone or showing an elevation of transaminases during the tapering phase. If patients did not have stable remission they were treated with mycophenolate mofetil (MMF) at a dose of 35 mg/kg/day

Results
76 patients had type I autoimmune hepatitis (AIH), 16–type II, 10–seronegative, 7–autoimmune sclerosing cholangitis (ASC), 1–overlap syndrome with hepatitis C, 2 had AIH as a part of the autoimmune

10.2.4. PUBLICATION #4

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ABSTRACT

EAPS Congress 2016

October 21-25, 2016

Invited Speaker Abstracts

1
EAPS-1570
Educational Symposium 1: Preventive health care

Is hip screening by ultrasound worthwhile

R. Schmid[1]
[1], *Baar, Switzerland*

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The ultrasound method of Prof. R. Graf (Austria) is gold standard for earliest detection of DDH in babies. It not only detects dislocation of the hip, but also quantifies the degree of dysplasia, using a geometric measurement system.
DDH can be healed quite simply, if detected early - which has an important impact on individual health and abilities.
Experiences from screening-like programs in Switzerland and from a help project in Mongolia (www.smopp.ch) including the data from a large screening study are presented and shall be the basis for analysis and discussion.

2
EAPS-1559
Educational Symposium 2: Child with asthma

Education of the asthmatic patient or his/her parent

M. Schöni[1]
[1], *Switzerland*

EDUCATION OF THE ASTHMATIC PATIENT OR HIS/HER PARENTS
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Education for asthmatic children and/or parents is essential for the understanding of the disease and the therapy. Whether it is done in educational classes (i.e. lessons for parents or children) or individually depends on the availability of sources, professionals and caregivers. Years of practicing teach us, that individual education seems to be far more efficient than learning classes. In individual counselling the health professional can teach and demonstrate on the individual patient the topics which must be covered: questions to the diagnosis itself, speculations or facts of the reason for asthma, preventive and clear actions to be taken in the individual case, testing for therapeutic interventions and modalities (i.e. choice of medication, device for inhalation, technique of inhalation etc.), and control of success of failure for the therapy.
In most cases the failure to get success in the leading and treating asthmatic children is based on wrong application of medication, bad or wrong technique of handling inhalation devices, compliance and adherence to and with medication, wrong medicaments and finally wrong diagnosis.
The personal and individual approach however is time consuming, needs personal engagement and at least an interpersonal contact that must be based on understanding each other (from language to empathy). It also needs control, repeated contacts and good follow-up. When being aware, that compliance with therapy in asthma ranges between 20-60% the personal approach is demanding.

3
EAPS-1578
Educational Symposium 3: Update in pulmonary hypertension

New treatment strategies in pediatric pulmonary hypertension

M. Beghetti[1]
[1], *Switzerland*

Pulmonary hypertension is a hemodynamic condition occurring rarely in newborn, infants and children. Nevertheless, it is associated with significant morbidity and mortality. When characterized by progressive pulmonary vascular structural changes, the disease is called pulmonary arterial hypertension (PAH). It results in increased pulmonary vascular resistance and eventual right ventricular failure and death. In the vast majority of cases, pediatric PAH is idiopathic or associated with congenital heart disease, and contrary to adult PAH, is rarely is associated with connective tissue, portal hypertension, HIV infection or thromboembolic disease. Although there is still no cure for PAH, quality of life and survival have been improved significantly with specific drug therapies. These treatments target the recognized pathophysiological pathways of PAH with Endothelin-1 receptor antagonists, Prostacyclin analogs, and Phosphodiesterase type 5 inhibitors whereas new pathways are currently explored. However beside sildenafil in Europe no medical therapies have been formally approved for pediatric PAH. Non medical therapies such as atrioseptostomy and Potts shunt have shown beneficial effects. Lung transplantation remain the final cure. Nevertheless, the management of pediatric PAH remains challenging, therapeutic strategy and treatment goals depending mainly on results from adult clinical trials and pediatric experts. We will discuss the current drug therapies available for the management of pediatric PAH.

4
EAPS-1566
Educational Symposium 4: Update in neurocritical care

Post-cardiac arrest management

H. Krishnan Kanthimathinathan[1]
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Paediatric cardiac arrest remains a devastating event. Good neurological survival is still unfortunately rare after out-of-hospital (1-4%) and in-hospital cardiac arrest (25-35%). Paediatric cardiac arrests differ from those in adults. They are predominately secondary to hypoxia rather than

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Conclusions
Optimising nutritional support for VLBW infants with early TPN and standardising enteral feeding protocol could minimise postnatal growth failure before discharge. Longer follow-up is necessary to evaluate the impact on neurodevelopmental and later health outcomes.

