UNIVERSITAT DE BARCELONA

Nutrition and cardiometabolic risk: a prospective population-based cohort study

Anna N. Funtikova

Nutrition and cardiometabolic risk: a prospective population-based cohort study

Anna N. Funtikova presents this thesis with the aim of obtaining the title of PhD within the Doctoral Program on Food and Nutrition at the Faculty of Pharmacy, University of Barcelona.

This work has been carried out at the Cardiovascular Risk and Nutrition Research Group, within the Epidemiology and Public Health Research Program at IMIM (Hospital del Mar Medical Research Institute), Barcelona.

Dr. Helmut Schröder (director)	Dr. María Izquierdo (tutor)	Anna N. Funtikova (PhD candidate)

Anna N. Funtikova
Barcelona, 2015

Посвящаю эту работу моим родителям за их веру в меня, любовь и терпение

Aknowledgements

First of all, I would like to thank the director of my thesis, Dr. Helmut Schröder, for his patience, attitude, efficiency, and love of his work. Thank you Helmut for all the papers you made me to publish. I always feel that I am lucky to have you as the director of my thesis.

Quería dar las gracias a la Dra. María Izquierdo por tu apoyo, por estar siempre dispuesta a resolver cualquier duda acerca de la elaboración de la tesis y por tus bromas tan alegres, al Ministerio de Educación, Cultura y Deporte de España por concederme una de las 50 becas para extranjeros para realizar este doctorado y al IMIM (Instituto Hospital del Mar de Investigaciones Médicas) por darme una beca para imprimir los ejemplares de esta tesis.

Quería agradecer especialmente a la jefa de nuestro grupo de investigación, la Dra. Montserrat Fito por tu bondad, tu comprensión y tu capacidad para encontrar soluciones a cualquier tipo de problema!

Gracias también al Dr. Jaume Marrugat por las actividades tan divertidas que has promovido en nuestro grupo y por tus útiles consejos. Gracias, Dr. Roberto Elosua por tu humor y por tus charlas siempre interesantes!

Un agradecimiento a la Dra. Maribel Covas por darme la bienvenida y aceptarme en tu grupo de investigación.

Al Dr. Javier Remesar por aconsejarme el grupo de investigación CARIN.

Special thanks to Gavin for his valuable advice during my work and revision of the English in my thesis.

Quisiera dar las gracias a mis compañeros del despacho. Primero a Álvaro! Llegamos el mismo día a trabajar, gracias por tus charlas, tus bromas, por enseñarme español informal y por el amor a mí pastelería! Muchas gracias a Marta, Ojcar, Carla, Sergi y Olga por el ambiente súper agradable en el despacho 122.12 y fuera del trabajo.

Gracias también a las secretarias, Yolanda, Esmeralda, Anna y Sandra, por vuestra ayuda y por demostrar tanto amor a mi pan Ruso!

Un especial agradecimiento a Juan por tu paciencia dándome explicaciones y enseñándome de forma súper comprensiva, contigo la estadística me parecía una ciencia bastante interesante. Y a Isaac por tu ayuda con R, por responder a las mismas preguntas cada vez y por todos los gráficos bonitos que me ayudaste a hacer!

Gracias a todos los miembros de los grupos CARIN y ULEC, sin vosotros mi doctorado no sería tan memorable!

Thank you very much to Dr. Renée de Mutsert for your help and support during my unforgettable research stay in the Netherlands.

Muchas gracias a Dr. Juan Sabaté por una estancia fantástica en EEUU. Por tu cuidado y la experiencia excelente que me ofreciste.

Gracias también a mis amigas: Sina y Rosa, por vuestro apoyo en los momentos más difíciles durante la escritura de esta tesis.

Y por último un agradecimiento especial a mi novio Guillermo, por tu apoyo y tu paciencia durante el año más duro de mi doctorado y por ayudarme a montar la tesis.

Самую большую благодарность я хотела бы выразить моим любимым родителям, моей сестре Ирине и моей единственной бабушке. Низкий Вам поклон за Ваше терпение, поддержку и веру в меня. Даже на расстоянии я чувствовала Вашу горячую безусловную любовь и искренние молитвы, все это стало самой сильной опорой для моего существования так далеко от родного дома..! Спасибо Вам за то, что Вы есть!

Preface

Why diet..? Sometimes we live our everyday lives without considering the things that happen to us every day, and the fact that they form the basis of our health and wellbeing. We never study or learn about them. We eat several times per day and rarely think about food as about our building material – the well-known phrase, “you are what you eat”, is not just metaphorical, but rather is literally true. Amino-acids from our food construct our body, carbohydrates give us energy and fats form our reserves. Of all possible variables, diet probably has the most powerful influence on our health, and modifying ones diet can produce visible effects in a relatively short period of time. The documentary movie “Super-size me” (2004) [1] is an extreme but resounding account of the rapid and harmful effects of modern diets on our health. It took just two weeks on a strict fast food diet to disrupt several important metabolic processes in a relatively young and healthy adult. While not a very scientific source of information, the movie attracted the attention of the general public and helped to highlight the importance of diet as more than just a fuel.

Peer-reviewed scientific studies confirm the importance of diet for health [2]. Moreover, clinical trials have demonstrated that a healthy diet is in itself a powerful and fast-acting tool for improving individual health [3, 4]. Thus, while a poor diet can seriously damage our health or even kill us, a healthy diet can serve as a natural approach for primary and even secondary prevention of common chronic diseases.

Diet is closely associated with common cardiovascular risk factors such as obesity, type 2 diabetes and hypertension. Since cardiovascular diseases are the number one cause of death worldwide, and that diet is one of the most powerful modifiable risk factors; this thesis describes epidemiological studies focused on the relationship between diet, cardiovascular risk factors and cardiovascular disease.

Abstract

Cardiovascular diseases (CVD) are the principal cause of mortality worldwide. Lifestyle plays a crucial role in preventing the development of CVD, and one of its key elements is diet, which directly affects cardiometabolic health and cardiovascular risk. Given the complexity of diet and also of cardiovascular disease etiology, a lot of recent research into the association between diet and disease have focused on dietary patterns, as this is currently the most holistic way to study dietary habits in the population. In this work, we examined dietary patterns and their components that are associated with cardiometabolic health. Data were obtained from two population-based cross-sectional surveys conducted in Girona (Spain) in 2000 and 2005. The first survey included 3,058 randomly selected free-living men and women aged 25 to 74 years. The second survey included 6,352 men and women aged 35 to 80 years. These cohorts were re-examined in 2010 and 2012, with a follow up rate of 80.6% and 78.0%, respectively. At baseline and follow-up, we collected data on diet, using a validated food frequency questionnaire (FFQ), and on cardiovascular risk. We performed validation studies of *a priori* and *a posteriori* dietary patterns. In the *a priori* analysis, we found that a Diet Quality Index derived from a short Diet Quality Screener had good ability to predict future cardiovascular health complications. The *a posteriori* analysis showed good reproducibility and modest validity of dietary patterns defined using cluster analysis of the FFQ data. In a further step, we analysed the impact of energy under-reporting

on dietary patterns and secular trends in dietary patterns. We concluded that energy under-reporting is a serious problem in nutritional epidemiology, especially when developing dietary guidelines for the general population, and more solid research is urgently needed in this area. To explore the association between diet and cardiovascular risk factors and CVD, we focused on abdominal obesity, a risk factor that had received increasing interest in recent years. Adherence to the Mediterranean diet and substitution of soft drinks with other caloric beverages, such as milk and juice, was associated with decreased abdominal adiposity. Finally, we studied the association between dairy products and cardiovascular events, and concluded that consumption of dairy products does not have an adverse impact on CVD incidence, and that frequent intake of yogurt was negatively associated with CVD incidence, presumably due to its prebiotic properties.

In conclusion, this PhD research shows a smaller increase in waist circumference among individuals with high adherence to the Mediterranean diet, and the opposite for soft drink consumption. Furthermore, the results support the hypothesis that yogurt consumption is cardioprotective. Finally, it underlines the need to control for energy misreporting in epidemiological studies, and to validate dietary patterns.

Resumen

Las enfermedades cardiovasculares (ECV) son la principal causa de mortalidad a nivel mundial. El estilo de vida juega un papel crucial en la prevención del desarrollo de las ECV, y uno de sus elementos clave es la alimentación, que afecta directamente a la salud cardiometabólica. Dada la multitud de variables que intervienen en los hábitos alimentarios y la complejidad de la etiología de las ECV, una gran cantidad de investigaciones recientes sobre la relación entre la dieta y la enfermedad se han centrado en estudiar los patrones dietéticos, ya que estos son, actualmente, la forma más holística para estudiar los hábitos alimentarios de la población. En este trabajo, hemos examinado qué patrones dietéticos y cuáles de sus componentes están asociados con la salud cardiometabólica. Los datos se han obtenido a partir de dos estudios transversales de base poblacional realizados en Girona (España) en 2000 y 2005. El primer estudio incluyó 3.058 hombres y mujeres, no institucionalizados, y seleccionados al azar de la población de entre 25 a 74 años. El segundo estudio incluyó 6.352 hombres y mujeres, no institucionalizados, de edades comprendidas entre los 35 a 80 años. Estas dos cohortes fueron re-examinadas en el año 2010 y en el año 2012, con una tasa de seguimiento del 80,6% y 78,0%, respectivamente. Al inicio y al final del estudio se recogieron los datos dietéticos, mediante un cuestionario de frecuencia de alimentos validado (CFA), y los datos sobre los factores de riesgo cardiovascular. Se realizaron estudios de validación de los patrones dietéticos *a priori* y *a posteriori*. En el análisis *a priori*, encontramos que el Índice de Calidad

Alimentaria derivado de un Cuestionario corto de Calidad de la Dieta tenía buena capacidad para predecir acontecimientos relacionados con la salud cardiovascular. El análisis *a posteriori* mostró una buena reproducibilidad y una validez modesta de los patrones dietéticos definidos mediante análisis de clústers de los datos del CFA. En una siguiente etapa, se analizó el impacto de la declaración inferior de los niveles reales de energía consumida sobre los patrones dietéticos y las tendencias seculares en los mismos. Llegamos a la conclusión de que la energía declarada por debajo de valores reales es un problema grave en la epidemiología nutricional, sobre todo en el desarrollo de guías alimentarias para la población en general, y se necesita con urgencia una investigación más sólida en este área. Para explorar la asociación entre la dieta y los factores de riesgo de ECV, nos hemos centrado en un factor de riesgo como la obesidad abdominal. El seguimiento de una dieta mediterránea y la substitución de las bebida gaseosas por otras bebidas calóricas, como la leche y el zumo, se asoció con una disminución de la adiposidad abdominal. Por último, estudiamos la asociación entre los productos lácteos y los episodios cardiovasculares, y llegamos a la conclusión de que el consumo de productos lácteos no tiene un impacto adverso sobre la incidencia de las ECV, y que el consumo frecuente de yogur se asoció negativamente con la incidencia de ECV.

En conclusión, esta investigación doctoral muestra un menor aumento de la circunferencia de la cintura entre los individuos que siguen una dieta mediterránea, y lo contrario para el consumo de bebidas gaseosas. Además, los resultados apoyan la hipótesis de que el consumo de yogur actúa como cardioprotector. Por último, subraya la necesidad de controlar la declaración errónea del consumo de energía en los estudios epidemiológicos, y de validar los patrones dietéticos.

Contents

1. Introduction

1.1. CVD trends and statistics

The 20th and 21st centuries have seen a significant improvement in health and health care. During this period, the main cause of death has changed from infection to non-communicable disease, including cardiovascular disease (CVD). The main reasons for this dramatic change are population aging, increased urbanization and globalization, which favor development of cardiovascular risk factors for the principle causes of mortality worldwide: ischemic heart disease and stroke. CVD is not only the most important cause of mortality, accounting for ~30% of deaths worldwide, but also has enormous economic costs, especially in developed countries, and results in significant disability [5]. Figure 1 shows CVD mortality in 2005 in Europe, where the lowest and highest rates were observed in French females and Russian males (109.88 and 1145.11 deaths per 100,000 cases, respectively) [6].

During the last 20 years, CVD mortality has decreased significantly in developed countries, but has remained consistent or even increased in middle and lower middle income countries (Figure 2) [7]. Figure 2 shows CVD mortality rates in North and South America, highlighting a steady decline in CVD mortality in almost all high income countries, such as Canada and the USA, and very slight or no decline in middle and lower middle income countries, such as Mexico, Panama, Paraguay.

A number of lifestyle factors play a major role in the development of CVD, such as physical activity, smoking, alcohol consumption and diet. Among these, diet has been shown to have a cause relationship with CVD and CVD risk factors [3, 8].

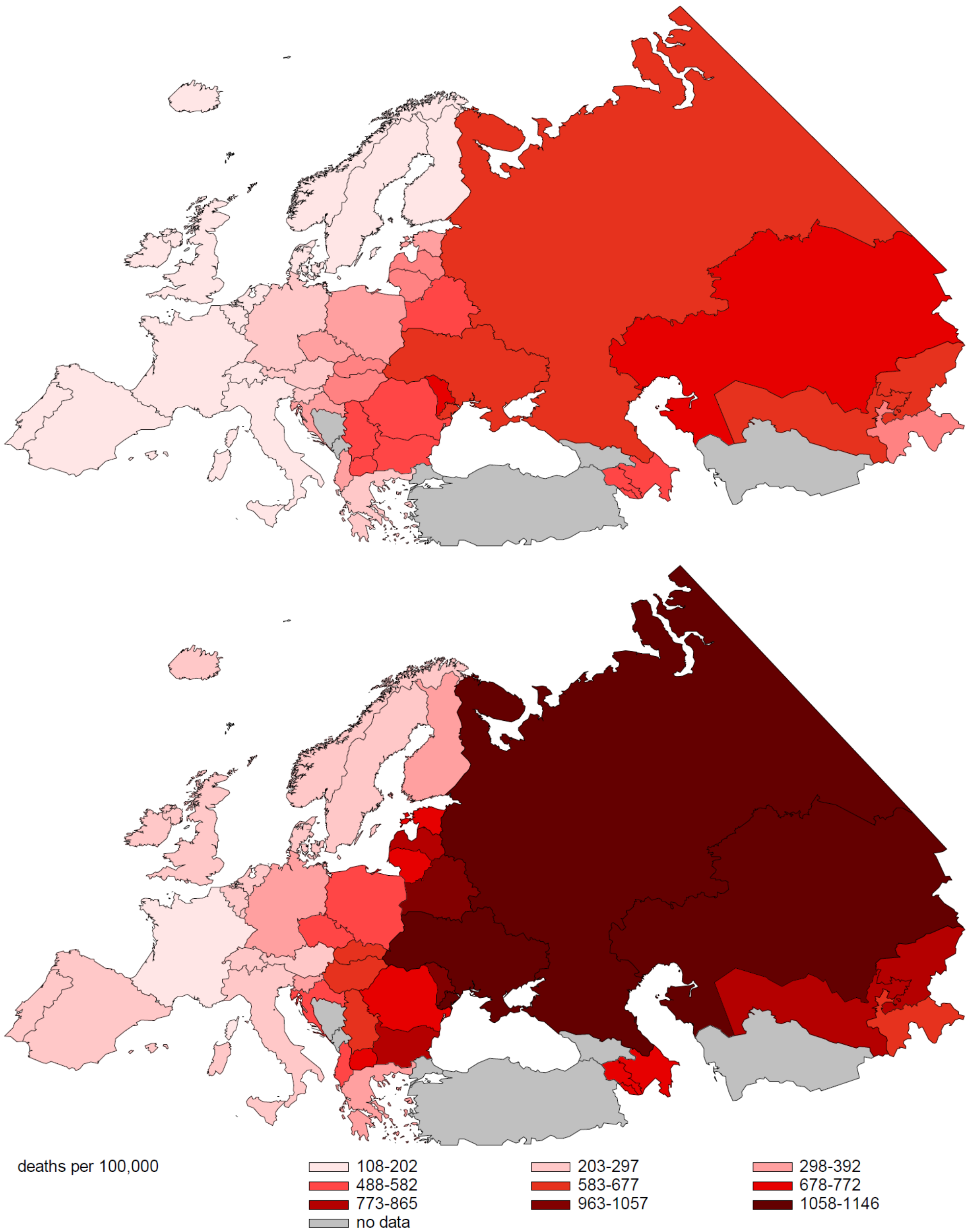


Figure 1. Age-standardized CVD mortality in Europe in 2005 (ICD-10: I00-I90). Top: females, bottom: males [6]. CVD mortality data for 2005 were not available for some countries, so data for 2003 (Armenia, Macedonia, Italy) or 2004 (Albania, Belgium, Azerbaijan, Portugal) are shown.