880
EAPS-1190
E-Poster Viewing
Gastroenterology and Nutrition

INCIDENCE OF INTESTINAL FAILURE AND PARENTERAL NUTRITION DEPENDENCE IN NEONATES FOLLOWING SURGERY FOR NECROTISING ENTEROCOLITIS IN A NEONATAL INTENSIVE CARE UNIT

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Background and aims
With the increasing survival of preterm babies, the complications of necrotising enterocolitis (NEC) have increased. One definition of intestinal failure is the need for parenteral nutrition (PN) for more than 3 months. The aim is to identify the incidence of intestinal failure and PN dependent in neonates following surgery for NEC.
Methods
Cases identified from Badger database between 2012 and 2014 were collected and reviewed.
Results
In these 3 years, 137 neonates had NEC diagnoses and 53 required surgery. The median gestational age was 26 weeks. The median birth weight was 785grams. The median duration of PN in 2012 was 49.5 days; 2013 was 44 days and 2014 was 52 days.
A total of 9 babies required PN for more than 3 months. 8 babies continuing on PN were transferred to Birmingham Children's Hospital (BCH). 1 baby established full enteral feeds after being on PN for more than 3 months. In addition, 1 baby developed intestinal failure but received less than 3 months of PN due to reorientation to palliative care. Therefore in our cohort, a total of 10 babies developed intestinal failure. Among these 8 babies, 2 babies became PN dependent and currently on home PN. 1 patient passed away. The median duration of PN for remaining 5 babies is 6 months.
Conclusions
The incidence of intestinal failure in neonates following surgery for NEC was 19% and incidence of PN dependent is 4%. This is important when counselling parents about the long term complications of surgically managed NEC cases.

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EAPS-1116
E-Poster Viewing
Gastroenterology and Nutrition

HHFNC and nCPAP and full oral feeding in BPD infants

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Background and aims
Infants born extremely prematurely and who develop bronchopulmonary dysplasia (BPD) may require respiratory support for many months, including when they could be able to take oral feeds (usually 34 weeks postmenstrual age (PMA)). Our aim was to test the hypothesis that full oral feeding in infants with BPD would be achieved earlier in those supported by humidified high flow nasal cannula (HHFNC) rather than nCPAP.
Methods
Data were compared from infants born prior to 33 weeks of gestational age between 2011 to 2013, who were extubated onto and supported by nCPAP until they required only low flow oxygen (nCPAP group) to those born between 2013 to 2015 who were extubated onto nCPAP and then transferred to HHFNC if they continued to require nCPAP for more than two weeks and had a supplementary oxygen requirement of less than 40% (nCPAP/HHFNC group).
Results
There were 72 infants in the nCPAP group and 44 infants in the nCPAP/HHFNC group. The postnatal age at trial of first oral feeds was earlier in the nCPAP/HHFNC group ($p=0.012$), but infants were a shorter time on nCPAP compared to nCPAP/HHFNC ($p=0.003$). On subgroup analysis, the age to achieve full oral feeds was earlier in the nCPAP/HHFNC group ($p<0.001$).
Conclusions
In infants with BPD who required respiratory support beyond 34 weeks PMA, use of nCPAP then HHFNC was associated in earlier establishment of full oral feeds.

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EAPS-0781
E-Poster Viewing
Gastroenterology and Nutrition

CHILDREN'S COGNITION INFLUENCED BY GESTATIONAL DIABETES AND MATERNAL LC-PUFAs IN DIET AND PLASMA

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Background and aims
Gestational diabetes (GD) alters placental transfer compromising key nutrients supply to the fetus, such as long chain polyunsaturated fatty acids (LC-PUFAs). These are crucial for cognitive development, especially arachidonic (AA) and docosahexaenoic (DHA) acids. We aimed to

determine if children's cognition is influenced by GD and LC-PUFAs dietary intake and plasma levels of pregnant women.

Methods

Children (n=88) were selected from the population-based PREOBE cohort, divided in control (n=59), maternal GD (n=29) and ultimately in subgroups of maternal pre-pregnancy body mass index (BMI) (normoweight, overweight and obese). Maternal plasma was analyzed at 40 weeks of gestation (WOG) to measure LC-PUFAs in the phospholipid fraction, also nutrient intake was collected at 34–40 WOG using standardized 7 day dietary records. Children's cognition was assessed at 18 months old using the Bayley III Cognitive Scale.

Results

Children from obese mothers with GD and a high dietary intake of AA and low of EPA, DHA, EPA:AA and DHA:AA ratios, showed a lower score on cognition than children from obese mothers without GD with same dietary intake. Regarding maternal plasma, children from obese mothers with GD and low plasma levels of EPA, AA, and EPA:AA ratio showed a lower score on cognition than children from obese mothers without GD with same low plasma levels.

Conclusions

Gestational diabetes decreases score on children's cognition if the mother has a BMI>30 and a low LC-PUFA (EPA, DHA and AA) intake. Obese pregnant women with GD should consider a high dietary intake of these FAs to enhance child cognition.

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EAPS-0457
E-Poster Viewing
Gastroenterology and Nutrition

The Role of Probiotics in Gastrointestinal manifestations of Children with Autism Spectrum Disorder.

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Background and aims

This study was designed to evaluate the role of probiotics supplementation in improving the gastrointestinal symptoms, the general and mental health of the autistic children aged 5 to 9 years old.

Methods

a case control interventional study where 30 autistic children aged 5 to 9 years old and 20 healthy controls of similar age and sex were enrolled . Gastrointestinal symptoms of autistic children were assessed with a modified six-item Gastrointestinal Severity Index(6-GSI)questionnaire, autistic symptoms were assessed with the Autism Treatment Evaluation Checklist(ATEC),and Gastrointestinal flora namely bifidobacterium and lactobacillus in stools were assessed by quantitative real time PCR ,before and after 3 months of supplementation of autistic children with a probiotic nutritional supplement formula(each gram contains 100 $x10^6$ colony forming units of different probiotic strains).

Results

After probiotic supplementation, the stool PCR of autistic children showed increases in the colony counts of Bifidobacteria and Lactobacilli levels, with a significant reduction in their body weight as well as significant improvements in the severity of autism (assessed by the ATEC), and gastrointestinal symptoms (assessed by the 6-GSI) compared to the baseline evaluated at the start of the study.

Conclusions

The use of probiotics seems to be helpful in reducing the severity of autism gastrointestinal symptoms and related abnormal behaviours as well as improving autistic children gut microbiota.

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E-Poster Viewing
Gastroenterology and Nutrition

FECAL MICROBIOTA PROFILE IN NEWLY-DIAGNOSED CROHN DISEASE IN CHILDREN: DATA FROM A MIDDLE EASTERN POPULATION

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Background and aims

Most reports on the microbiome in Crohn's disease (CD) are from Western populations. **The aim** is to describe fecal microbiota in a population of children from Saudi Arabia.

Methods

All children were ethnically homogenous with dietary lifestyle different from the West. The age ranged from 0.5 to 17 years at presentation. The diagnosis of CD was confirmed according to standard criteria. Controls were children who have no inflammation or infection. Stool samples (10 CD and 10 controls) were collected at presentation before any treatment and immediately frozen in -80^0 C. Samples were shipped in dry ice to MR DNA, Shallowater, TX, USA where Amplicon pyrosequencing (bTEFAP®) using 16 S primers was performed. Bioinformatics analysis was performed to assess microbial diversity as well as genera and species associated with CD.

Results

Both alpha beta diversities were reduced in CD stools. CD-associated bacterial genera included Spirochaeta and Bacillus that were significantly more abundant in CD stool ($p < 0.0001$), whereas Intistinibacter and Holdemanella were significantly depleted ($p< 0.0001$). At the species level, several Fusobacterium mortiferus and Psychrobacter pulmonis were significantly more abundant in CD stools whereas many species were significantly depleted ($p < 0.001$). These include Roseburia inulivorans, Blutia luti, Peptostreptococcus anerobius, Peptoclostridium difficile, Intistinibacter bartelitii, Dialister spp, and Bacteroides spp.

Conclusions

This study reveals fecal dysbiosis in CD Saudi children similar to Western descriptions suggesting a minor role of lifestyle and ethnicity.

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E-Poster Viewing
Gastroenterology and Nutrition

POTENTIAL ACTIVITY OF S100B LEVEL TOWARDS METABOLIC SYNDROME IN OBESE CHILDREN

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Background and aims

Background and aim: The epidemic of pediatric obesity is followed by increased rates of associated metabolic complications such as pediatric type 2 diabetes mellitus and pediatric metabolic syndrome. Adipose tissue is a good source of S100B which has been linked lately to metabolic syndrome, moreover to insulin insensitivity. This case control study has been designed to evaluate the significance of S100B as an early predictor for metabolic syndrome in obese children and to study its relation to the different parameters of the disease.