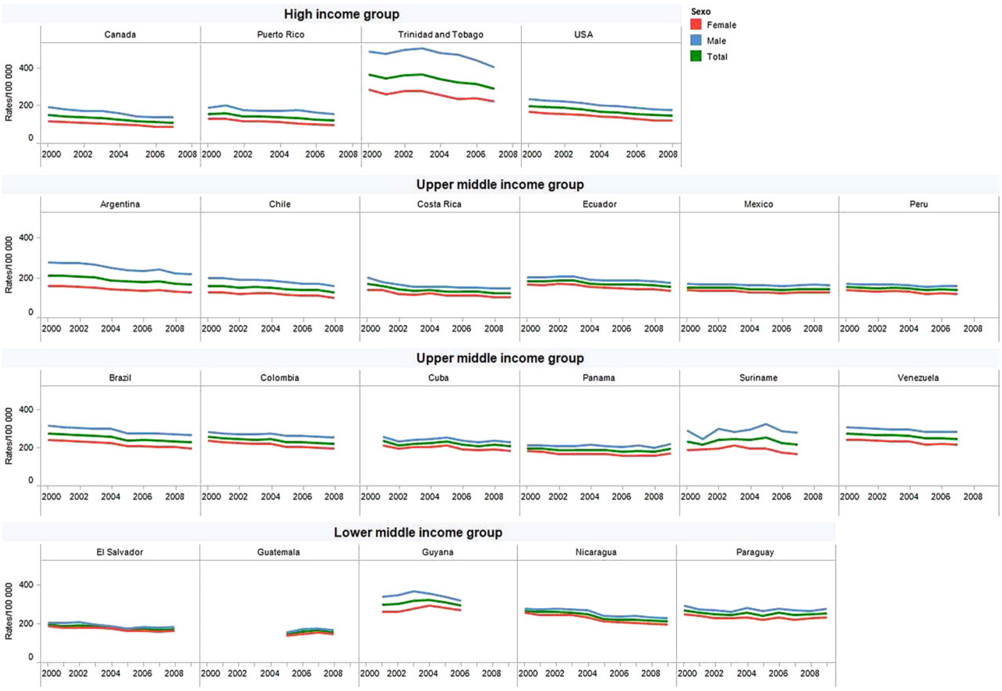


Figure 2. Trends in mortality due to cardiovascular diseases (ICD-10 I00–I99) (age-adjusted rates/100,000). Selected countries in North and South America, 2000 to the latest available year [7].

1.2. Approaches to studying diet

In nutritional epidemiology, the relationship between diet and disease has typically been studied by testing for association between a health outcome of interest and a single nutrient or food. While useful, this approach has several limitations. First, people do not eat nutrients in isolation or just one food item all the time, but rather complete meals with a complex combination of foods and nutrients that interact with each other. A classic example of interaction between nutrients is improved absorption of iron in the presence of vitamin C. Nutrients can also be inter-correlated (e.g. potassium and magnesium), which can make it difficult to distinguish between their effects [9]. Furthermore, a large number of different nutrients and foods, consumed together in a given diet, may show statistically significant associations with a health outcome of interest just by chance because certain food items are usually closely related to certain dietary pattern, which can be related to the health outcome. Therefore, while the effect of a single nutrient or food can be too small to detect, the overall effect of the nutrients or foods in a specific dietary pattern can be large enough to be detected by practicable study designs. Therefore, to overcome these limitations, we decided to analyze the association between the entire diet, or dietary pattern, and health outcomes of interest. This approach is a better approximation of reality, where people consume a combination of different nutrients and foods. Since dietary patterns cannot be directly measured as nutrients and foods, special statistical methods based on dietary data can be used to represent typical dietary patterns in a study population. Two major groups of methods are currently used to reconstruct dietary patterns, *a priori* methods and *a posteriori* methods, and are described below.

1.2.1. *A priori* methods for constructing dietary patterns

A priori methods include all possible types of indexes and scores, most of which can be divided into two types. The first type is based on dietary recommendations, and describes diet quality, in general or specific terms, for a certain health status. The second type of index is based on the principles of a traditional diet, and describes the level of adherence to that diet.

Construction of a specific index is a complex task, requiring thorough knowledge of healthy nutrition. Depending on the objective, the index must be designed to capture specific aspects of dietary habits in the study population. Population-specific dietary guidelines, food pyramids, and the results of multiple clinical studies are among the sources of information required to construct indexes/scores to describe diet quality or adherence to a dietary pattern that is beneficial for prevention of a certain disease. Examples of this type of index/score include the Healthy Eating Index (HEI), the Healthy Food Diversity Index, the Dietary Approach to Stop Hypertension (DASH) score, the Dutch Healthy Diet Index, the Healthy Lifestyle-Diet Index, the US Healthy Food Diversity Index, and the Alternate Healthy Eating Index (AHEI), as well as the Diet Quality Index (DQI), which was developed in our group [10].

The second type of *a priori* dietary patterns is used to analyze adherence to a certain diet, such as the Mediterranean, Nordic, Vegetarian, or Vegan diets. Examples of this type of dietary index/score include the Mediterranean Diet Score (MDS), Modified Mediterranean Dietary Score, Baltic Sea Dietary Score. Vegetarian and Vegan diets are normally defined by non-consumption of certain types of animal foods, depending on the strictness of the diet's definition. Many studies have demonstrated the beneficial effect of these diets for various health problems, making them popular not only in the original region, but also in other populations.

Methods focused on *a priori* dietary patterns are quick and easy to use instruments for measuring diet quality and level of adherence to a specific diet. One of their disadvantages is that they cannot capture new dietary patterns arising in the population, although they can measure trends in diet quality or adherence to a certain dietary pattern. Also, the validation of methods focused on *a priori* dietary patterns is rather laborious, in that they must be validated or at least adapted for every target population, especially in populations that are very different.

1.2.2. *A posteriori* methods for constructing dietary patterns

A posteriori dietary patterns are data-driven dietary patterns constructed using various statistical methods. The main advantage of this type of dietary patterns is that it allows us to define new dietary patterns arising in the target population, which is difficult to do with *a priori* dietary patterns.

Several statistical methods can be used to construct *a posteriori* dietary patterns, the most popular being cluster and principal component analysis (PCA). Cluster analysis groups individuals with similar dietary habits into relatively homogeneous clusters, and each individual in a sample is assigned to just one cluster or dietary pattern. Study participants can be categorized according to various criteria, such as the amount of food consumption [11], frequency of food consumption [12], or percentage of total energy consumption [13]. Cluster analysis is a very convenient form of dietary pattern analysis and provides readily interpretable results, although it has several weaknesses. During cluster definition, the investigator must take several subjective decisions, including defining the number of clusters and their names, and criteria for cluster definition.

PCA is another popular method for constructing *a posteriori* dietary patterns. Unlike clusters, principal components are mathematical functions of the variables of interest, and not of the sample participants. PCA uses existing dietary data and defines common dietary patterns based on correlations between food groups or food items. The final value of each principal component is a summary score, such that each individual can belong to all principal components, or dietary patterns, but has different score in each one. In comparison with cluster analysis, the main disadvantage of PCA is the difficulty in interpreting the final dietary patterns.

1.2.3. Validation of dietary patterns

Analysis of dietary patterns measures various parameters of diet. The utility and quality of the measuring method is expressed in terms of its validity and reproducibility. A method's validity describes how well it measures what it intends to measure, and its reproducibility shows whether it gives comparable results each time it is used. A population's dietary habits and dietary patterns undergo constant change, and valid and reproducible methods are required to track these changes within and between populations. Few studies have explored the validity and reproducibility of dietary patterns, and *a posteriori* dietary patterns are studied less than *a priori* ones. Results vary according to the method and the objective of the study. While dietary patterns will clearly differ between studies and populations, both *a priori* and *a posteriori* dietary patterns shows reasonable validity and reproducibility [14-16]. In a recent study of *a priori* dietary patterns, Collins et al. [17] analyzed the reproducibility and validity of the Australian Recommended Food Score (ARFS) and compared the data from the ARFS with food data from the Australian Eating Survey (AES) food frequency questionnaire (FFQ). Their analysis of reproducibility and validity showed medium to high correlation of the ARFS with the AES FFQ [reproducibility: median correlation for ARFS food groups, 0.66 (95% CI 0.48, 0.84), what is considered to be a strong correlation].

The validity and reproducibility of *a posteriori* dietary patterns constructed using cluster analysis has been less well studied than other methods. In a recent validation study, Dekker et al. [18] explored the internal stability and reproducibility of dietary patterns defined using cluster analysis from three consecutive surveys over a 10-year period. They defined two dietary patterns in each survey, a high-fiber bread pattern and low-fiber bread pattern. These patterns showed many similarities between surveys and high reproducibility over the 10-year period. The patterns were stable over time and only the low-fiber bread group showed notable changes in consumption (>5% of total energy). This demonstrates the importance of studies focused on the reproducibility and validity of *a posteriori* dietary patterns, in that they also reflects dietary trends and transitions in population.

1.3. Dietary patterns and CVD: Background

1.3.1. *A priori* dietary patterns

Various *a priori* indices have been developed to date [19, 20], some of which are widely used and have formed the basis for the development of new ones. The most popular *a priori* dietary patterns and their association with health are explained in more detail below.

1.3.1.1. Mediterranean diet

The Mediterranean diet is a traditional dietary pattern, and has been the primary focus of research in this field, partly because natives of olive-growing Mediterranean regions stand out for their long life expectancy. The Seven Countries Study showed that the specific dietary pattern of these regions is key to their longevity and reduced CVD risk, compared to North European countries [21]. The specific dietary pattern of this region was named the Mediterranean diet, which is a plant-based diet characterized by abundant consumption of monounsaturated fatty acids (MUFA) in the form of extra virgin olive oil, and abundant consumption of nuts, legumes, seasonal fruits and vegetables and whole grain cereals, moderate consumption of dairy products, fish and wine, and low consumption of meat. Some investigators suggest that the concept of the

Mediterranean diet should include the entire lifestyle of the Mediterranean population, which is characterized by physical activity, sunny climate, after-lunch napping and sociality, which play an important role in preventing chronic non-communicable diseases.

Since the Mediterranean diet was initially described, many indexes have been developed with the aim of quantifying the level of adherence to the Mediterranean dietary pattern. The first Mediterranean diet index, the MDS, was developed by Trichopoulou et al. (1995) [22] to reflect the traditional diet of older Greeks, including eight dichotomized components: high consumption of vegetables, legumes, fruit, nuts and cereals, and a high ratio of monounsaturated:saturated fat; low consumption of dairy and meat products; and moderate consumption of ethanol. In a meta-analysis of 18 cohort studies and 2,190,627 participants, Sofy et al., [23] found that a 2-point increase in adherence to the MDS was associated with a 10% (Relative risk (RR) = 0.90, 95%CI 0.97,0.93) decrease in risk of CVD incidence and mortality. In the Greek EPIC study, a 2-point increase in the MDS was associated with a 25% (Hazard ratio (HR) = 0.75, 95%CI 0.57, 0.98) and 19% (HR = 0.81, 95%CI 0.67, 0.99) decrease in risk of coronary heart disease (CHD) mortality among women and men, respectively [24].

Many intervention studies using the Mediterranean diet have been conducted, where the health effects of this diet were compared to those of other diets. In a meta-analysis of six intervention studies, Nordmann et al. [25] calculated the weighted mean difference between the effect of the Mediterranean diet and low-fat diets on CVD risk factors over 2 years, and found that the Mediterranean diet group achieved more favorable cardiovascular profile than the low-fat diet group. The Mediterranean diet group had greater reductions in body weight (-2.24 kg, 95%CI -3.9,-0.6), systolic (-1.70 mm Hg, 95%CI -3.4,-0.1) and diastolic (-1.47 mm Hg, 95%CI -2.1,-0.8) blood pressure, total cholesterol (-7.35 mg/dL, 95% CI -10.3, -4.4), C-reactive protein (-0.97 mg/L, 95%CI -1.5, -0.5), and fasting plasma glucose (-3.83 mg/dL, 95%CI -7.0, -0.6).

The most noteworthy results regarding the protective effect of the Mediterranean diet against CVD mortality and morbidity were obtained by the PREDIMED study [3]. Estruch et al. [3] compared two Mediterranean diet intervention groups, one with consumption of one liter/week of virgin olive oil, and another with consumption of 30 g/day of nuts, and a control group on a low-fat diet. They showed a 30% (RR = 0.70, 95%CI 0.54, 0.92) decrease in the risk of cardiovascular events in the virgin olive oil group and a 28% (RR = 0.72, 95%CI 0.54, 0.96) decrease in the nuts group, in comparison with the low-fat control diet.

1.3.1.2. Dietary Approach to Stop Hypertension

Another dietary pattern widely known to be beneficial for CVD risk is the DASH diet, which was created according to dietary recommendations aimed at lowering blood pressure [26]. The DASH dietary pattern is characterized by high intake of plant-based foods, such as fruits and vegetables, and low intake of fat and cholesterol.

Various intervention studies have demonstrated the beneficial effect of the DASH diet on blood pressure and other CVD risk factors. Chen et al. [27] reported that their DASH intervention group had lower 10-year CHD risk after 8 weeks of intervention than a fruit and vegetable group (RR = 0.89, 95% CI 0.75, 0.90) and a control group (RR = 0.82, 95% CI 0.81, 0.97). However, these results were ethnicity-specific, with African-Americans showing greater reduction of 10-year CHD risk than Caucasians (p-value for interaction 0.038).

Another study evaluated the effect of the DASH diet combined with restriction of sodium [4], a well-known risk factor for CVD but not originally a component of the DASH intervention. The trial included three groups on the DASH diet and three groups on a control diet, with three levels of sodium consumption for each group: high, intermediate and low. After the intervention, the groups on the DASH diet had lower blood pressure than the control groups at all sodium levels, and the DASH diet had a greater effect in the intermediate and high sodium groups than in the low sodium group.

Application of the DASH dietary pattern in epidemiological studies required the development of a DASH-style diet score consisting of eight food components that reflect adherence to the DASH diet: fruit, vegetables, whole grain, nuts and legumes, low-fat dairy, red and processed meats, sweetened beverages, and sodium [28]. In a meta-analysis of seven cohort studies, Salehi-Abargouei et al. [29] reported 20% (RR = 0.80, 95%CI 0.74, 0.86) lower risk for CVD in the highest n-tile of the DASH-style diet score than in the lowest n-tile (here, n represents tertile, quartile, etc., depending on the study). Regarding CVD mortality, in the Dietary Pattern Methods Project based on three large cohorts, Liese et al., [30] analyzed the DASH score together with three other dietary scores: HEI 2010, AHEI 2010 and alternative MDS. They showed that high diet quality scores were associated with 19-28% (HRs = 0.81-0.72) lower risk of CVD mortality in women and 14-26% (HRs = 0.86-0.74) lower risk in men. In a separate analysis for each diet score, the highest quintile of the DASH score, which represents better diet quality, was associated with 17% (HR = 0.83, 95%CI 0.76, 0.91) and 22% (HR = 0.78, 95%CI 0.71,0.85) lower risk of CVD in men and women, respectively, in comparison with the lowest quintile [31]. Additionally, in a recent meta-analysis [32] of cohort studies, the highest diet quality category of the DASH was associated with a 20% (RR = 0.80, 95%CI 0.76, 0.85) decrease in cardiovascular mortality and incidence, in comparison with the lowest diet quality category.

The DASH dietary pattern demonstrates a protective effect against CVD, and is shown to be an effective alternative to medication in lowering high blood pressure.

1.3.1.3. Healthy Eating Index

The HEI is another *a priori* tool for evaluating dietary patterns based on dietary recommendations. The HEI was constructed according to the Dietary Guidelines for Americans, which is based on scientific knowledge and aims to promote healthy dietary habits and to reduce the burden of chronic diseases in the population of the United States [33]. The HEI evaluates adherence to the guidelines and, following updates to the American Dietary Guidelines, there are three versions of this index: the HEI, HEI-2005 and HEI-2010 [33-35]. The index uses amounts of foods and nutrients consumed per 1000 kcal energy intake. The latest version, which reflects the 2010 Dietary Guidelines for Americans, contains nine components that ensure adequate intake of nutrients: total fruit (includes fruit juices), whole fruit (all forms except juices), total vegetables, greens & beans, whole grains, dairy, total protein foods, seafood & plant proteins, and fatty acids; and three components in moderation: refined grains, sodium and empty calories.

Furthermore, the AHEI is a version of the HEI that evaluates consumption of foods and nutrients that are associated with risk of chronic diseases [36]. The AHEI uses absolute intake instead of the nutrient density approach used in the HEI, and has two versions: the AHEI and the AHEI-2010 [36, 37]. The latest version consists of eleven components: vegetables, fruits, whole grains, sugar-sweetened beverages & fruit juice, nuts & legumes, red/processed meat, trans-fat, long-chain fats, polyunsaturated fatty acids (PUFA), sodium and alcohol.

Many epidemiological studies have used these indices to analyze the association between diet quality and health [32]. A recent meta-analysis by Schwingshackl and Hoffmann [32] analyzed the association between the HEI, AHEI and DASH scores and cardiovascular mortality in 12 cohort studies, and found that the highest diet quality category of the HEI-2010 and the AHEI-2010 were associated with 20% (RR = 0.80, 95%CI 0.76, 0.85) and 26% (RR = 0.74, 95%CI 0.72, 0.77) lower risk of CVD (combined mortality and incidence) than the lowest diet quality category. Subgroup analysis showed that newer versions of these indices (i.e. HEI-2005, HEI-2010 and AHEI-2010) were more strongly associated with health outcomes than the older versions. In the Multi-Ethnic Study of Atherosclerosis, Harmon et al. [31] showed that, among various indices, the HEI-2010 showed the strongest association with decreased risk of CVD mortality in men, with a HR of 0.74 (95%CI 0.69, 0.81) for the 5^{th} vs. the 1^{st} quintile. In contrast, the AHEI-2010 showed the strongest association with CVD mortality in women, with a HR of 0.76 (95%CI 0.69, 0.83) for the 5^{th} vs. the 1^{st} quintile.

The HEI and the AHEI are currently widely used in nutritional epidemiology studies, and there is sufficient scientific evidence to confirm their utility and efficacy in identifying people at risk of developing CVD.

1.3.2. *A posteriori* dietary patterns

The association between dietary patterns and CVD has been studied extensively over the past 10 years. Cluster and PCA analyses are frequently used to study *a posteriori* dietary patterns. Most studies identify two common dietary patterns, a Prudent or healthy dietary pattern, and a Western or unhealthy dietary pattern. The Prudent dietary pattern is characterized by abundant consumption of vegetables, fruit, whole grain, plant oils, low-fat dairy, fish and poultry. The Western dietary pattern is characterized by increased intake of refined grain, red meat & derivatives, fast food, sugared carbonated beverages, high-fat dairy, fried foods, snacks, sweets and deserts. While some studies define more than two dietary patterns or use different names for these patterns, the names of the patterns reflect the same idea in most studies: more and less healthy dietary patterns.

1.3.2.1.Principal component analysis and factor analysis

Hou et al. [38] recently reported the results of a meta-analysis of 12 cohort studies including 409,780 participants that evaluated the association between CHD risk and dietary patterns defined using PCA and factor analysis. They found that the highest category of the Prudent dietary pattern was associated with 20% (Summary Relative Risk Estimate (SRRE) = 0.80, 95%CI 0.74, 0.87) lower risk of CHD than the lowest category, and that the Western dietary pattern was not associated with risk of CHD. However, subgroup analysis showed a 45% increase in CHD risk in participants from US cohorts following a Western dietary pattern. In another recent meta-analysis, Li et al. [39] analyzed the association between CVD and stroke mortality and dietary patterns defined using factor analysis and PCA. They observed a significant inverse association between the Prudent dietary pattern and risk of CVD mortality (SRRE 0.81, 95%CI 0.75, 0.81), but no significant association with stroke mortality (SRRE = 0.89, 95%CI 0.77, 1.02). Adherence to the Western dietary pattern was not associated with risk of CVD and stroke mortality. Martínez-González et al. [40] obtained similar results in the PREDIMED study, in which the authors analyzed the association between various CVD events and mortality and Western and Mediterranean dietary patterns defined using factor analysis. The third and fourth quartiles of the

Mediterranean dietary pattern were strongly associated with decreased risk of a primary CVD event [Odds Ratio (OR), 0.60 (95%CI 0.42, 0.83) for Q3; 0.52 (95%CI 0.36, 0.74) for Q4], myocardial infarction [OR, 0.58 (95%CI 0.34, 0.97) for Q3; 0.41 (95%CI 0.23, 0.75) for Q4] and CVD mortality [OR, 0.56 (95%CI 0.30, 1.05) for Q3; 0.37 (95%CI 0.18, 0.75) for Q4], compared to the first quartile. In contrast, the Western dietary pattern was not associated with higher risk of CVD events and mortality. Neither of these dietary patterns were associated with risk of stroke in this analysis.

1.3.2.2. Cluster analysis

Although less widely used than PCA, cluster analysis has also been used to provide evidence of an association between dietary patterns and CVD incidence and mortality. In the Framingham Offspring/Spouse Study, the heart-healthy dietary pattern in women was associated with 40% (p=0.02) lower risk of subclinical CVD [41], and the Empty Calorie dietary pattern was associated with more than two-fold risk of carotid atherosclerosis, compared to the heart-healthy dietary pattern (OR = 2.28 95%CI 1.12, 4.62) [42]. In a Portuguese cohort study [43], a “red meat and alcohol” dietary pattern was associated with 98% (OR = 1.98, 95%CI 1.17, 3.12) and 91% (OR = 1.91, 95%CI 1.35, 2.92) higher risk of acute myocardial infarction in women and men, respectively, compared to a “healthy” dietary pattern. Brunner et al. [44] found that a healthy dietary pattern was associated with 21% (HR = 0.79, 95%CI 0.51, 0.98) lower risk of fatal and non-fatal CHD after 15 years of follow-up, compared to an unhealthy dietary pattern in a fully adjusted model. They also found that the healthy dietary pattern was associated with 26% (HR = 0.74, 95%CI 0.58, 0.94) lower risk of type 2 diabetes, which is a known strong risk factor of CVD development, compared to an unhealthy dietary pattern.

There is strong scientific evidence from cohort studies and large randomized clinical trials (RCTs) regarding the protective and beneficial effects of healthy dietary patterns on CVD risk factors, and CVD incidence and mortality. There is considerable overlap between the components of healthy-type dietary patterns (e.g. the Mediterranean, DASH and HEI *a priori* patterns, and the Prudent and healthy *a posteriori* patterns), in that they are more plant-based and have a lower emphasis on animal-based foods. Analysis of individual elements of diet in some studies indicates that they have a weaker effect on health outcomes than whole dietary patterns [8, 22]. Therefore, dietary patterns are a more effective way to analyze the association between diet and health, as it is a more holistic approach and closer to reality.

1.4. Association between food and food groups and CVD risk factors: Background

Many studies have evaluated the association between CVD and different foods, including soft drinks, red and processed meat, fish, dairy products, olive oil, nuts, and fruits and vegetables.1.4.1. Soft drinks

1.4.1. Soft drinks

Many scientific studies have demonstrated that excessive consumption of sugar, especially in the form of soft drinks, is associated with metabolic diseases such as obesity and type 2 diabetes [45, 46]. In a meta-analysis, Malik et al. [47] showed that increased consumption of soft drinks is associated with increased adiposity in both adults and children. Daily consumption of 12 oz. (≈ 355 ml) of soft drinks for one year was associated with a 0.05 kg/m^2 (95%CI 0.03, 0.07) increase in BMI and a 0.12 kg (95%CI 0.10, 0.14) increase in weight in children and adults, respectively. Recently, Xi et al. [48] published a meta-analysis on the association between soft drinks and risk of CHD, stroke and hypertension. They found that high consumption of soft drinks, compared to low consumption, was directly associated with hypertension (RR = 1.10, 95%CI 1.06, 1.15, random-effects analysis) and CHD (RR = 1.17, 95%CI 110, 1.24, fixed-effect analysis). Also, a one-serving per day increase in soft drink consumption was associated with higher risk of hypertension (p for trend < 0.05) and CHD (RR = 1.17, 95%CI 1.10, 1.24). They found no association with stroke. Additionally, in a recent review by Keller et al. [49], highlighted the fact that soft drink consumption was associated with increased levels of total cholesterol, triglycerides, fasting blood glucose and the ratio of visceral to subcutaneous adipose tissue.

1.4.2. Red and processed meat

Red and processed meat is another food group, which has been shown by many studies to have a negative impact on health. Pan et al. [50] analyzed the association between red and processed meat and CVD mortality in two large cohorts from the USA, the Health Professionals Follow-up Study and the Nurses' Health Study. They found that a 1 serving/day increase in total red meat, unprocessed red meat, and processed red meat consumption were associated with increased CVD mortality [HRs (95% CIs): 1.16 (1.12-1.20), 1.18 (1.13-1.23) and 1.21 (1.13-1.31), respectively]. The EPIC study [51] showed that >40 g/day of processed meat was associated with increased risk of CVD mortality in a dose dependent manner.

Consumption of >160 g/day was associated with 72% (HR = 1.72, 95%CI 1.29, 2.30) higher risk of CVD mortality than low consumption (<20 g/day). However, the test for a similar association for unprocessed red meat did not reach statistical significance. A recent meta-analysis of large cohort studies with almost 2,000,000 participants and 4 years of follow-up showed that a 0.5 serving/day increase in consumption of red meat was associated with 30% (HR = 1.30, 95%CI 1.21, 1.41) higher risk of type 2 diabetes[52].

1.4.3. Fish

In contrast to the negative impact of red meat on health, another important source of animal protein and fat, fish, has been shown to be beneficial for cardiovascular health. Fish, especially fatty fish, is a good source of omega-3 PUFA, whose protective effect on CVD risk has been demonstrated in many studies [53]. In a meta-analysis of 13 cohorts, He et al. [54] showed that consumption of fish at least once per week is associated with 15% (RR = 0.85, 95%CI 0.76, 0.96) lower risk of CHD. In a large national pregnancy cohort, Strom et al. [55] showed that individuals who never consumed fish had 189% (HR = 2.89, 95%CI 1.39, 5.99) higher risk of CVD than those who consumed fish every week. Similar results were found for omega-3 PUFA, with high-risk estimates for the lowest intake group (HR = 1.91, 95%CI 1.26, 2.89). A previous meta-analysis of ten RCTs confirmed the beneficial effects of omega-3 PUFA on CVD [56], with 19% lower risk of cardiac death (RR = 0.81, 95%CI 0.69, 0.95) in patients receiving omega-3 PUFA dietary supplements.

1.4.4. Milk and dairy products

Milk and dairy products are another animal product whose impact on cardiovascular health has been widely studied, although results remain inconclusive to date. Recent studies show no association between milk and dairy consumption and risk of CVD, or even an inverse association [57]. For some years, dairy product were thought to be harmful for cardiovascular health because of their high saturated fatty acids (SFA) content. However, a meta-analysis by Soedamah-Muthu et al. [58] indicated an inverse association between milk consumption (200 ml/day) and risk of CVD (RR = 0.94, 95%CI 0.89, 0.99). In a cohort of women, Patterson et al. [59] showed that the highest quartile of dairy consumption was associated with 23% (HR = 0.77, 95%CI 0.63, 0.95) lower risk of myocardial infarction than the lowest quartile after 11.6 years of follow-up, and this association was partially explained by cheese consumption. In a Japanese cohort with 24 years of follow-up, a 100g/day increase in total dairy consumption was associated with decreased risk of CVD mortality in women (HR = 0.86, 95%CI 0.74, 0.99); there was no significant association in men [60]. Additionally, there is considerable evidence of the beneficial impact of dairy on CVD risk factors, such as type 2 diabetes. A meta-analysis of twelve cohort studies showed that high intake of dairy products was associated with 11% (RR = 0.89, 95%CI 0.82, 0.96) lower risk of type 2 diabetes than low intake, and this association was explained by consumption of low-fat dairy, cheese and yogurt [61].

1.4.5. Olive oil

Many studies have demonstrated the beneficial health effects of another popular dietary source of lipids, olive oil. Olive oil is an abundant source of MUFAs, which have been shown to be very beneficial for cardiovascular health [53]. Two recent meta-analyses have confirmed the beneficial effect of olive oil on CVD incidence and mortality. Schwingshackl and Hoffmann [62] showed that the top tertile of olive oil consumption was associated with 40% (RR = 0.60, 95%CI 0.47, 0.77) lower risk of stroke, 28% (RR = 0.72, 95%CI 0.57, 0.91) lower CVD incidence, and 30% (RR = 0.70, 95%CI 0.48, 1.03) lower CVD mortality, compared to the bottom tertile. Interestingly, neither dietary consumption MUFA, the MUFA:SFA ratio, nor oleic fatty acid were as strongly associated with these outcomes as olive oil. In another meta-analysis of Martínez-González et al. [63] all cohorts and the PREDIMED clinical trial consistently reported an inverse association between stroke incidence and a 25 g/day increase in consumption of olive oil (RR = 0.76, 95%CI 0.67, 0.86). Ultimately, in a combined analysis of all studies, a 25 g/day increase in olive oil consumption was associated with 18% (RR = 0.82, 95%CI 0.70, 0.96) lower risk of CVD incidence.

1.4.6. Nuts

Another healthy source of dietary fat is nuts. In a recent meta-analysis of observational and clinical studies, Afshin et al. [64] showed that four weekly servings (1 serving = 28.4 g) of nuts was associated with 24% (RR = 0.76, 95%CI 0.69, 0.84) lower risk of fatal ischemic heart disease (IHD), and 22% (RR = 78, 95%CI 0.67, 0.92) lower risk of non-fatal IHD. There was no significant association with stroke. In a combined analysis of four large US cohort studies, Kelly and Sabaté [65] found that participants who consumed nuts at least four times per week had 37% (RR = 0.63, 95%CI 0.51, 0.83) lower risk of CHD than those who rarely or never consumed nuts.

1.4.7. Fruits and vegetables

Almost all healthy dietary patterns include increased consumption of fruit and vegetables. Official dietary recommendations for public health strongly recommend consumption of fruit and vegetables to decrease CVD risk [66, 67]. A meta-analysis of cohort studies showed that a 1-serving increase in consumption of vegetables and fruit was associated with 5% (RR = 0.95, 95%CI 0.92, 0.99) and 7% (RR = 0.93, 95%CI 0.89, 0.96) lower risk of CVD, respectively [68]. A subsequent meta-analysis of cohort studies including more than 200,000 participants showed that individuals who consumed 3-5 and >5 servings of fruit and vegetables per day was associated with 7% (RR = 0.93, 95%CI 0.86, 1.0) and 17% (RR = 0.83, 95%CI 0.77, 0.89) lower risk of CHD than those who consumed <3 servings per day [69]. Finally, the results from the EPIC-Heart study demonstrated that consumption of at least 8 servings of fruit and vegetables per day was associated with 22% (RR = 0.78, 95%CI 0.65, 0.95) lower risk of fatal IHD than consumption of ≤3 servings per day [70]. Together with these findings, several RCTs have shown the beneficial effects of fruit and vegetables consumption on cardiovascular health [71], although not all studies have shown strong, convincing evidence. It seems likely that fruit and vegetables have a synergistic beneficial effect on health in combination with other healthy foods in the context of a healthy dietary pattern.

2. Rationale

CVD is the most important cause of premature death worldwide [5]. There is abundant evidence to support the effect of diet quality on the development of CVD [3, 8]. To assess diet quality, various dietary indexes based on dietary recommendations or traditional dietary habits, have been developed [22, 33, 36]. Several of these indexes are derived from the full-length FFQ, and are associated with CVD development [37, 72]. As far as we are aware, only one short diet quality screener has been examined to assess its predictive validity, i.e. the ability of its score to predict risk of future CVD events [73]. Therefore, we decided to perform a simulation study of the predictive validity of the DQI, which is derived from the short Diet Quality Screener (sDQS) developed by our group.

There is even less validation evidence for dietary patterns defined using cluster analysis than that for dietary indexes, even though cluster analysis is a widely used approach to define dietary patterns [14]. While dietary patterns defined using cluster analysis are easy to interpret, it is difficult to compare them between different populations, samples and dietary data sources. Thus, it is important to perform validation studies of dietary patterns defined using cluster analysis. Consequently, the second manuscript from this PhD research deals with the validity and reproducibility of dietary patterns defined using cluster analysis of various sources of dietary data.

In nutritional epidemiology, all dietary assessment methods are biased by energy under-reporting. This is an important challenge for observational studies, and affects all types of diet analysis, including dietary patterns. According to a review by Devlin et al. [14], energy under-reporting in dietary patterns defined using cluster analysis is a very important but understudied topic in the field of nutritional epidemiology. Therefore, in this study we address the question of the impact of energy under-reporting on the construction of dietary patterns using cluster analysis. We also investigate the impact of energy under-reporting on secular trends in dietary patterns derived from cluster analysis. Secular trends in dietary patterns constructed using cluster analysis have mainly been studied in adolescents [74, 75], and to date only two studies have been conducted in adults, as far as we are aware. The impact of energy under-reporting on secular trends in dietary patterns has not yet been explored in European populations [76, 77].

We then investigate one of the fastest growing risk factors for CVD, abdominal obesity, and its association with diet. The prevalence of abdominal obesity has been found to increase rapidly between successive surveys in all populations studied [78-81]. The prevalence of abdominal obesity in the US adult population increased significantly from 46.4% (95%CI 42.1, 50.8) in 1999-2000 to 54.2% (95%CI 51.3, 57.0) in 2011-2012 [79], while that of general obesity did not [82]. The high prevalence of abdominal obesity is also a problem of the current generation of children and adolescents [83, 84]. In addition to its high prevalence, waist circumference, which is a surrogate marker of abdominal obesity, has increased disproportionately with respect to general obesity in many countries [85, 86]. The increasing prevalence of abdominal obesity is a major concern for public health because abdominal obesity is strongly linked to CVD [87, 88]. Thus, identifying factors that lead to the development of abdominal obesity is a research priority.