10.2.5. PUBLICATION #5

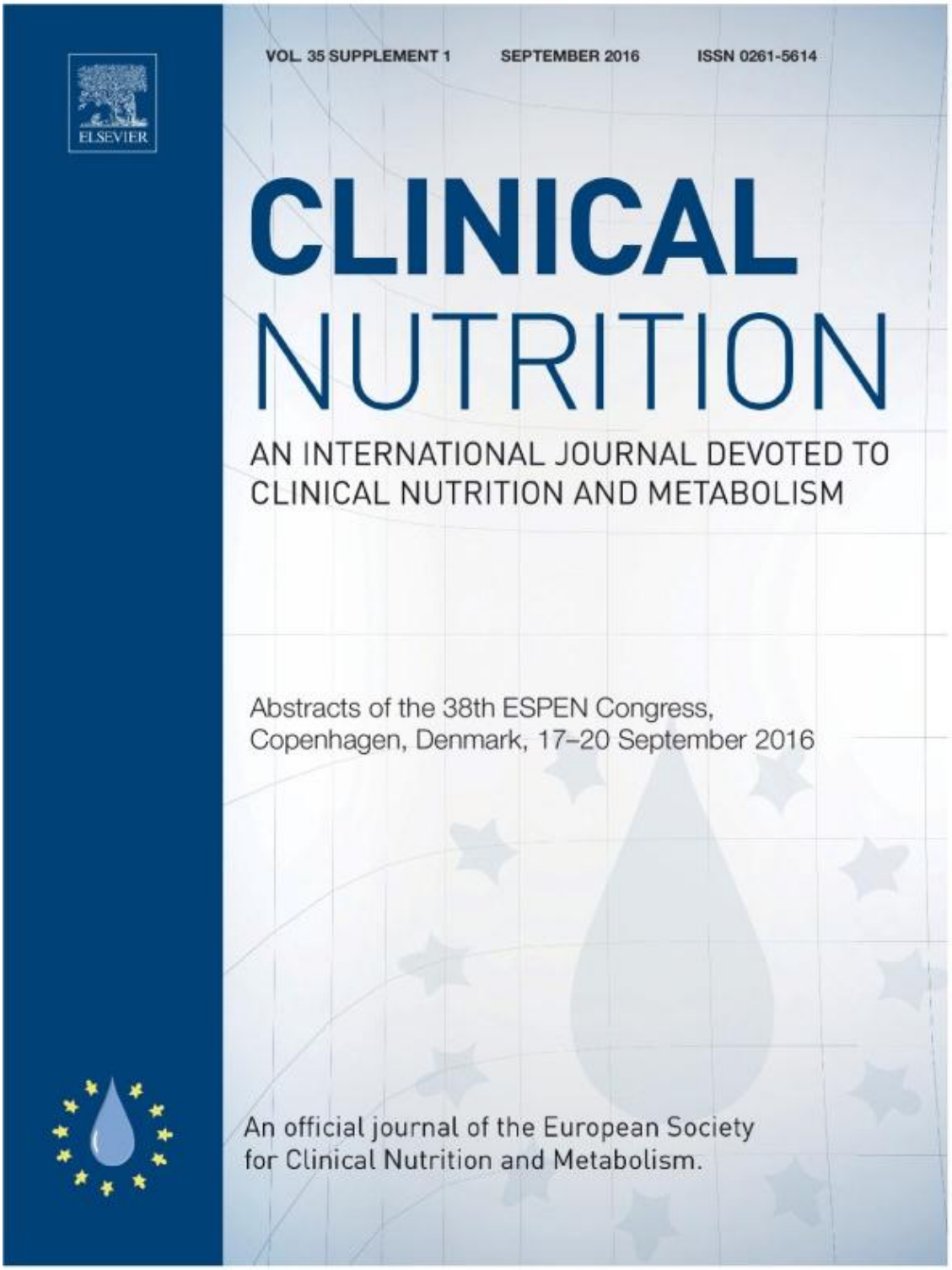

Table 1: Plasma levels of 25-hydroxy vitamin D3 after oral dose of the novel vitamin D nanoemulsion and marketed form

	Nano-VD	Oil-VD	Nano-alone	Oil-alone	p-value*
Basal	18.19 ± 0.88	13.19 ± 3.15	13.74 ± 2.67	14.77 ± 3.99	p < 0.05
1 hour	31.68 ± 3.14	14.77 ± 3.87	13.44 ± 2.16	13.60 ± 4.54	p < 0.001
6 hours	48.00 ± 1.41	14.58 ± 4.47	11.95 ± 0.14	14.50 ± 3.42	p < 0.001
24 hours	61.00 ± 3.29	9.69 ± 1.82	17.99 ± 0.04	13.75 ± 3.30	p < 0.001
p-value**	p < 0.001	p > 0.05	p < 0.001	p > 0.05	

*P by Kruskal-Wallis test, **P by Friedman-ANOVA test

Methods: Twenty-four, adult male albino rats (289 ± 43 g) were housed in single cages and fed AIN-93M diets ad libitum. All single oral doses were delivered by gavage. The four treatment groups (n = 6 rats each) were: a) a 3 mL of VDN solution containing 27 ug/mL VD (3.240 IU), b) the nanoemulsion without VD, c) the same VD dose using the commercial VD with 3 mL of canola oil, and d) a 3 mL dose of canola oil without VD. Serial blood samples (n = 10) were automatically withdrawn (0, 0.5, 1, 2, 4, 6, 8, 10, 12, and 24 h) from surgically inserted carotid catheter in each rat using an automated blood sampling device. The zero time represents the basal level after recovery from surgery and just before ingestion of treatment. Plasma 25-hydroxy vitamin D (25OHVD) was measured by ELISA.
Results: At all time-points, VDN treatment showed higher levels 25OHVD compared to the commercial formulation or controls ($p < 0.05$). The novel VDN formulation tripled serum 25OHVD levels within 24 h (61.0 ± 3.3 vs 18.2 ± 0.9, $p < 0.001$), while the commercial preparation did not in the same period.
Conclusion: Bioavailability of liposoluble VD can be increased using novel pea protein-based nanoemulsion. Further research should address the underlying mechanisms.