Diet is one of the main risk factors for abdominal obesity [89]. Caloric beverages represent a significant part of total dietary energy intake [90], and, of these, soft drinks have received most attention in the scientific community in relation to health outcomes. Several large prospective cohort studies have demonstrated the negative effect of soft drink consumption on CVD risk factors and CVD incidence [47, 48, 91, 92]. In their RCT, Maersk et al. [93] showed a significant increase in visceral adipose tissue in a group consuming soft drinks compared to groups consuming milk (31%, 95%CI 10, 53; p = 0.05), diet soft drinks (23%, 95%CI 3, 43; p = 0.14) or water (24%, 95%CI 4, 44; p = 0.10). This issue is of particular concern in Spain where soft drink consumption increased significantly [94, 95]; Spain now has the highest per capita consumption of soft drinks of all southern European countries [96]. Therefore, we examined whether increased consumption of soft drinks is associated with abdominal obesity in the adult population in Spain.

While soft drink consumption is known to be linked to abdominal obesity and other CVD risk factors, little is known about the association between dietary patterns and abdominal adiposity. The Mediterranean dietary pattern has received a lot of attention recently, and the most prominent results regarding the beneficial effect of this diet were obtained from the PREDIMED study [3], a 5-year intervention with the Mediterranean diet in individuals with high cardiovascular risk. In this study, the Mediterranean diet intervention group had up to 30% lower risk of major cardiovascular events than the low-fat diet group. Further, this study not only showed that the intervention group had less abdominal obesity than the control group [97], but also that the Mediterranean diet was associated with a less negative effect of abdominal fat accumulation, regardless of the level of abdominal adiposity [98]. However, before the presentation of data from this PhD research, little was known about impact of the Mediterranean diet on abdominal obesity. Some cross-sectional studies have found an inverse association [99], while others have not [100], and there was insufficient evidence from long-term cohort studies. Thus, we explored the association between abdominal obesity and the Mediterranean diet in 10-year Spanish population-based cohort study.

In the past five years, yogurt consumption has been a focus of research because of its probiotic properties. Numerous studies in animals and humans have shown that probiotics protect against cardiovascular risk factors such as obesity, type 2 diabetes and dyslipidemia [101]. Two intervention studies of the effect of yogurt consumption on CVD risk factors have shown its beneficial effects on the lipid profile of diabetic and healthy participants [102, 103]. The few prospective studies that have examined the effect of yogurt and other probiotic dairy foods on CVD incidence and mortality have provided inconsistent results, and not all have specifically focused on the effects of yogurt [104-107]. Thus, the final aim of this PhD research was to investigate whether frequent yogurt consumption is associated with risk of CVD events and mortality in the Spanish population.

3. Objectives

The general aim of this PhD project was to study the association between diet and cardiometabolic health. To achieve this aim, we defined the following specific objectives:

- Validity, reproducibility, and energy under-reporting.
    - To determine the predictive validity of the sDQS-derived DQI using simulation studies
    - To analyze the validity and reproducibility of dietary patterns derived from FFQ data and 24-hour recall as a reference method using cluster analysis.
    - To explore the impact of energy under-reporting on dietary patterns derived using cluster analysis.
    - To analyze the effect of energy under-reporting on secular trends in dietary patterns derived using cluster analysis.
- Diet and abdominal obesity
    - To examine the association between changes in consumption of caloric beverages and changes in waist circumference.
    - To determine the risk of 10-year incidence of abdominal obesity, defined according to waist circumference thresholds, as a function of caloric beverage consumption and changes in beverage consumption patterns.
    - To explore the association between adherence to the Mediterranean diet and changes of waist circumference.
    - To determine the risk of 10-year incidence of abdominal obesity, defined according to waist circumference thresholds, as a function of adherence to the Mediterranean diet.

    - To assess whether the final results varied according to the various strategies used to construct the Mediterranean diet index.
- Diet and CVD events
    - To explore the association between yogurt consumption and incidence of CVD.

4. Methods

4.1. Study population

Data were obtained from two population-based surveys from the Registre Gironí del Cor (REGICOR), conducted in Girona, north-east Spain, in 2000 and 2005, with re-examinations in 2009/10 and 2012, respectively. The baseline survey conducted in 2000 examined a randomly selected population-based sample of 3,058 men and women aged 25–74 years (participation rate: 71.0 %), using a two-stage sampling method. All 2,715 non-institutionalized participants still residing in the catchment area were invited for re-examination in 2009/10, of whom 2,181 attended (follow-up participation rate: 80.3 %).

The baseline survey of 2005 was conducted in a random sample of participants from the city of Girona (~70,000 inhabitants) and three surrounding rural towns, and included 6,352 non-institutionalized men and women aged >34 years (participation rate: 72.0%). In all surveys, selected participants were contacted by letter, and informed about the aims of the study and the tests to be performed. Participants were asked to fast for at least 10 hours before their appointment at the health examination site. They were provided with telephone number for inquiries. Participants, who provided a phone number, were contacted 1 week before the examination to confirm attendance. Participants who presented with acute myocardial infarct at baseline were excluded from the study. Hospitalized participants or those with missing data for key variables, such as physical activity, anthropometric measurements, or fasting glucose, also were excluded from the study. All participants gave signed informed consent to participate in the study, which was approved by the local

Clinical Research Ethics Committee (CEIC-PSMAR, Barcelona, Spain). The results of the examination were sent to all participants.

During 63,814.88 person-years of follow-up, representing a median of 6.7 years per person, 336 CVD events were confirmed.

4.2. Measurements

All examinations were performed by a team of trained nurses and interviewers using the same standard questionnaires and measurement methods in all surveys. A calibrated precision scale was used for weight measurement with participants in underwear. Readings were rounded to the nearest 200 g. Height was measured in the standing position and rounded to the nearest 0.5 cm. Body mass index (BMI) was calculated as weight divided by squared height (kg/m^2).

Blood pressure was measured using a periodically calibrated mercury sphygmomanometer. A cuff adapted to upper arm perimeter (obese, young, adult) was selected for each participant. After a 5-min rest, two measurements were taken at least 20 min apart, and the lowest value was recorded for the study.

Blood was drawn after 10-14 h fasting, with less than 60-s duration. Aliquots of serum were stored at –80°C. Total cholesterol and triglyceride concentrations were determined enzymatically (Roche Diagnostics, Basel, Switzerland). High-density lipoprotein cholesterol (HDL-cholesterol) was measured as cholesterol after precipitation of Apo protein B-containing lipoproteins with phospho-tungstic-Mg^{2+} (Boehringer, Mannheim, Germany). Analyses were performed using a Cobas Mira Plus autoanalyzer (Roche Diagnostics, Basel, Switzerland). Quality control was performed using External Quality Assessment – the WHO Lipid Program (WHO, Prague, Czech

Republic) and Monitrol-Quality Control Program (Baxter Diagnostics, Dudingen, Switzerland). Inter-assay coefficients of variation were 2.5%, 4.5%, and 3.2% for total cholesterol, HDL-cholesterol, and triglycerides, respectively. Low-density lipoprotein cholesterol was calculated using the Friedewald equation when triglycerides were <3.4 mmol/l (300 mg/dl). The Minnesota leisure-time physical activity (LTPA) questionnaire validated for the Spanish population was used to assess the amount of LTPA performed during the previous year [108, 109]. A structured standard questionnaire was used to record smoking habits, and demographic and socio-economic data. Maximum educational level attained was dichotomized into primary school, and secondary school or university.

Data on energy intake and food consumption were collected using a validated FFQ [110, 111] consisting of 166 food items, including alcoholic and non-alcoholic beverages. Participants were asked to estimate their usual intake during the preceding year, and had to choose from 10 frequency categories, ranging from never or less than once per month to >6 times per day. Medium servings were defined by natural (e.g. 1 apple, 1 slice) or household units (e.g., 1 teaspoon, 1 cup).

All dietary and energy intake and expenditure measurements were performed at both baseline and follow-up using the same protocol.

5. Results

○ Paper I. Validity of a sDQS-derived DQI to predict changes in anthropometric and cardiovascular risk factors: a simulation study.

- Increased DQI at baseline was predictive of decreased BMI and waist circumference at follow-up.
- Baseline DQI was inversely associated with serum triglyceride levels, total cholesterol, and the ratio of triglycerides to HDL-cholesterol.
- Baseline DQI was directly associated with levels of HDL-cholesterol at follow-up.

○ Paper II. Modest validity and fair reproducibility of dietary patterns derived using cluster analysis.

- Two dietary patterns, “fruit & vegetables” and “meat” were identified in all dietary data sources: FFQ baseline, FFQ follow-up and 24-hour dietary recall.
- In cross-tabulation analysis, >60% of participants were consistently categorized in clusters derived from the two FFQs (reproducibility), and from the FFQ at follow-up and 24-hour dietary recall (validity).
- Spearman correlation analysis showed reasonable reproducibility and lower validity, especially for the “fruit & vegetables” dietary pattern.
- The *κ* statistic showed fair validity and reproducibility of the dietary patterns defined using cluster analysis.

○ Paper III. Effect of energy under-reporting on secular trends in dietary patterns in a Mediterranean population.

- Three dietary patterns were identified using cluster analysis in both surveys: “healthy”, “mixed” and “western”.
- The “mixed” dietary pattern was the most prevalent in both surveys. Exclusion of energy under-reporters resulted in a

marked decrease in the number of participants with a "mixed" dietary pattern in the REGICOR 2000 survey.

- After excluding energy under-reporters from the analysis of dietary patterns, we observed higher consumption of healthy foods in the "mixed" pattern in the REGICOR 2000 survey, and of unhealthy foods in the REGICOR 2005. The "healthy" dietary pattern did not change significantly, and the "western" was slightly affected.
- After excluding energy under-reporters from the analysis of secular trends, changes in food groups between surveys increased in the "mixed" and "western" dietary patterns, but remained at the same level in the "healthy" dietary pattern. Those changes were characterized by an increase in consumption of unhealthy foods.

○ Paper IV. Soft drink consumption is positively associated with increased waist circumference and 10-year incidence of abdominal obesity in Spanish adults.

- Consumption of soft drinks was positively associated with changes in waist circumference. Each 100 kcal increase in soft drink consumption was associated with a 1.1 cm increase in waist circumference over 10 years of follow-up.
- Substitution of 100 kcal of soft drinks with 100 kcal of whole milk and juice was associated with a 1.3 cm and 1.1 cm decrease in waist circumference, respectively, over 10 years of follow-up.
- Increased and sustained consumption of soft drinks was associated with a 1.5 cm and 1.2 cm increase in waist circumference, respectively, compared with non-consumption over 10 years of follow-up.

- Increased consumption of soft drinks at baseline was associated with increased odds of 10-year incidence of abdominal obesity at follow-up.

o Paper V. Impact of the Mediterranean diet on changes in abdominal fat and 10-year incidence of abdominal obesity in the Spanish population.

- A higher Mediterranean diet index was inversely associated with waist circumference.
- Greater adherence to the Mediterranean diet (top tertile of the index) was associated with decreased risk of 10-year incidence of abdominal obesity, although this association did not reach statistical significance.
- Two Mediterranean diet indexes, one based on the tertile distribution of food group consumption in the study population, and another based on dietary recommendations, had similar results in the analysis of their association with waist circumference and incidence of abdominal obesity.

o Paper VI. Yogurt consumption is negatively associated with cardiovascular disease events in Spanish adults

- Daily consumption of any type of yogurt was associated with decreased risk of cardiovascular events. The association with incidence of stroke was stronger than that for incidence of myocardial infarction and angina.

6. Papers

1st paper

Validity of a sDQS-derived DQI to predict changes in anthropometric and cardiovascular risk factors: a simulation study. European Journal of Clinical Nutrition 2012; 66(12): 1369-71.

European Journal of Clinical Nutrition (2012) **66,** 1369–1371

www.nature.com/ejcn

SHORT COMMUNICATION

Validity of a short diet-quality index to predict changes in anthropometric and cardiovascular risk factors: a simulation study

A Funtikova[1], JM Baena-Díez[2], C Koebnick[3], SF Gomez[1,4], M-I Covas[1,5], A Goday[6] and H Schröder[1,7]

Coronary heart disease prevention in the primary care setting, where time is extremely limited, requires valid instruments that efficiently screen for unhealthy lifestyle habits. Identification of the individuals who would most benefit from dietary intervention is particularly important in this context. We used dietary intake data derived from a full-length food frequency questionnaire to simulate responses to our previously validated short dietary quality screener. We determined the prospective association of the resulting diet-quality index (DQI) with changes in anthropometric and cardiometabolic risk variables in 2181 men and women in a 10-year follow-up. Multiple linear regression analyses revealed that a higher DQI score at baseline related directly ($P = 0.002$) to high-density lipoprotein cholesterol (HDL-C) and inversely ($P < 0.016$) to waist circumference (WC), triacylglycerides (TG), the TG to HDL-C ratio and the total cholesterol to HDL-C ratio at follow-up. A low DQI score is predictive for an increase in WC and the development of an unfavourable cardiometabolic profile.

European Journal of Clinical Nutrition (2012) **66,** 1369–1371; doi:10.1038/ejcn.2012.131; published online 26 September 2012

Keywords: predictive validity; dietary screener; cardiometabolic risk

INTRODUCTION

Coronary heart disease (CHD) is the main cause of premature mortality worldwide.[1] There is resounding evidence for a pivotal impact of unhealthy diets on the development of CHD.[2] Dietary indexes based on nutritional guidelines or traditional dietary habits have been developed to estimate diet quality. Those that predict cardiometabolic risk and mortality[3,4] are also more generally useful for the identification of individuals with unhealthy dietary habits.[5,6] However, the administration of detailed and time-consuming dietary assessment methods is a burden in time-limited primary care settings and only a few short dietary assessment instruments are available that provide valid information on total diet quality.[7–9] Furthermore, the predictive validity (for example, how well the short screener predicts future health outcomes) was not addressed in these validation studies.[3,4,7–9] We recently established the construct and concurrent validity of a short diet-quality index (DQI) derived from the short dietary quality screener (sDQS).[8] The objective of the present study was to determine the predictive validity of the DQI in a simulation study using data from a long-term population study.

METHODS

Data were obtained from a population-based survey conducted in Girona (Spain) in 2000 and 2009. The baseline survey in 2000 examined a random sample of 3052 men and women aged 25–74 years (participation rate: 71.0%). In 2009, the 2715 noninstitutionalized participants still residing in the catchment area were invited for re-examination, and 2181 attended (80.3%).

Dietary intake data derived from a validated full-length food frequency questionnaire (FFQ)[10,11] were employed to simulate responses that would have been obtained using the sDQS. The DQI derived from the simulated data was calculated, as described in the validation study.[8]

The sDQS asked for the usual food intake of 18 food items grouped in 3 food categories over the past year. The first food category contains major food items such as bread, pasta and vegetables, the second category is devoted to meat and other food items from animal origin and the third category contains fish, legumes and nuts. Food frequency consumption was arranged in 3 frequency response categories.

Blood was withdrawn after 10–14 h of fasting. Serum-sample aliquots were stored at −80 °C. Glycaemia was measured in an aliquot of serum. Total cholesterol (TC) and triacylglycerides (TG) concentrations were determined enzymatically (Roche Diagnostics, Basel, Switzerland). High-density lipoprotein cholesterol (HDL-C) was measured after precipitation of apoprotein B-containing lipoproteins with phosphotungstic-Mg^{++} (Boehringher, Mannheim, Germany). Analyses were performed in a Cobas Mira Plus autoanalyzer (Roche Diagnostics). The External Quality Assessment-WHO Lipid Program (World Health Organization, Prague, Czech Republic) and Monitrol-Quality Control Program (Baxter Diagnostic, Dudingen, Switzerland) were used for quality control. Low-density lipoprotein cholesterol was calculated by the Friedewald equation, whenever TG values were <300 mg/dL.

A calibrated precision scale was used for weight measurement. Readings were rounded up to 200 g. Height was measured in the standing position and rounded up to the nearest 0.5 cm. Weight was divided by height squared (kg/m^2) to establish the body mass index (BMI).

Measurement of waist circumference (WC) was performed midway between the lowest rib and the iliac crest in the horizontal positions. The measurement, taken in centimetres, was rounded to the nearest 0.5 cm.

[1]Cardiovascular Risk and Nutrition Research Group (CARIN-ULEC), Research Program in Inflammatory and Cardiovascular Disorders (RICAD), Institut de Recerca Hospital del Mar (IMIM), Biomedical Research, Barcelona, Spain; [2]Centro de Salud La Marina; IDIAP Jordi Gol, Barcelona, Spain; [3]Department of Research and Evaluation, Kaiser Permanente Southern California, Pasadena, CA, USA; [4]Fundación THAO, Barcelona, Spain; [5]CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Madrid, Spain; [6]Department of Endocrinology and Nutrition, Hospital del Mar, Barcelona, Spain and [7]CIBER Epidemiologia y Salud Pública (CIBERESP), Madrid, Spain. Correspondence: Dr H Schröder, Cardiovascular Risk and Nutrition Research Group (CARIN-ULEC), Research Program in Inflammatory and Cardiovascular Disorders (RICAD), Institut de Recerca Hospital del Mar (IMIM), Biomedical Research, Park, c/Doctor Aiguader 88, 08003 Barcelona, Spain.
E-mail: hschroeder@imim.es
Received 22 May 2012; revised 22 August 2012; accepted 26 August 2012; published online 26 September 2012

Anthropometric measurements, laboratory tests and dietary assessment by the FFQ were performed at baseline and at follow-up.

Linear modelling procedures were used to estimate the general characteristics, according to the tertile distribution of the sDQS-derived DQI. Multiple linear regression analysis was performed to determine the association between scoring for the sDQS at baseline and the anthropometric and cardiometabolic risk variables calculated at follow-up.

The main findings were essentially similar for men and women. Therefore, we present stratified results adjusted for sex only when appropriate. Differences were considered significant if $P<0.05$. The SPSS for Windows version 15 (SPSS, Inc., Chicago, IL, USA) was used for all statistical analysis.

RESULTS

Men had higher DQI scores (Table 1). Participants with higher scores on the DQI were older, had less education, were more likely to smoke and spent more time in leisure physical activities than their peers with a low DQI score (Table 1). An increment of 10 units in the DQI was associated with a decrease of 1.17 kg/m^2 in BMI and 3.25 cm in WC at follow-up (Table 2, Model 1). These associations were attenuated after adjusting for baseline levels of BMI and WC (Table 2, Model 2). Fully adjusted linear regression models revealed an inverse association of the DQI with follow-up serum TG levels and TC/HDL-C and TG/HDL-C ratios. The opposite was observed for HDL-C (Table 2, Model 2).

DISCUSSION

The pivotal role of healthy dietary habits in primary and secondary CHD prevention has been emphasized by international health organizations.[12] Cross-sectional data from previous validation studies have demonstrated the potential usefulness of short dietary screeners to identify individuals at cardiovascular risk.[7] However, prospective data on the predictive validity of DQIs derived from short screeners have not been published previously.

The present DQI validation study, using simulated sDQS data, indicates a reasonable capacity to predict cardiometabolic changes. Specifically, the simulated sDQS-derived DQI correctly predicted an increase in WC and the development of an unfavourable cardiometabolic risk profile over 10 years.

Epidemiological evidence associated increases of 1 mg/dl of HDL-C and 1 point in the TC to HDL-C ratio with a 3.5% reduction and 36.7% increase, respectively, in the risk of myocardial infarction at population scale.[13] In this study, a 10-point increment in the DQI was related to a 1.97 mg/dl HDL-C increase and a 0.15 decrease in the TC to HDL-C ratio. Therefore, the association between the DQI and these CHD risk predictors can be considered relevant.

A strength of this study is the population-based prospective design with relatively long follow-up. The simulation of responses can be considered a limitation of the present work. However, data from a previously conducted validation study in 102 men and women[8] revealed a good correlation ($r=0.51$) and low cross-misclassification (6.9%) between the DQI derived from the sDQS (actual response) and the DQI derived from a validated full-length FFQ. The sDQS was developed for the estimation of diet quality in a Spanish population. A further limitation is that we do not have the data needed to establish the degree of repeatability of the sDQS. The external validity of the sDQS should be addressed in future studies, but is achievable if minor adjustments are made to accommodate cultural differences in dietary habits.

In conclusion, our study provides evidence for the predictive validity of the sDQS-derived DQI. Together with the demonstrated construct and concurrent validity of the sDQS, the study results

Table 1. General characteristics of the study population, according to tertile distribution of the diet quality index (DQI)[a]

Variables	*Tertile distribution of DQI*						
	1st		*2nd*		*3rd*		*P-value*
Men (%)	54.9	51.9; 57.8	47.7	44.7; 50.7	42.7	39.5; 46.0	<0.001
Age (years)	45.8	45.0; 46.6	51.1	50.3; 51.9	54.1	53.2; 54.9	<0.001
Low education[b] (%)	56.0	52.6; 59.4	63.7	60.3; 67.0	72.0	68.4; 75.7	<0.001
LTPA (METs · min/d)	242.5	221.3; 263.6	284.6	263.8; 305.3	305.5	283.1; 327.8	<0.001
Smokers[c] (%)	64.9	61.8; 68.0	75.6	72.6; 78.7	85.3	82.1; 88.6	<0.001

Abbreviations: LTPA, leisure-time physical activity; METs, metabolic equivalents. [a]Mean and CI 95%. [b]No secondary school. [c]Never smokers and ex-smokers up to 1 year.

Table 2. Multiple linear regression models of the association between a 10-point increase over baseline diet quality values and cardiovascular risk factors at follow-up

CRF	*Model 1*[a]			*Model 2*[b]		
	B	*95% CI*	*P-value*	*B*	*95% CI*	*P-value*
BMI (kg/m^2)	−1.127	−1.702; −0.551	<0.001	−0.180	−0.478; 0.117	0.235
WC (cm)	−3.254	−4.778; −1.730	<0.001	−1.431	−2.587; −0.274	0.015
TC (mg/dl)	−0.200	−5.008; 4.607	0.935	−0.380	−4.591; 3.832	0.860
HDL-C (mg/dl)	1.977	0.517; 3.438	0.008	1.937	0.696; 3.178	0.002
LDL-C (mg/dl)	−0.482	−4.636; 3.671	0.820	−0.710	−4.372; 2.952	0.704
TG[c] (mg/dl)	−0.032	−0.058; −0.007	0.013	−0.029	−0.051; −0.006	0.012
TC/HDL-C	−0.153	−0.271; −0.03	0.011	−0.150	−0.249; −0.050	0.003
TG/HDL-C[c]	−0.048	−0.080; −0.016	0.003	−0.048	−0.075; −0.021	<0.001
FG[c] (mg/dl)	−0.010	−0.020; <0.001	0.065	−0.008	−0.016; <0.01	0.075

Abbreviations: BMI, body mass index; CI, confidence interval; CRF, cardiovascular risk factors; FG, fasting glucose, HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; TC, total cholesterol; TC/HDL-C, ratio total cholesterol/HDL-cholesterol; TG, triacylglycerides; TG/HDL-C, ratio triacylglycerides/HDL-cholesterol; WC, waist circumference. [a]Model 1: adjusted for age, sex, baseline smoking, education, baseline leisure-time physical activity. [b]Model 2: additionally adjusted for the baseline variable of the corresponding CRF. [c]Log-transformed.

indicate the usefulness of this tool for the identification of individuals who would most benefit from dietary intervention. Furthermore, it can be efficiently implemented in time-limited settings such as primary care.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This research was supported by a grant (2FD097-0297-CO2-01) from Fondo Europeo de Desarrollo Regional (FEDER); by portions of grants from Spain's Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III FEDER (PI11/01900), Red HERACLES (RD06/0009), and by a joint contract (CP03/00115) with the Instituto de Salud Carlos III and the Health Department of the Catalan Government (Generalitat de Catalunya). The CIBERESP is an initiative of the Instituto de Salud Carlos III, Madrid, Spain. We appreciate the English revision made by Elaine Lilly, PhD (Writers First Aid).

REFERENCES

1 The World Health Organization. *Causes of Death 2008 Summary Tables*. Health Statistics and Informatics Department, World Health Organization, Geneva, Switzerland, 2011.
2 Srinath Reddy K, Katan MB. Diet nutrition and the prevention of hypertension and cardiovascular diseases. *Public Health Nutr* 2004; **7**: 167–186.
3 Toft U, Kristoffersen LH, Lau C, Borch-Johnsen K, Jorgensen T. The dietary quality score: validation and association with cardiovascular risk factors: the Inter99 study. *Eur J Clin Nutr* 2007; **61**: 270–278.
4 McNaughton SA, Dunstan DW, Ball K, Shaw J, Crawford D. Dietary quality is associated with diabetes and cardio-metabolic risk factors. *J Nutr* 2009; **139**: 734–742.
5 Kourlaba G, Panagiotakos DB. Dietary quality indices and human health: a review. *Maturitas* 2009; **62**: 1–8.
6 Wirt A, Collins CE. Diet quality-what is it and does it matter? *Public Health Nutr* 2009; **12**: 2473–2492.
7 Schröder H, Fito M, Estruch R, Martinez-Gonzalez MA, Corella D, Salas-Salvado J *et al.* A short screener is valid for assessing Mediterranean diet adherence among older Spanish men and women. *J Nutr* 2011; **141**: 1140–1145.
8 Schröder H, Benitez Arciniega A, Soler C, Covas MI, Baena-Diez JM, Marrugat J. Validity of two short screeners for diet quality in time-limited settings. *Public Health Nutr* 2012; **15**: 618–626.
9 Neuhouser ML, Kristal AR, McLerran D, Patterson RE, Atkinson J. Validity of short food frequency questionnaires used in cancer chemoprevention trials: results from the Prostate Cancer Prevention Trial. *Cancer Epidemiol Biomarkers Prev* 1999; **8**: 721–725.
10 Schröder H, Covas MI, Marrugat J, Vila J, Pena A, Alcantara M *et al.* Use of a three-day estimated food record, a 72-hour recall and a food-frequency questionnaire for dietary assessment in a Mediterranean Spanish population. *Clin Nutr* 2001; **20**: 429–437.
11 Benítez-Arciniega AA, Mendez MA, Baena-Díez JM, Rovira Martori MA, Soler C, Marrugat J *et al.* Concurrent and construct validity of Mediterranean diet scores as assessed by an FFQ. *Public Health Nutr* 2011; **14**: 2015–2021.
12 The World Health Organization. Diet, nutrition and the prevention of chronic diseases. Report of a Joint WHO/FAO Expert Consultation 2003; WHO Technical Report Series no. 916. WHO, Geneva, Switzerland.
13 Stampfer MJ, Sacks FM, Salvini S, Willett WC, Hennekens CH. A prospective study of cholesterol, apolipoproteins, and the risk of myocardial infarction. *N Engl J Med* 1991; **325**: 373–381.

2nd paper

Modest validity and fair reproducibility of dietary patterns derived by cluster analysis. Nutrition Research 2015; 35(3): 265-8.

NUTRITION RESEARCH 35 (2015) 265–268

Available online at www.sciencedirect.com

ScienceDirect

www.nrjournal.com

Communication

Modest validity and fair reproducibility of dietary patterns derived by cluster analysis

Anna N. Funtikova [a,b,c], Alejandra A. Benítez-Arciniega [d], Montserrat Fitó [a,e], Helmut Schröder [a,b,*]

[a] Cardiovascular Risk and Nutrition Research Group (CARIN-ULEC), Program of Research in Epidemiology and Public Health, IMIM (Institut de Recerca Hospital del Mar), Barcelona, Spain
[b] CIBER Epidemiology and Public Health (CIBERESP), Instituto de Salud Carlos III, Spain
[c] Food and Nutrition PhD program, University of Barcelona, Barcelona, Spain
[d] Faculty of Medicine, Autonomous University of Mexico State, Toluca, Mexico
[e] CIBER Physiopathology of Obesity and Nutrition (CIBEROBN), Instituto de Salud Carlos III Spain

ARTICLE INFO

Article history:
Received 29 July 2014
Revised 30 December 2014
Accepted 30 December 2014

Keywords:
Validation
A posteriori dietary patterns
Repeatability
Cluster analysis
Food frequency questionnaire
Reproducibility

ABSTRACT

Cluster analysis is widely used to analyze dietary patterns. We aimed to analyze the validity and reproducibility of the dietary patterns defined by cluster analysis derived from a food frequency questionnaire (FFQ). We hypothesized that the dietary patterns derived by cluster analysis have fair to modest reproducibility and validity. Dietary data were collected from 107 individuals from population-based survey, by an FFQ at baseline (FFQ1) and after 1 year (FFQ2), and by twelve 24-hour dietary recalls (24-HDR). Repeatability and validity were measured by comparing clusters obtained by the FFQ1 and FFQ2 and by the FFQ2 and 24-HDR (reference method), respectively. Cluster analysis identified a "fruits & vegetables" and a "meat" pattern in each dietary data source. Cluster membership was concordant for 66.7% of participants in FFQ1 and FFQ2 (reproducibility), and for 67.0% in FFQ2 and 24-HDR (validity). Spearman correlation analysis showed reasonable reproducibility, especially in the "fruits & vegetables" pattern, and lower validity also especially in the "fruits & vegetables" pattern. κ statistic revealed a fair validity and reproducibility of clusters. Our findings indicate a reasonable reproducibility and fair to modest validity of dietary patterns derived by cluster analysis.

1. Introduction

Dietary patterns are increasingly used by researchers studying the relationship between diet and diseases [1]. Cluster analysis is one of the methods used to define dietary patterns. Although this method easily defines comprehensive dietary patterns [2], it has a limited comparability of patterns between different data sources, time points, and study samples [3]. This requires careful analysis of the validity and reproducibility of the patterns defined using cluster analysis.

Abbreviations: 24-HDR, 24-hour dietary recalls; FFQ, food frequency questionnaire.

* *Corresponding author*. Cardiovascular Risk and Nutrition Research Group (CARINULEC), Institut de Recerca Hospital del Mar (IMIM), Biomedical Research Park, c/Doctor Aiguader 88, 08003 Barcelona, Spain. Tel.: + 34 933 160 720; fax: +34 933 160 796.
E-mail address: hschroeder@imim.es (H. Schröder).

http://dx.doi.org/10.1016/j.nutres.2014.12.011

NUTRITION RESEARCH 35 (2015) 265–268

The hypothesis of the study was that the dietary patterns derived by cluster analysis have fair to modest reproducibility and validity. In the present study, we aimed to analyze the validity and reproducibility of dietary patterns derived from a food frequency questionnaire (FFQ) and 24-hour recall as a reference method.

2. Methods and materials

This validation study included 150 men and women, aged 30 to 80 years, a consecutively selected sample from a population-based cross-sectional survey carried out in Girona (Spain) in 2005. A total of 107 (71.3%) participants completed the FFQ at baseline (FFQ1) and at 1-year follow-up (FFQ2) and provided at least ten 24-hour dietary recalls (24-HDR). The general characteristics of participants did not differ from the initial sample [4]. All participants were duly informed and provided signed consent to participate in the study. The project was approved by the local Ethics Committee (CEIC-PSMAR, Barcelona, Spain).

Dietary intake data were determined by the FFQ [5] at baseline and follow-up. The 24-HDR (reference method for validity analysis) were collected monthly over a 12-month period by a trained telephone interviewer. At least ten 24-HDR, including minimum 5 weekdays and 1 weekend day, were required for inclusion in analysis. Participants were not alerted to the dates when they would be interviewed.

The K-mean cluster algorithm was used to derive dietary patterns from FFQ1, FFQ2, and 24-HDR. All food items from the FFQ and 24-HDR were combined into 45 food groups according to similarities in their nutritional content. The larger number of food items contained in the 24-HDR was grouped according to the FFQ food groups to be able to run the same cluster analysis for both instruments. Clusters were based on consumption of food groups in grams. Several runs of cluster formation were performed to establish the best cluster configurations. Criteria for cluster solutions were nutritional meaningfulness and a reasonable sample size (ie, every cluster contained at least 5% of the study sample). This solution was confirmed by the tree diagram resulting from the Ward method of cluster analysis. Finally, discriminant function analysis was carried out to examine the stability and classification ability of the cluster solution. The optimal cluster solutions derived from FFQ1and FFQ2 contained 3 clusters. The 24-HDR solution contained 2 clusters. One of the FFQ clusters had less than 5% of the population (1 participant in FFQ1 and 4 participants in FFQ2); these 5 individuals were not included in the cluster analysis of FFQ data.

2.1. *Statistical analyses*

Mean values and proportions of general characteristics are presented according to cluster membership (Table 1). To test the hypothesis of the study, we used contingency tables for cross-tabulation analysis between clusters derived from (*a*) the FFQ1 and FFQ2 (cluster reproducibility) and (*b*) the FFQ2 and 24-HDR (cluster validity) to identify participants who were categorized consistently (the same cluster) and inconsistently (the opposite cluster). Relative agreement was appraised by Spearman correlation coefficient and Cohen κ coefficient [6]. Spearman correlation analysis of food group consumption was used to determine reproducibility (FFQ1-FFQ2) and validity (FFQ2–24-HDR) of dietary patterns among participants with concordant classification between the dietary data sources (Table 2). κ statistic values were calculated, comparing clusters derived from FFQ1, FFQ2, and 24-HDR. Differences were considered significant if $P < .05$. The SPSS for Windows version 15 (SPSS, Inc., Chicago, IL) was used for statistical analysis.