Disclosure of Interest: A. Almajwal Grant/Research Support from: none, Consultant for: none, Speaker Bureau of: none, Shareholder of: none, Paid Instructor at: none, Other: none, M. Abulmeaty: None declared, J. Andrade: None declared, M. Elsadek: None declared, S. Razak: None declared

MON-LB274
OUTCOME AFTER PREOPERATIVE IMMUNONUTRITION IN HEAD AND NECK SQUAMOUS CELL CARCINOMA PATIENTS

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Rationale: Perioperative disease-related malnutrition results in compromised wound healing, reduced immunologic functions, increased susceptibility to infections and decreased tolerance to further treatment, leading to a prolonged hospital stay and a poorer prognosis. Patients with head and neck squamous cell carcinoma (HNSCC) are specifically at risk for nutritional deficiencies. The aim of this study was to evaluate the effect of a preoperative immunonutrition (IN) (Oral Impact®) in HNSCC patients undergoing elective oncologic surgery on postoperative outcome, concerning wound healing, local and systemic infection rates, general complications, length of hospital stay (LOS), and rate of re-admissions for postoperative complications.
Methods: Single centre, retrospective study to compare short-term complications (within first 30 days after surgical intervention) of HNSCC patients before (control group) and after implementation (intervention group) of preoperative IN, which was given during 5 days preoperatively. The local wound complications were graded using the Buzby`s classification, Dindo's grading system was used for classifying general complications, and the total LOS and number of readmissions were analyzed.
Results: The control group included 209 and the intervention group 202 patients, respectively. The LOS was significantly lower in the intervention group (median 6 vs 8 days). This was also significant after multivariate adjustment (fully adjusted mean difference −5.65 days (95%CI −7.74 to −3.56), $p < 0.001$). Local wound infections were significantly less frequent in the intervention group receiving IN (7.4% vs 15.3%, adjusted OR 0.30 (95%CI 0.13 to 0.70), $p = 0.006$).
Conclusion: Data about the impact of preoperative IN on postoperative outcome in HNSCC patients are scarce. Preoperative IN reduces significantly wound infections and shortens the hospital stay. In subgroups as patients with previous CRT and patients with an extensive surgery, the effect was even more enhanced. Further randomized controlled trials may be needed to confirm our results and to give evidence based recommendations.

Disclosure of Interest: None declared

MON-LB275
PRE-PREGNANCY BODY MASS INDEX AND GESTATIONAL DIABETES ON MATERNAL FATTY ACID PROFILE AND PLACENTAL TRANSFER

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Rationale: Gestational diabetes (GD) may modify maternal fatty acid (FA) availability during pregnancy and may also alter placental transfer compromising fetus supply of key nutrients such as long chain (LC) polyunsaturated fatty acids (PUFAs), especially arachidonic (AA) and docosahexaenoic (DHA) acids. A high body mass index (BMI) increases the risk for GD and is also related to an altered FA profile, hence we aimed to determine the maternal alterations in FA profile and FA placental transfer caused by GD and if they change according to maternal BMI.
Methods: Pregnant women (n = 179) were selected from the population-based PREOBE cohort, divided in control (n = 135) and women with GD (n = 44), these last were ultimately divided according to their pre-pregnancy BMI (normoweight, overweight and obese). Maternal plasma and umbilical

cord samples were analyzed at delivery (40 weeks of gestation) to measure PUFAs in the phospholipid fraction. Placental FA transfer was analyzed calculating ratios between FAs in the umbilical cord of the offspring and FAs in maternal plasma.

Results: Women with gestational diabetes showed higher plasma levels of AA (10.46% vs 9.32% of total detected FAs), eicosapentaenoic acid (EPA) (0.38% vs 0.30%), DHA (5.17% vs 4.33%), PUFAs (44.25% vs 43.17%), n3 PUFAs (6.12% vs 5.13%), LC-n3 PUFAs (6.01% vs 5.01%), and LC-n6 PUFAs (15.12% vs 14.18%) and lower n6:n3 (6.49% vs 7.83%) and LCn6:LCn3 (2.61% vs 3.00%) ($p < 0.05$). A BMI ≥ 25 in these women implicated a higher ($p < 0.05$) LCn6:LCn3 (2.89% vs 2.37%). Regarding FA placental transfer, women in control group showed higher transfer ratios in AA (2.02 vs 1.73), EPA (0.70 vs 0.46), DHA (1.57 vs 1.33), PUFAs (1.00 vs 0.95), n3 PUFAs (1.44 vs 1.22), n6 PUFAs (0.94 vs 0.91), LC-n3 PUFAs (1.47 vs 1.24) and LC-n6 PUFAs (1.85 vs 1.66) (among others) and a lower transfer in n6: n3 (0.68 vs 0.78) ($p < 0.05$). BMI didn't modify placental transfer ratios.