3. Results

We identified 2 dietary patterns, "fruits & vegetables" and "meat," common to all dietary data sources, FFQ1, FFQ2, and

Table 1 – General characteristics of the clusters from FFQ1 at baseline, FFQ2 at follow-up, and multiple 24-HDR [a]

Variables	FFQ1			FFQ2			24-HDR		
	Fruits & vegetables n = 66	Meat n = 40	*P* [b]	Fruits & vegetables n = 72	Meat n = 30	*P* [b]	Fruits & vegetables n = 72	Meat n = 34	*P* [b]
Women (%)	65.2	22.5	<.001	55.6	40.0	.152	62.5	20.6	<.001
Age (y)	61.7 (1.4)	52.5 (1.8)	<.001	60.0 (1.4)	54.1 (2.1)	.022	61.0 (1.3)	52.5 (2.0)	.001
Education [c] (%)	56.1	75.0	.050	59.7	71.0	.278	54.8	79.4	.014
LTPA	273	236	.752	264	276	.681	263	282	.538
(METs ·min/d)	164, 414	139, 481		159, 419	161, 457		160, 409	152, 457	
Smokers [d] (%)	6.1	33.3	<.001	12.5	24.1	.147	9.7	30.3	.008
BMI (kg/m^2)	27.6 (0.52)	27.8 (0.68)	.829	27.3 (0.5)	28.4 (0.8)	.264	27.6 (0.5)	27.7 (0.7)	.903
Obesity (%)	30.3	20.5	.273	26.8	30.0	.740	26.8	26.5	.975

BMI, body mass index; LTPA, leisure-time physical activity; METs, metabolic equivalents.

[a] Means and SDs for continues variables (age and BMI); proportions for categorical variables (women, education, smokers, obesity); median and 25th and 75th percentiles in LTPA.

[b] *P* values were obtained by analysis of variance, Mann-Whitney *U*, and Pearson χ^2 for normal continuous, nonnormal continuous, and categorical variables, respectively.

[c] More than secondary school education.

[d] Active smokers or ex-smokers less than 1 year.

Table 2 – Spearman correlations of food groups consumption in dietary patterns between FFQ1 and FFQ2 (reproducibility) and between FFQ2 and 24-HDR (validity) among participants with concordant cross-classification

Food group	FFQ1-FFQ2 reproducibility				FFQ2–24-HDR validity				FFQ1–24-HDR validity			
	Fruits & vegetables		Meat		Fruits & vegetables		Meat		Fruits & vegetables		Meat	
	52 (51.0%) participants		16 (15.7%) participants		54 (52.4%) participants		15 (14.6%) participants		53 (50.0%) participants		21 (19.8%) participants	
	r	P	*r*	P	*r*	P	*r*	P	*r*	P	*r*	P
Raw vegetables	0.585	<.001	0.818	<.001	0.476	<.001	0.689	.004	0.240	.084	0.500	.021
Cooked potatoes	0.592	<.001	0.335	.204	0.339	.012	0.308	.264	0.223	.109	−0.243	.288
Fried potatoes	0.549	<.001	0.779	<.001	0.259	.059	0.358	.190	0.457	.001	0.272	.233
Poultry, rabbit	0.456	.001	0.088	.745	0.032	.817	0.145	.607	0.369	.007	0.049	.834
Red meat	0.397	.004	0.165	.542	0.170	.218	0.350	.201	0.510	<.001	0.204	.375
Fresh fish	0.257	.066	0.774	<.001	0.190	.169	0.618	.014	0.540	<.001	0.365	.104
Rice, pasta	0.408	.003	0.071	.795	0.266	.052	0.646	.009	0.070	.617	0.010	.964
Bread	0.342	.013	0.103	.704	0.327	.016	−0.064	.820	0.304	.027	0.562	.008
Fruits	0.237	.091	−0.082	.762	0.292	.032	−0.018	.950	0.383	.005	0.395	.077
Nuts	0.437	.001	0.354	.178	0.256	.062	0.174	.534	0.531	<.001	−0.070	.764
Olive oil	0.231	.099	0.374	.154	0.174	.207	0.443	.098	0.008	.955	0.242	.291
Desserts	0.474	<.001	0.574	.020	0.333	.014	0.283	.306	0.480	<.001	0.329	.145
Wine	0.649	<.001	0.591	.016	0.713	<.001	0.703	.003	0.628	<.001	0.763	<.001
Soft drinks	0.307	.027	0.165	.542	−0.003	.980	0.256	.044	0.083	.557	0.510	.018
Coffee	0.477	<.001	0.400	.124	0.449	.001	0.545	.036	0.527	<.001	0.408	.066
Whole fat dairy	0.076	.594	0.762	.001	0.294	.031	0.350	.201	0.266	.054	0.531	.013
Skimmed dairy	0.440	.001	0.494	.052	0.450	.001	0.514	.050	0.470	<.001	0.696	<.001

24-HDR. Discrimination function analysis revealed a Wilk λ of .797, $P < .001$; .886, $P = .001$; and .947, $P = .020$ for cluster solutions of the 24-HDR, FFQ2, and FFQ1, respectively. The names reflect the food group with the highest mean intake in the pattern (online supplementary material, Table 3). The main findings were essentially similar for men and women. Therefore, results were not stratified.

In both the FFQ1 and 24-HDR, the "fruits & vegetables" pattern had a higher proportion of older and less educated participants than the "meat" pattern (Table 1). The "fruits & vegetables" pattern also had more women and nonsmokers. The FFQ2 patterns had the same trends, but most of them are not significant. Cluster membership remained the same for 67.7% of participants in FFQ1 and FFQ2 clusters (cluster reproducibility) and for 67.0% in FFQ2 and 24-HDR (cluster validity). The 2 × 2 tables are available in the online supplementary material (Table 4A-C).

We ran a Spearman correlation analysis of food group consumption comparing dietary patterns of FFQ1 and FFQ2 (reproducibility), FFQ2 and 24-HDR (validity), and FFQ1 and 24-HDR in the participants with concordant classification (Table 2). Wine, coffee, and skimmed dairy products consumption showed moderate ($r > 0.400$) to strong ($r > 0.600$) correlation between dietary data sources in both analyses. In the reproducibility analysis of the "fruits & vegetables" dietary pattern, strong and moderate correlation was observed in 1 and 9 of the 17 groups, respectively. In comparing the "meat" pattern between FFQs, 1 food group had very strong correlation ($r > 0.800$); and 6 food groups had a significant strong or moderate correlation. In the validation analysis of "fruits & vegetables," only wine showed a strong correlation; and 3 groups were moderately correlated. In the "meat" dietary pattern, there was strong and moderate significant Pearson correlation in 4 and 2 food groups, respectively. In the comparison of FFQ1 vs 24-HDR, we found 7 food groups with moderate and 1 group with strong correlation in the "fruits & vegetables" dietary pattern; in the "meat" pattern, there were 2 food groups with strong and 4 with moderate correlation.

κ statistic revealed fair validity (24-HDR vs FFQ1 $\kappa = 0.24$, $P = .014$; 24-HDR vs. FFQ2 $\kappa = 0.23$, $P = .020$) and reproducibility (FFQ1 vs. FFQ2 $\kappa = 0.034$, $P < .001$) of the clusters.

4. Discussion

We identified "fruits & vegetables" and "meat" dietary patterns from the dietary data collected by FFQ1, FFQ2, and 24-HDR. The patterns had similar lifestyle and social characteristics, and food content between dietary data sources. We found reasonable reproducibility of the patterns but only modest to fair validity.

Validity of a posteriori dietary patterns has not been extensively studied [7,8]. Only a few studies have analyzed this topic using cluster analysis [9–12]. Two of them analyzed internal validity [11,12], where the "heart-healthy" pattern was associated with the most favorable nutrient profile for cardiovascular health and the "empty energy" was associated with the poorest diet quality. They also showed good reproducibility across similar population groups (80% of new participants were classified consistently). In the present study, the "fruits & vegetables" pattern showed better reproducibility than the "meat" pattern; but the opposite occurred in the validity analysis. Interestingly, the "fruits & vegetables" dietary pattern in FFQ1 had better correlation, compared to the same pattern in FFQ2, with the analogous dietary pattern of 24-HDR.

Another study validated dietary patterns between FFQ and 3-day diary [9]. In every dietary data source, they defined "healthy" and "unhealthy" patterns, which had similar food content to our "fruits & vegetables" and "meat" patterns, respectively. Concordant cluster membership was somewhat lower (59%) compared to the present study.

A study in Canadian adults found that the k-means cluster approach, applying the percentage of total energy method, showed the best reproducibility in comparison than with other methods [10]. We used the consumption-in-grams method and also obtained a good level of reproducibility.

Our study had some limitations. Both 24-HDR and the FFQ collect data retrospectively and are prone to similar limitations, such as recall bias. Additionally, we had no data on objective measures of food intake, such as biomarkers of nutrient intake. Food intake on the FFQ was previously validated by our team (16 food groups; ρ ranging from 0.19 to 0.69), but more detailed food groups were used for the cluster analysis (eg, raw and cooked vegetables) than for the validity study (all vegetables). Cluster analysis is prone to arbitrary decisions during the process of cluster definition. Small sample size makes it difficult to reach a high level of significance. The defined patterns are population specific, and further studies are needed to reproduce these findings in other populations. The main strength of the study is the methodology used to collect dietary data: the validated and reproducible FFQ and multiple 24-HDR.

The results of this study show that the cluster analysis provides dietary patterns with modest validity and fair reproducibility, as we previously hypothesized. Further research is needed to reduce the subjectivity of the cluster approach to permit the comparison of the results between the different time points, dietary data sources, and study samples.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.nutres.2014.12.011.

Conflict of interests

Authors have no conflict of interest to be disclosed.

Acknowledgment

The authors appreciate the English revision by Elaine Lilly (Writers First Aid). This research was supported by a grant (2FD097-0297-CO2-01) from Fondo Europeo de Desarrollo Regional; by scholarship for PhD training from a national program for university professors formation, FPU, of Spain's Ministry of Education (AP2010-3198); by portions of grants from Spain's Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III Fondo Europeo de Desarrollo Regional (PI11/01900), and Red HERACLES (RD06/0009); and by a joint contract (CES09/030) with the Instituto de Salud Carlos III and the Health Department of the Catalan Government (Generalitat de Catalunya). The CIBERESP is an initiative of the Instituto de Salud Carlos III, Madrid, Spain.

REFERENCES

[1] Hu FB. Dietary pattern analysis: a new direction in nutritional epidemiology. Curr Opin Lipidol 2002;1:3–9.

[2] Wirfalt AK, Jeffery RW. Using cluster analysis to examine dietary patterns: nutrient intakes, gender, and weight status differ across food pattern clusters. J Am Diet Assoc 1997;3:272–9.

[3] Devlin UM, McNulty BA, Nugent AP, Gibney MJ. The use of cluster analysis to derive dietary patterns: methodological considerations, reproducibility, validity and the effect of energy mis-reporting. Proc Nutr Soc 2012;4:599–609.

[4] Benitez-Arciniega AA, Mendez MA, Baena-Diez JM, Rovira Martori MA, Soler C, Marrugat J, et al. Concurrent and construct validity of Mediterranean diet scores as assessed by an FFQ. Public Health Nutr 2011;11:2015–21.

[5] Schroder H, Covas MI, Marrugat J, Vila J, Pena A, Alcantara M, et al. Use of a three-day estimated food record, a 72-hour recall and a food-frequency questionnaire for dietary assessment in a Mediterranean Spanish population. Clin Nutr 2001;5:429–37.

[6] Altman DG. Practical statistics for medical research. London: Chapman and Hall; 1991.

[7] Khani BR, Ye W, Terry P, Wolk A. Reproducibility and validity of major dietary patterns among Swedish women assessed with a food-frequency questionnaire. J Nutr 2004;6:1541–5.

[8] Nanri A, Shimazu T, Ishihara J, Takachi R, Mizoue T, Inoue M, et al. Reproducibility and validity of dietary patterns assessed by a food frequency questionnaire used in the 5-year follow-up survey of the Japan Public Health Center-Based Prospective Study. J Epidemiol 2012;3:205–15.

[9] Bountziouka V, Tzavelas G, Polychronopoulos E, Constantinidis TC, Panagiotakos DB. Validity of dietary patterns derived in nutrition surveys using a priori and a posteriori multivariate statistical methods. Int J Food Sci Nutr 2011;6:617–27.

[10] Lo SG, Yasui Y, Csizmadi I, McGregor SE, Robson PJ. Exploring statistical approaches to diminish subjectivity of cluster analysis to derive dietary patterns: the Tomorrow Project. Am J Epidemiol 2011;8:956–67.

[11] Millen BE, Quatromoni PA, Copenhafer DL, Demissie S, O'Horo CE, D'Agostino RB. Validation of a dietary pattern approach for evaluating nutritional risk: the Framingham Nutrition Studies. J Am Diet Assoc 2001;2:187–94.

[12] Quatromoni PA, Copenhafer DL, Demissie S, D'Agostino RB, O'Horo CE, Nam BH, et al. The internal validity of a dietary pattern analysis. The Framingham Nutrition Studies. J Epidemiol Community Health 2002;5:381–8.

3rd paper

Effect of energy under-reporting on secular trends of dietary patterns in a Mediterranean population. PLoS One 2015; 10(5): e0127647.

PLOS | ONE

RESEARCH ARTICLE

Effect of Energy Under-Reporting on Secular Trends of Dietary Patterns in a Mediterranean Population

Anna N. Funtikova[1,2,3], Santiago F. Gomez[1,4], Montserrat Fitó[1,5], Roberto Elosua[6], Alejandra A. Benítez-Arciniega[7], Helmut Schröder[1,2]*

1 Cardiovascular Risk and Nutrition Research Group (CARIN-ULEC), IMIM (Hospital del Mar Medical Research Institute), Barcelona, Spain, **2** CIBER Epidemiology and Public Health (CIBERESP), Instituto de Salud Carlos III, Madrid, Spain, **3** PhD program "Foods and Nutrition", University of Barcelona, Barcelona, Spain, **4** Fundación THAO, Barcelona, Spain, **5** CIBER Physiopathology of Obesity and Nutrition (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain, **6** Cardiovascular Epidemiology and Genetics (EGEC-ULEC), IMIM (Hospital del Mar Medical Research Institute), Barcelona, Spain, **7** Faculty of Medicine, Autonomous University of Mexico State, Toluca, Mexico

* hschroeder@imim.es

CrossMark click for updates

OPEN ACCESS

Citation: Funtikova AN, Gomez SF, Fitó M, Elosua R, Benítez-Arciniega AA, Schröder H (2015) Effect of Energy Under-Reporting on Secular Trends of Dietary Patterns in a Mediterranean Population. PLoS ONE 10(5): e0127647. doi:10.1371/journal.pone.0127647

Academic Editor: Michael Müller, University of East Anglia, UNITED KINGDOM

Received: June 20, 2014

Accepted: April 17, 2015

Published: May 29, 2015

Data Availability Statement: All relevant data are within the paper.

Funding: This research was supported by a grant (2FD097-0297-CO2-01) from Fondo Europeo de Desarrollo Regional (FEDER); by a scholarship for PhD formation from the national program of university professors formation, FPU, from Ministry of Education of Spain (AP2010-3198); by portions of grants from Spain's Ministry of Health (Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III: FEDER [PI11/01900], FEDER [CB06/02/0029] and Red Investigación Cardiovascular, Programa HERACLES

Abstract

Background

Diet is an important factor in the prevention of chronic diseases. Analysis of secular trends of dietary patterns can be biased by energy under-reporting. Therefore, the objective of the present study was to analyse the impact of energy under-reporting on dietary patterns and secular trends in dietary patterns defined by cluster analysis.

Design and methods

Two cross-sectional population-based surveys were conducted in Spain, in 2000 and 2005, with 3058 and 6352 participants, respectively, aged 25 to 74 years. Validated questionnaire was used to collect dietary data. Cluster analysis was run separately for all participants, plausible energy reporters (PER), and energy under-reporters (EUR) to define dietary patterns.

Results

Three clusters, "healthy", "mixed" and "western", were identified for both surveys. The "mixed" cluster was the predominant cluster in both surveys. Excluding EUR reduced the proportion of the "mixed" cluster up to 6.40% in the 2000 survey; this caused secular trend increase in the prevalence of the "mixed" pattern. Cross-classification analysis of all participants and PER' data showed substantial agreement in cluster assignments: 68.7% in 2000 and 84.4% in 2005. Excluding EUR did not cause meaningful (≥15%) changes in the "healthy" pattern. It provoked changes in consumption of some food groups in the "mixed" and "western" patterns: mainly decreases of unhealthy foods within the 2000 and increases of unhealthy foods within the 2005 surveys. Secular trend effects of EUR were similar to

[RD12/0042], [RD06/0009]), and by a joint contract (CP03/00115) between the Instituto de Salud Carlos III and the Health Department of the Catalan Government (Generalitat de Catalunya), and AGAUR (2014 SGR 240). The CIBERESP and CIBEROBN are an initiative of the Instituto de Salud Carlos III, Madrid, Spain.

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

those within the 2005 survey. Excluding EUR reversed the direction of secular trends in consumption of several food groups in PER in the "mixed" and "western" patterns.

Conclusions

EUR affected distribution of participants between dietary patterns within and between surveys, secular trends in food group consumption and amount of food consumed in all, but not in the "healthy" pattern. Our findings emphasize threats from energy under-reporting in dietary data analysis.