Conclusion: Gestational diabetes alters maternal FA profile and this may vary according to pre-pregnancy BMI. In spite of maternal weight, GD decreases placental transfer of crucial FAs (e.g. AA and DHA) involved in children development.

Disclosure of Interest: None declared

MON-LB276
EFFECTS OF A NUTRITIONAL PROGRAM FOR OVERWEIGHT AND OBESE ADULTS WITH INTELLECTUAL AND DEVELOPMENT DISABILITIES IN COMMUNITY RESIDENCES

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Rationale: One third of the population with intellectual and development disabilities (IDD) is overweight or obese. This comorbidity associated to IDD leads to an increase in their cardiovascular risk.

Aim: Analyze the effectiveness of a nutritional program in improving anthropometric parameters and cardiovascular risk factors.

Methods: Multicenter and interventional study of people with IDD overweight/obese living in community residences in Catalonia. Followed for 1 year (2014-2015). Intervention divided in two parts:Educational sessions on healthy lifestyle for the residences' staff, the participants themselves and their families. 5 dietetic appointments. In the first visit a low calorie diet and physical activity plan was provided. Variables: anthropometry, biochemistry, dietary and exercise questionnaires.

Results: n:234; 61.5% women. Mean age 47 years ± 12.3. 14 participants abandoned the study. Nutritional status: *Baseline*: 35.5% overweight; 64.5% obesity. *Final*: 53% overweight, 42% obesity and 5% normal weight. 64% lost weight; average weight loss 5.1% ± 3.6 kg. It was found that > 2% weight loss was significantly associated with a decrease in waist circumference, systolic blood pressure, total cholesterol and LDL cholesterol. 69.5% increased exercise duration from 23.9 minutes/week to 90 m/w ($p < 0.001$). At baseline, 65.8% exceeded their nutritional requirements on weekdays and 90.2% on weekends/holidays (361-593 extra kcals), no significant changes were found at this respect.

Conclusion: An intervention in IDD patients entails a clinically significant weight loss and an increase in physical activity. A positive impact in cardiovascular risk factors is observed with modest weight loss.

Disclosure of Interest: None declared

MON-LB277
CHILDREN DIAGNOSED WITH AN ANORECTAL MALFORMATION: DO THEY NEED NUTRITIONAL SUPPORT?

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Rationale: Children diagnosed with an anorectal malformation (ARM) are often known with chronic constipation, faecal incontinence and treated with laxatives. The aim of this prospective study is to understand the nutritional condition and food intake compared with the resting energy expenditure (REE) and recommended daily allowences (RDA) in order to improve the nutritional support for the individual patient.

Methods: Included are 14 children (1-10 years). They were seen in the out-patient clinic. Antroprometric measurements (weight, height) are done according the standard procedure. Growthcharts height for age (HfA) and BMI are used. REE is based on the standard equation WHO and compared with the intake of energy (IE). Parents are asked to keep a three days food record. Food intake is compared with RDA of protein (P), fibre (F), fluids (Fl), calcium (Ca).

Results: Included are 9 boys (mean age 6.4 y) and 5 girls (mean age 4.8 y). Mean HfA and BMI in boys are higher than in girls: resp. 0.44 SDS, 0.49 SDS and −1.52 SDS, −0.24 SDS. The mean difference between REE en IE is 33%: boys 33.9% and girls 32.5%. Mean intake of P is 2.4 gr/ kg bodyweight/day (range 1.5-3.0). Mean intake of Fl and Ca in comparison with RDA are higher in boys than in girls: resp. −0.6%, 8.1% and −13.1%, −13.4%. Mean intake of F in comparison with RDA in boys and girls are almost the same: resp. −32.3% and −33.7%.

Conclusion: The population is small and heterogeneus: 60% boys and 40% girls. The nutritional condition of girls is not good: mean HfA −1.52 SDS and mean BMI −0.24 SDS. IE is just 33% above REE. P is sufficient in boys and girls. F is deficient in every case. The nutritonal intake of girls shows a deficiency of Ca en Fl. More research has to be done in order to recognise the determinants influencing the nutritional intake and condition and to optimize the nutritonal support of the individual patients, especially the girls.