Introduction

Diet is a key factor in the prevention of chronic diseases [1]. Identification and promotion of healthy diets is paramount for evaluation and planning of national dietary intervention programs. Dietary pattern analysis takes into account the entire food intake and the interactions between all consumed nutrients, and has become widespread for exploring diet-disease relationships. An example of this approach is cluster analysis, which creates easily interpretable dietary patterns that are mutually exclusive [2]. This makes cluster analysis an interesting tool to explore secular trends of dietary patterns in populations. Secular trends in dietary patterns performed with cluster analysis have been analysed mainly among adolescents [3,4]. To date, only one study has been performed in the adult population [5].

All dietary assessment methods are biased by implausibly low self-reported energy intakes, a major challenge for nutritional epidemiologists [6]. Furthermore, energy under-reporting of foods seems to be selective [7]. This in turn affects dietary pattern analysis, which is based on biased dietary assessment methods. Few studies have investigated this issue and results to date are inconsistent [8].

Although the effect of energy under-reporting on secular trends of dietary pattern is unknown, it is well known that energy under-reporters tend to report healthier food choices. Additionally, it is reasonable to assume that the proportion of energy under-reporters will differ between dietary patterns. Therefore, we hypothesized that energy under-reporting might bias the time trends of dietary patterns.The aim of the present study was to explore the impact of energy under-reporting, first, on post-hoc dietary pattern analysis and, second, on secular trends of dietary patterns obtained by cluster analysis.

Materials and Methods

Study design and participants

Data were obtained from two population-based cross-sectional surveys of the REGICOR study (Registre Gironí del Cor) conducted in Girona (Spain) in 2000 and 2005 [9]. The 2000 survey examined a random population-based sample of 3058 men and women aged 24 to 77 years (participation rate: 71.0%); the second survey included 6352 non-institutionalized men and women older than 33 years (participation rate: 72.0%). No participants were repeated in the second survey. The present study selected only REGICOR participants aged 35 to 74 years and excluded individuals with extreme energy intake values (defined as <800 and >4200 kcal/day for men and <600 and >3500 kcal/day for women)[10]. Based on the characteristics of participants reporting extreme energy intakes according to the criteria of Willett [10], especially age and BMI, it is reasonable to assume that these values are actual outliers. Furthermore, cluster analysis is very sensitive to outliers. Therefore, we decided to exclude extreme energy intakes to avoid biased cluster formation. The exclusion of extreme intakes reduced the number of energy over-reporters; however, other surveys reported similar or even smaller proportions of energy over-reporters to the proportion defined in our study [11–13]. In total, 7373 participants (2188 from 2000 and 5185 from 2005) remained. Exclusion criteria affected the following numbers of REGICOR 2000 and REGICOR 2005 participants: age, 518 and 665; missing values in energy intake, 1 and 28; extremely low energy intake, 112 and 30; and extremely high energy intake, 239 and 444, respectively. The project was approved by the local Ethics Committee (CEIC—PSMAR, Barcelona, Spain). All participants received the results of their examination.

Anthropometric data

Measurements were performed by a team of trained nurses and interviewers who used the same standard methods in both surveys. Weight was measured by a calibrated precision scale and rounded up to the nearest 200 g. Height was measured in the standing position and rounded up to the nearest 0.5 cm. Weight was divided by height squared (kg/m^2) to establish the BMI. Obesity was considered with BMI $\geq$ 30 kg/m^2.

Dietary intake data

A validated [14,15] FFQ was administered by a trained interviewer to collect food consumption data. This 165-item food list, including alcoholic and non-alcoholic beverages, asked participants for their usual intakes over the preceding year. Individuals chose from 10 frequency categories, ranging from never or less than once per month to 6 or more times per day. Medium servings were defined by natural (e.g., 1 apple, 1 slice) or household units (e.g., 1 teaspoon, 1 cup).

Overall diet quality was determined by the modified Mediterranean diet score (mMDS) [16]; the published Pearson correlation for the energy-adjusted mMDS vs. multiple recalls was 0.48 [15]. The mMDS was calculated according to sex-specific, energy-adjusted tertile distribution of food consumption in the study population. For cereals, fruits, vegetables, legumes, fish, olive oil and nuts the lowest tertile was coded as 1, medium as 2, and highest as 3. For meat (including red meat, poultry and sausages) and for dairy products the score was inverted, with the highest tertile coded as 1 and lowest as 3. Moderate red wine consumption (up to 20 g) was included as a favourable component in the Mediterranean diet score, with a score of 3. Exceeding this upper limit or reporting no red wine consumption was coded as 0. Total mMDS scores ranged from 10 to 30.

Implausible energy-reporting

Implausible energy reporters were identified by the revised Goldberg method [17,18]. Basal metabolic rate (BMR) was calculated using the Mifflin equation [19]:

$$\mathrm{BMR} = (\mathrm{Weight}_{\mathrm{kg}} \times 9.99) + (\mathrm{Height}_{\mathrm{cm}} \times 6.25) - (\mathrm{Age}_{\mathrm{y}} \times 4.92) + 5 \text{ (among men)}$$

$$\mathrm{BMR} = (\mathrm{Weight}_{\mathrm{kg}} \times 9.99) + (\mathrm{Height}_{\mathrm{cm}} \times 6.25) - (\mathrm{Age}_{\mathrm{y}} \times 4.92) + 161 \text{ (among women)}$$

The index of variability (S) in components of energy balance was determined. The coefficients of variability (CV) in components of energy balance were approximate values for these CV parameters derived by pooling the means of several studies [20]. The applied values for intra-individual variations in repeated measures of energy intake (CV^2_{wEI}), BMR (CV^2_{wBMR}), and physical activity level (CV^2_{tP}), were 23%, 8.5%, and 15%, respectively [20]. The number of recording days was set to 365 because the FFQ captured one year of estimated food intake. An individual physical activity level (PAL) was calculated and categorized according to quintile distribution of self-reported leisure-time physical activity (LTPA): sedentary = 1.35 (1st quintile), light = 1. 55 (2nd quintile), moderately active = 1.75 (3rd quintile), active = 1.85 (4th quintile), and vigorous = 2.2 (5th quintile). Participants with BMR above or below the upper and lower 95% confidence interval limit of 1.96 standard deviations for plausible energy intake were characterized as implausible energy reporters.

The following formula was used:

$$Cut - off = PAL \times \exp\left[\pm 1.96 \times \frac{(S/100)}{\sqrt{n}}\right]$$

where

$$S = \sqrt{\left[\frac{CV_{wEI}^2}{d} + CV_{wBMR}^2 + CV_{tP}^2\right]}$$

Dietary patterns

The K-mean cluster algorithm, a non-hierarchical cluster analysis based on Euclidean distances, was used to derive dietary patterns. All individuals were placed in groups/clusters based on highest similarity and shortest distance to the cluster centre inside of the group and highest diversity and largest distance between cluster centres outside of the group.

The 165 food items of the FFQ were combined into 48 food groups according to similarities in their nutritional content. We used two methods to define clusters, based on absolute intakes of food groups and based on percentage of energy contribution of every food group. The results of both approaches did not differ significantly (not shown), therefore, according to the initial aims of the study we preferred the method using absolute intakes of food groups. To define the best cluster solution, several runs of cluster formation were performed. Criteria for cluster solutions were nutritional meaningfulness and a reasonable sample size (every cluster contained at least 5% of the study population). The final cluster solutions contained 7 and 5 clusters for 2000 and 2005, respectively. In both surveys, 3 meaningful clusters were retained and the rest of the clusters were removed as outliers due to insufficient size. In total, 32 (1.5%) and 20 (0.4%) participants in 2000 and 2005, respectively, were removed from further analysis. The same procedure was applied for separate cluster analyses in plausible energy reporters and in energy under-reporters. Among plausible energy reporters, 3 clusters remained after reaching a 5-cluster solution in both surveys and excluding from further analysis 10 (0.6%) and 15 (0.4%) participants in 2000 and 2005, respectively. Among energy under-reporters, 3 clusters remained after 6- and 5-cluster solutions in 2000 and 2005, respectively; 13 (2.2%) and 18 (1.3%) energy under-reporting participants, respectively, were excluded from further analysis.

We also performed cluster analysis in energy over-reporters, but, due to low prevalence of these participants (4.98% in the REGICOR 2000 and 4.96% in the REGICOR 2005), the cluster solutions were inconsistent. We joined the plausible energy reporters with energy over-reporters, and it resulted in similar clusters with those defined in only plausible energy reporters group. Therefore, we decided to include the energy over-reporters in the plausible energy reporters group. With fewer than 5% energy over-reporters, hardly comparable with the total proportion of plausible reporters, the combined group will be called "plausible energy reporters", without forgetting that it includes energy over-reporters for purposes of the cluster analysis.

We also performed cluster analysis with data pooled from both surveys. As explained above, we defined a three-cluster solution in the set of data with all participants and in the set of data with only plausible energy reporters.

Other variables

LTPA was measured by the validated Minnesota LTPA questionnaire administered by a trained interviewer [21,22]. Reported smoking habits and demographic and socioeconomic variables were obtained from structured standard questionnaires administered by trained personnel. Participants were categorized as never-smokers and ever-smokers. Maximum education level attained was elicited and recorded for analysis as primary school versus secondary school or university.

Statistical analysis

A univariate general linear model was used to define mean values of food consumption and other variables according to the cluster distribution. To define the p-value for linear trend, we used a univariate general linear model for continuous, logistic regression for categorical, and Kruskal-Wallis H test for non-parametric variables.

To compare characteristics of the clusters between surveys and between different categories of energy reporters, we used Student t-test for continuous, χ^2 test for categorical, and Mann-Whitney U test for non-parametric variables.

Contingency tables were used for the cross-classification of clusters of all participants and clusters of plausible energy reporters. The proportion of subjects consistently categorized (same cluster) was calculated.

Fifteen percentage difference was considered as meaningful difference in food group consumption between different groups of participants[23].

Differences were considered significant if $p \leq 0.05$. Statistical analysis was performed using SPSS version 18.0. (SPSS Inc. Chicago, Ill., USA) and R.

Results

Three clusters were identified for each survey, according to main food consumption characteristics: "healthy", "mixed", and "western". The distribution of the mMDS indicated the construct validity of these clusters. Significantly higher mMDS index scores were found in "healthy" cluster members, followed by mixed and western cluster members (Table 1). Therefore, the clusters were labelled according to the diet quality of every cluster measured by the mMDS.

In both surveys, the "mixed" cluster was the most prevalent, followed by the "western" and "healthy" clusters (Table 1). The highest proportion of energy under-reporting was found in the "mixed" cluster and the lowest in the "western" cluster in both surveys. Excluding energy under-reporters or analysing only this subgroup produced cluster solutions similar to the original data set (Table 1). Excluding energy under-reporters strongly decreased the proportion of the "mixed" cluster. Therefore, in plausible energy reporters "western" and "healthy" clusters had higher proportion of participants and the "mixed"—lower proportion in comparison with the original data set in the REGICOR 2000 survey. This was not the case in the REGICOR 2005 survey (Table 1). Cross-classification of individuals according to the original clusters and those obtained after excluding energy under-reporters showed that 68.7% in 2000 and 84.4% in 2005 were consistently placed into the same cluster.

Age and the proportion of women decreased across clusters (from "healthy" to "western") in both surveys (Table 1). The opposite was observed for educational level and smoking. These findings were similar for cluster solutions of plausible and energy under-reporters (Table 1). The proportion of women increased in the "mixed" cluster of plausible and energy under-reporters of the 2000 survey, and was significantly higher compared to their 2005 peers. It is

Table 1. General characteristics of clusters in the REGICOR 2000 and 2005 surveys.[a]

Variables	REGICOR 2000				REGICOR 2005			
	Healthy	Mixed	Western	p^b	Healthy	Mixed	Western	p^b
Proportion of participants, N (%)								
Clusters with all participants	558 (25.9)	945 (43.8)	653 (30.3)		1276 (24.7)	2349 (45.5)	1540 (29.8)	
Energy under-reporters[c]	80 (14.3)	460 (48.7)	57 (8.73)		173 (13.6)	1071 (45.6)	108 (7.01)	
Clusters with energy plausible reporters	616 (39.0)	101 (6.40)	861 (54.6)		988 (25.9)	1806 (47.3)	1021 (26.8)	
Clusters with energy under-reporters	112 (19.1)	240 (40.9)	235 (40.0)		304 (22.7)	574 (42.9)	459 (34.3)	
Age, years								
All participants	57.3	55.3*	49.3	<0.001	58.0	56.4	49.2	<0.001
	56.4, 58.2	54.7, 56.0	48.5, 50.1		57.4, 58.6	56.0, 56.8	48.7, 49.7	
Plausible reporters	57.3	55.6	50.4*	<0.001	57.7	55.3	48.8	<0.001
	56.5, 58.1	53.5, 57.6	49.7, 51.0		57.0, 58.3	54.8, 55.7	48.1, 49.4	
Under-reporters	59.3	57.6*	52.0	<0.001	57.2	59.4	51.3	<0.001
	57.3, 61.2	56.2, 58.9	50.7, 53.3		56.0, 58.3	58.5, 60.2	50.4, 52.3	
Women, %								
All participants	65.6	55.4	26.3*	<0.001	67.7	54.3	32.9	<0.001
Plausible reporters	66.9	70.3*	34.5*	<0.001	69.5	57.6	28.6	<0.001
Under-reporters	61.6	67.1*	26.0	<0.001	58.2	57.8	24.6	<0.001
High education[d], %								
All participants	24.7**	25.7**	38.6**	<0.001	48.8	51.9	59.5	<0.001
Plausible reporters	24.8**	21.8**	37.0**	<0.001	48.9	51.5	60.2	<0.001
Under-reporters	17.9**	22.5**	30.2**	0.008	48.0	49.1	63.6	<0.001
LTPA[e], METs min/d								
All participants	227*	195**	203	0.001	272	218	215	<0.001
	113, 405	92.1, 336	97.0, 371		174, 451	113, 378	109, 397	
Plausible reporters	196**	173	153**	0.01	249	171	203	<0.001
	91.3, 330	84.0, 294	74.9, 293		133, 393	87.0, 292	100, 367	
Under-reporters	377	262	335	<0.001	392	287	395	<0.001
	223, 519	157, 433	191, 496		259, 603	179, 483	237, 603	
Smoking[f], %								
All participants	12.5**	17.8**	35.8**	<0.001	38.2	50.1	61.3	<0.001
Plausible reporters	11.9**	12.9**	31.8**	<0.001	38.2	48.1	64.9	<0.001
Under-reporters	5.4**	12.9**	32.8**	<0.001	46.4	45.3	63.4	<0.001
Obesity, %								
All participants	28.7	29.6*	27.0**	0.48	28.1	25.3	18.6	<0.001
Plausible reporters	25.5	26.7	22.6*	0.20	25.5	19.6	18.9	<0.001
Under-reporters	40.2	40.4	40.0**	0.96	42.4	33.1	26.4	<0.001
Energy, kcal								
All participants	2626	1914*	2852	<0.001	2647	1975	2858	<0.001
	2585, 2667	1882, 1946	2814, 2891		2621, 2673	1956, 1994	2835, 2882	
Plausible reporters	2594**	2604**	2671**	0.008	2754	2359	2983	<0.001
	2551, 2637	2498, 2710	2634, 2706		2726, 2782	2338, 2380	2956, 3011	
Under-reporters	1835	1576	1826**	0.85	1908	1572	1933	0.32
	1763, 1906	1527, 1625	1777, 1876		1870, 1946	1544, 1600	1902, 1964	
mMDS, points								
All participants	20.8**	19.2*	18.7	<0.001	21.4	19.7	18.6	<0.001
	20.5, 21.1	19.0, 19.4	18.5, 18.9		21.2, 21.5	19.5, 19.8	18.4, 18.8	
Plausible reporters[g]	20.5**	19.2	18.6	<0.001	21.3	19.4	18.5	<0.001

(Continued)

Table 1. (*Continued*)

Variables	REGICOR 2000				REGICOR 2005			
	Healthy	Mixed	Western	p^b	Healthy	Mixed	Western	p^b
	20.3, 20.8	18.6, 19.8	18.4, 18.8		21.1, 21.5	19.3, 19.6	18.3, 18.7	
Under-reporters[g]	20.5*	19.4	19.7	0.03	21.4	19.8	19.4	<0.001
	19.9, 21.1	19.0, 19.8	19.3, 20.1		21.1, 21.8	19.6, 20.1	19.1, 19.7	

LTPA, leisure-time physical activity; METs, metabolic equivalents; mMDS, modified Mediterranean diet score.

*p≤0.05

**p <0.001 for differences between the REGICOR 2000 and 2005 surveys.

[a]Values are means and 95% C.I. or percentages (if specified).

[b]Polynomial contrasts were used to obtain *p* for linear trend in normal distributed continues variables (age, energy, mMDS), Kruskal-Wallis H test was used to obtain *p* value for non-parametric variables (LTPA), χ^2 test was used to obtain *p* for linear trend for categorical variables (women, high education, smoking, obesity).