Disclosure of Interest: None declared

MON-LB278
MACULAR CAROTENOIDS ARE POSITIVELY RELATED TO ACADEMIC ACHIEVEMENT IN PRE-ADOLESCENT CHILDREN

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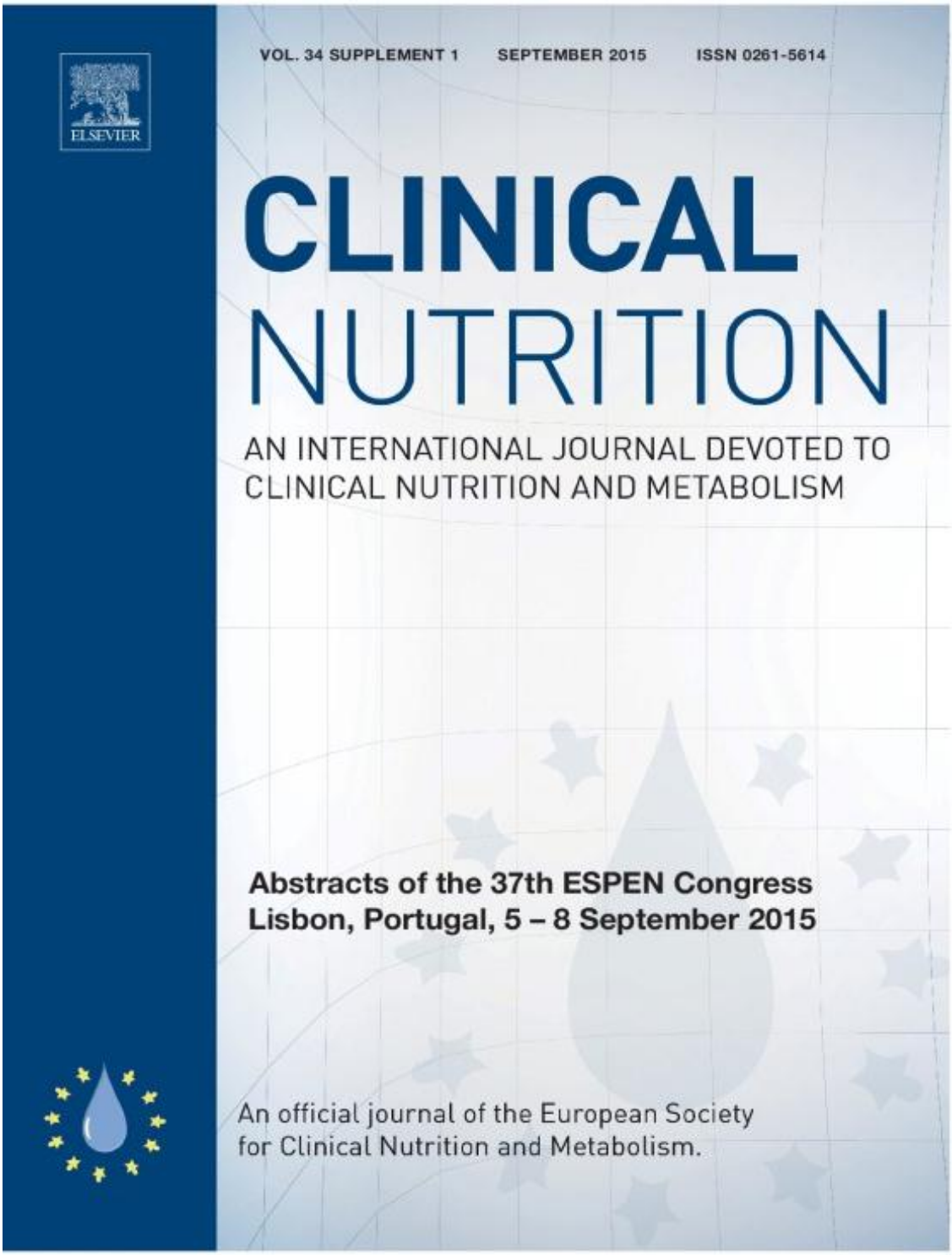
10.2.6. PUBLICATION #6



Median PG-SGA numerical score was 4 points and 26% of the patients scored ⩾9 points. 73% of the patients had low HGS, of which 31% were classified as Stage B or C. In contrast to all four patients using the Pt-Global webtool, 66% of patients using the paper version needed assistance with completing the PG-SGA SF, mainly because of not being able to write due to the shunt, or visual impairment.

Conclusion: More than one-third of haemodialysis patients were malnourished or suspected to be malnourished and in about one-quarter the PG-SGA numerical score indicated critical need for improved symptom management and/or nutrient intervention options. Frequency of low HGS was higher than that of malnutrition. Use of the PG-SGA and HGS provides a complete assessment of nutritional status in haemodialysis patients.

Disclosure of Interest: M. Kuijken: None declared, S. Lichthart: None declared, L. Wit-van der Werf: None declared, M. Donders: None declared, A. Gastel: None declared, G. Berg: None declared, H. Jager-Wittenaar Other: Co-developer of the PG-SGA based Pt-Gobal app

MON-LB018

IMPACT OF MATERNAL PRE-PREGNANCY BMI IN CHILDREN'S BMI PERCENTILE AND FATTY ACID PROFILE AT 6 MONTHS AND 3 YEARS OF AGE

A. de la Garza Puentes[1,2], L. Pereira de Almeida[1], R. Montes[1,2], A.M. Chisaguano[1,3], I. Salas[1], A.I. Castellote[1,2], M.T. Segura[4], L. García-Valdés[4], F.J. Torres-Espínola[4], J. Florido[5], C. Padilla[5], C. Campoy[4,6], M.C. López-Sabater[1,2]. *[1]Department of Nutrition and Food Science, University of Barcelona, Barcelona, [2]CIBER Phisiopathology of Obesity and Nutrition (CIBERobn), Institute of Health Carlos III, Madrid, Spain; [3]Faculty of Health and Science, National University of Chimborazo, Chimborazo, Ecuador; [4]Centre of Excellence for Paediatric Research EURISTIKOS, [5]Department of Obstetrics and Gynecology, [6]Department of Paediatrics, University of Granada, Granada, Spain*

Rationale: Pre-pregnancy Body Mass Index (BMI) is associated with children's health through life span, possibly leading to diseases such as obesity which is related to imbalances in n-6:n-3 ratio, n-3 and n-6 long-chain polyunsaturated fatty acids (LC-PUFAs) that are fundamental in childhood neurodevelopment and risk of diseases. The aim was to determine if maternal pre-pregnancy BMI alters fatty acid (FA) profile and/or BMI percentile of children.

Methods: Mothers and their infants from a Spanish population were included. Infant cheek cell samples and BMI percentiles were collected at 6 months and 3 years of age. Children were divided according to maternal pre-pregnancy BMI: 17 children from normoweight (group 1), 12 from overweight (group 2) and 6 from obese (group 3). All mothers had appropriate weight gain. FAs were assessed by glycerophospholipids determination and analyzed by fast gas chromatography. Statistical analyses with SPSS 20.0 using ANOVA Bonferroni's *post-hoc* and Student's T Test.

Results: At 6 months, children from groups 1 (75%) and 2 (50%) tended to be at 15.1–84.9th BMI percentile and group 3 (57%) at ⩽15th BMI percentile. Groups had few children tending to ⩾85th BMI percentile, group 3 had the most (14%). The n6:n3 and LC-n6:n3 ratios were significantly higher ($p < 0.05$) in group 3 than in group 1, maintaining at 3 years of age. Without significant differences, PUFA n-6 and LC-PUFA n-6 were higher in groups 2 and 3 than in group 1. The PUFA n-3 and LC-PUFA n-3 were higher in children from group 1 than group 3.

Conclusion: Children in group 3 have a FA profile and BMI percentile altered at 6 months when compared to group 1. As this relation remains at 3 years of age, a normoweight pre-pregnancy BMI might be important on children's outcome. There is a suggestion of the importance of a complete nutritional assessment in children as they may have an altered FA profile with normal/low BMI percentile.

Disclosure of Interest: None declared

MON-LB019

DOES WATERCRESS INFLUENCE PHASE ANGLE IN BREAST CANCER PATIENTS DURING RADIOTHERAPY?

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Rationale: A large number of clinical trials propose phase angle (PA) as a useful prognostic role in breast cancer (BC), since prognosis is associated with changes on cell membrane integrity and alterations in fluid balance. Several authors suggest that PA can be an important tool to evaluate disease progression. Recent studies identified several active isothiocyanates in watercress extract that may have a significant anticarcinogenic activity than PEITC from watercress alone. Several watercress components have antigenotoxic effects *in vitro* resulting in reduced DNA damage and have antiproliferative effects. These cell effects of watercress supplementation may further prove useful in modulating cancer progression and disease recurrence. This longitudinal nutrition intervention clinical trial aimed to evaluate the effect on phase angle of a nutrition intervention supplemented with watercress (IG) *vs* a control group (CG) that maintained their *ad libitum* diet.

Methods: 31 BC pts referred for radiotherapy (RT) with curative intent were randomized (IG n = 16; CG n = 15). The intervention group was extensively and comprehensively instructed on how to ingest 100 g of watercress daily, during RT. PA was assessed before RT and after treatment.

Results: Before RT, overall mean value of PA was 6.1° (IG 6.1° *vs* CG 6.2°) and, at the second time point, PA was 6.3° (IG 6.3° *vs* CG 6.5°), with no statistically significant differences between groups, although with a trend for significance ($p < 0.09$). All patients were above the 5th percentile according to sex/age in both assessments. During the study, in the IG there was a trend for a positive difference with a difference between the end of RT and the start of intervention.

Conclusion: There was a trend for watercress supplementation (100 g/d) to positively influence PA during RT treatment in BC patients, that may aid RT efficacy and increase the potential for a favourable treatment outcome and disease prognosis.

Disclosure of Interest: None declared