[c]The proportion of energy under-reporters in the clusters of "all participants".

[d]More than secondary school education.

[e]Median and 25th and 75th percentiles.

[f]Active smokers or ex-smokers less than 1 year.

[g] mMDS for plausible and energy under-reporters were calculated on the base of tertile distribution of food group consumption in all participants (according to survey).

doi:10.1371/journal.pone.0127647.t001

important to note the considerable increase in the proportion of smokers from REGICOR 2000 to REGICOR 2005.

Obesity prevalence decreased across clusters only in the REGICOR 2005 survey (Table 1). The prevalence of obesity in nearly all clusters of plausible energy reporters decreased in comparison with all reporters in both surveys. More obese individuals were found in all clusters of energy under-reporters compared to plausible energy reporters.

Diet quality measured by the mMDS decreased across clusters in both surveys, independently of energy reporting status and it was significantly higher among members of the "mixed" cluster in 2005 and of the "western" cluster in both surveys in energy under-reporters, compared to their plausible-reporter peers (Table 1).Overall dietary pattern characteristics identified by cluster analysis in all participants, plausible energy reporters and energy under-reporters were similar for both REGICOR surveys, 2000 and 2005 (Table 2). An inverse linear trend across clusters was observed for cooked and raw vegetables, pulses, cooked potatoes, fresh fish, olive oil, citrus and other fruits, nuts and low fat dairy (Table 2). A direct linear trend was found in fried potatoes, red meat, sausages, white bread, pastry, wine, fast food, soft drinks and high fat dairy.

Energy under-reporting and dietary patterns within surveys

The amount of food consumption within the same survey was affected after excluding energy under-reporters from analysis (Fig 1). The "healthy" pattern did not have any meaningful changes in food consumption after exclusion of energy under-reporters, both in the REGICOR 2000 and 2005 surveys. Most of the changes occurred in the "mixed" pattern. The decreases occurred in consumption of such unhealthy food, as sausages, white bread, pastry, soft drinks and fast food, and such healthy food group, as pulses; and increases in such healthy food groups, as low fat dairy and citrus fruits, in the REGICOR 2000 survey. In the REGICOR 2005 survey the opposite was true. Energy consumption increased meaningfully only in the "mixed" pattern in both surveys (Fig 1). The "western" pattern was slightly affected by excluding energy

Table 2. Characteristics of food groups and nutrients according to clusters of surveys in 2000 and 2005.

Variables	Healthy		Mixed		Western		*P*-trend
	2000	2005	2000	2005	2000	2005	
Cooked vegetables, g/4.2 MJ							
All participants	61.6	56.9	37.4	37.3	27.8	26.5	<0.001[a]
	58.6, 64.7	55.3, 58.4	35.0, 39.7	36.2, 38.5	25.0, 30.7	25.1, 28.0	<0.001[b]
Plausible reporters	53.1	53.7	39.0	32.9	27.4*	25.5	<0.001[a]
	50.3,56.0	52.2,55.3	32.1,46	31.8,34.1	25.0,29.7	24.0,27.1	<0.001[b]
Under-reporters	53.7*	72.5	60.9**	45.4	32.7	30.1	<0.001[a]
	46.0,61.4	68.5,76.5	55.6,66.2	42.5,48.3	27.4,38	26.9,33.4	<0.001[b]
Raw vegetables, g/4.2 MJ							
All participants	191	179	123*	113	86.9	82.7	<0.001[a]
	182, 199	174, 184	116, 129	109, 117	79.4, 94.5	78.3, 87.1	<0.001[b]
Plausible reporters	175	178	113	102	89.5*	80.5	<0.001[a]
	168,183	173,183	94.8,131	98,106	83.4,95.6	75.5,85.5	<0.001[b]
Under-reporters	159*	201	171**	131	107*	88.2	<0.001[a]
	137,180	190,212	157,186	123,139	92.5,122	79.1,97.3	<0.001[b]
Cooked potatoes, g/4.2 MJ							
All participants	34.5	33.9	26.7	27.1	24.1*	21.8	<0.001[a]
	32.0, 36.9	32.5, 35.3	24.8, 28.5	26.1, 28.1	21.9, 26.3	20.6, 23.1	<0.001[b]
Plausible reporters	32.6	32.0	26.0	26.4	23.8*	21.5	<0.001[a]
	30.4,34.7	30.4,33.5	20.8,31.3	25.2,27.5	22.0,25.6	20.0,23.0	<0.001[b]
Under-reporters	34.7	35.1	32.7	29.5	23.7	24.9	0.004 [a]
	28.5,40.9	32.1,38.1	28.4,36.9	27.3,31.7	19.4,28.0	22.4,27.3	<0.001 [b]
Fried potatoes, g/4.2 MJ							
All participants	2.15	2.12	3.59*	3.02	6.63**	5.55	<0.001[a]
	1.73, 2.57	1.89, 2.34	3.27, 3.92	2.85, 3.18	6.24, 7.02	5.34, 5.75	<0.001[b]
Plausible reporters	2.36	2.1	3.83	3.2	5.79	5.93	<0.001[a]
	1.96,2.75	1.85,2.35	2.86,4.80	3.01,3.38	5.46,6.13	5.69,6.18	<0.001[b]
Under-reporters	3.39*	1.95	2.44	2.28	4.93	5.35	0.013 [a]
	2.38,4.39	1.47,2.43	1.75,3.12	1.93,2.63	4.24,5.63	4.96,5.74	<0.001[b]
Pulses, g/4.2 MJ							
All participants	34.7	34.9	34.1**	29.4	29.8*	27.6	<0.001[a]
	32.7, 36.8	33.9, 35.9	32.5, 35.6	28.7, 30.2	27.9, 31.6	26.7, 28.5	<0.001[b]
Plausible reporters	32.8	33.6	28.4	28.0	29.3*	27.6	0.001[a]
	31.2,34.4	32.6,34.7	24.4,32.5	27.2,28.7	27.9,30.7	26.6,28.6	<0.001[b]
Under-reporters	59.5**	36.1	31.3	31.3	36.4*	32.2	<0.001[a]
	54.1,64.9	33.7,38.4	27.6,35	29.6,33.0	32.7,40.2	30.3,34.1	0.013 [b]
Red meat, g/4.2 MJ							
All participants	29.7*	31.8	39.0	38.6	47.2	45.5	<0.001[a]
	28.0, 31.4	30.7, 32.9	37.6, 40.3	37.8, 39.5	45.6, 48.8	44.5, 46.5	<0.001[b]
Plausible reporters	30.4	31.8	34.7	37.6	45.4*	47.8	<0.001[a]
	28.9,32.0	30.6,33	30.9,38.5	36.7,38.5	44.1,46.7	46.7,49.0	<0.001[b]
Under-reporters	36.1	36.1	33.1*	37.8	48.4*	43.9	<0.001[a]
	31.7,40.5	33.5,38.6	30.1,36.1	35.9,39.6	45.4,51.4	41.8,46.0	<0.001[b]
Sausages, g/4.2 MJ							
All participants	5.92*	6.49	7.08*	7.58	10.2	10.2	<0.001[a]
	5.38, 6.45	6.13, 6.85	6.67, 7.49	7.31, 7.84	9.66, 10.7	9.88, 10.5	<0.001[b]
Plausible reporters	6.31	6.27	5.70*	7.69	9.28**	10.7	<0.001[a]

(*Continued*)

Table 2. (*Continued*)

Variables	Healthy		Mixed		Western		*P*-trend
	2000	2005	2000	2005	2000	2005	
	5.79,6.84	5.88,6.66	4.41,6.99	7.40,7.98	8.84,9.73	10.4,11.1	<0.001[b]
Under-reporters	5.74**	7.88	5.24**	7.08	9.82	9.16	<0.001[a]
	4.68,6.80	7.06,8.71	4.51,5.96	6.48,7.68	9.09,10.6	8.48,9.83	<0.001[b]
Fresh fish, g/4.2 MJ							
All participants	31.2	28.9	20.5	20.2	16.4*	15.2	<0.001[a]
	29.6, 32.7	28.1, 29.7	19.3, 21.6	19.6, 20.8	15.0, 17.8	14.5, 16.0	<0.001[b]
Plausible reporters	26.9	27.8	19.8	17.9	16.5*	15.0	<0.001[a]
	25.7,28.2	27.0,28.7	16.6,23.0	17.3,18.5	15.4,17.6	14.2,15.9	<0.001[b]
Under-reporters	29.0*	34.9	27.6	24.0	20.8*	17.3	0.003 [a]
	24.6,33.5	33.0,36.9	24.5,30.6	22.6,25.4	17.7,23.9	15.7,18.9	<0.001[b]
Rice & Pasta, g/4.2 MJ							
All participants	24.8*	27.2	28.3	28.5	24.9*	27.9	0.911 [a]
	22.9, 26.6	26.1, 28.3	26.9, 29.7	27.6, 29.3	23.2, 26.6	26.9, 28.9	0.359 [b]
Plausible reporters	25.5	27.5	26.8	26.6	24.8*	27.8	0.537 [a]
	24.0,27.0	26.2,28.7	23.1,30.4	25.6,27.5	23.6,26.1	26.6,29.0	0.723 [b]
Under-reporters	25.7	27.9	31.4	31.2	29.5	31.1	0.261 [a]
	20.2,31.1	25.7,30.2	27.7,35.1	29.5,32.9	25.7,33.2	29.3,33.0	0.035 [b]
White bread, g/4.2 MJ							
All participants	17.8	18.0	26.8	25.9	24.6	24.0	<0.001[a]
	16.1, 19.4	16.9, 19.0	25.5, 28.0	25.1, 26.7	23.1, 26.1	23.0, 25.0	<0.001[b]
Plausible reporters	19.9*	17.5	21.8*	27.5	25.7**	22.8	<0.001[a]
	18.4,21.3	16.3,18.6	18.3,25.3	26.6,28.3	24.6,26.9	21.7,23.9	<0.001[b]
Under-reporters	26.8**	13.3	21.9	24.5	28.2	26.5	0.615 [a]
	22.4,31.2	10.9,15.6	18.9,24.9	22.8,26.2	25.1,31.2	24.6,28.4	<0.001[b]
Olive oil, g/4.2 MJ							
All participants	12.5	12.4	11.3**	13.2	8.56*	9.30	<0.001[a]
	11.9, 13.2	12.1, 12.8	10.8, 11.8	12.9, 13.5	7.97, 9.16	8.95, 9.65	<0.001[b]
Plausible reporters	12.7	12.2	10.5*	13.1	9.21	8.78	<0.001[a]
	12.1,13.3	11.7,12.6	9.05,11.9	12.8,13.4	8.72,9.7	8.37,9.19	<0.001[b]
Under-reporters	8.84**	13.56	15.1	13.8	8.45*	9.80	0.678 [a]
	7.34,10.3	12.7,14.4	14.0,16.1	13.1,14.4	7.42,9.49	9.09,10.5	<0.001[b]
Pastry, g/4.2 MJ							
All participants	2.92*	2.09	4.30*	3.57	6.78	7.49	<0.001[a]
	2.24, 3.59	1.70, 2.48	3.78, 4.82	3.28, 3.85	6.15, 7.40	7.14, 7.84	<0.001[b]
Plausible reporters	3.37**	2.1	3.06*	4.30	6.40**	8.34	<0.001[a]
	2.74,4.01	1.64,2.56	1.5,4.61	3.96,4.64	5.86,6.93	7.88,8.79	<0.001[b]
Under-reporters	1.77	1.47	3.35	2.86	5.16	4.5	<0.001[a]
	0.225,3.32	0.809,2.14	2.29,4.4	2.37,3.34	4.09,6.23	3.96,5.04	<0.001[b]
Citrus fruits, g/4.2 MJ							
All participants	152**	110	85.3*	74.0	46.8*	41.0	<0.001[a]
	145, 160	106, 114	79.5, 91.1	71.0, 77.0	39.8, 53.9	37.3, 44.7	<0.001[b]
Plausible reporters	142**	115	109**	65.7	52.1**	38.8	<0.001[a]
	135,148	111,119	92.6,125	62.5,69	46.6,57.6	34.5,43.1	<0.001[b]
Under-reporters	200**	100	85.9	95.9	44.5	41.9	<0.001[a]
	181,219	90.8,109	73.2,98.6	89.4,103	31.6,57.3	34.5,49.2	<0.001[b]

(*Continued*)

Table 2. (*Continued*)

Variables	Healthy		Mixed		Western		*P*-trend
	2000	2005	2000	2005	2000	2005	
Other fruits, g/4.2 MJ							
All participants	222**	151	129**	108	81.3*	74.9	<0.001[a]
	213, 231	147, 156	122, 136	105, 112	73.2, 89.5	70.6, 79.1	<0.001[b]
Plausible reporters	209**	156	139**	104	88.1**	68.8	<0.001[a]
	201,217	151,161	118,159	100,108	81.1,95.2	63.7,73.9	<0.001[b]
Under-reporters	206**	129	164*	135	84.1*	73.6	<0.001[a]
	186,226	120,139	150,177	128,142	70.3,97.8	65.6,81.6	<0.001[b]
Nuts, g/4.2 MJ							
All participants	6.42*	7.60	4.46	4.51	4.32	4.22	<0.001[a]
	5.86, 6.99	7.26, 7.94	4.02, 4.89	4.26, 4.76	3.80, 4.85	3.91, 4.54	<0.001[b]
Plausible reporters	6.05	8.18	5.01	4.63	4.50	4.08	<0.001[a]
	5.51,6.58	7.79,8.58	3.7,6.33	4.34,4.92	4.04,4.95	3.69,4.47	<0.001[b]
Under-reporters	3.82*	5.15	5.52	4.71	3.53	4.04	0.715 [a]
	2.52,5.13	4.5,5.81	4.63,6.41	4.23,5.19	2.63,4.43	3.5,4.57	0.010 [b]
Wine, g/4.2 MJ							
All participants	17.0	15.8	33.8*	28.3	46.6**	29.9	<0.001[a]
	13.1, 20.9	13.7, 17.9	30.8, 36.8	26.8, 29.9	43.0, 50.2	28.0, 31.8	<0.001[b]
Plausible reporters	17.3	15.2	22.5	25.6	44.7**	31.7	<0.001[a]
	13.7,20.8	13.0,17.4	13.8,31.3	23.9,27.2	41.7,47.7	29.5,33.9	<0.001[b]
Under-reporters	16.1	20.0	20.9	24.0	58.4**	41.9	<0.001[a]
	7.05,25.1	15.1,24.9	14.8,27.1	20.4,27.5	52.1,64.6	37.9,45.8	<0.001[b]
Softdrinks, g/4.2 MJ							
All participants	6.06	5.24	10.8	11.5	21.1*	28.7	<0.001[a]
	3.01, 9.11	3.07, 7.42	8.46, 13.1	9.91, 13.1	18.2, 23.9	26.7, 30.7	<0.001[b]
Plausible reporters	6.41	5.01	7.37	11.56	19.3**	33.0	<0.001[a]
	3.41,9.42	2.57,7.46	-0.05,14.8	9.75,13.4	16.7,21.8	30.6,35.4	<0.001[b]
Under-reporters	3.13	5.40	7.82	8.36	17.0*	25.2	<0.001[a]
	-2.98,9.23	0.91,9.89	3.65,12.0	5.10,11.6	12.8,21.2	21.5,28.9	<0.001[b]
High fat dairy, g/4.2 MJ							
All participants	30.5	31.6	55.0*	46.1	59.4	62.6	<0.001[a]
	24.7, 36.3	28.1, 35.1	50.6, 59.5	43.5, 48.6	54.0, 64.7	59.4, 65.7	<0.001[b]
Plausible reporters	33.8	30.0	52.4	55.4	65.5	59.3	<0.001[a]
	28.4,39.2	26.1,34	39,65.7	52.4,58.3	61.0,70.1	55.4,63.1	<0.001[b]
Under-reporters	38.1*	22.5	36.6	36.2	51.9	58.3	0.089 [a]
	24.9,51.2	15.5,29.5	27.7,45.6	31.1,41.3	42.8,60.9	52.6,64	<0.001 [b]
Low fat dairy, g/4.2 MJ							
All participants	104	107	81.9*	91.4	30.1*	37.7	<0.001[a]
	96.9, 112	102, 112	76.2, 87.6	88.0, 94.8	23.2, 37.0	33.4, 41.9	<0.001[b]
Plausible reporters	101	108	96.8	73.6	39.5*	33.1	<0.001[a]
	94.4,107	104,113	81.1,113	70.1,77.2	34.1,44.9	28.4,37.8	<0.001[b]
Under-reporters	69.1**	107	149	139	29.7**	46.2	0.001[a]
	50.8,87.3	96.5,118	137,162	131,147	17.1,42.3	37.3,55.1	<0.001[b]
Fast food, g/4.2 MJ							
All participants	0.13	0.1	0.30*	0.17	0.69	0.63	<0.001[a]
	-0.03,0.28	0.02, 0.18	0.19, 0.42	0.10, 0.23	0.55, 0.83	0.55, 0.70	<0.001[b]

(*Continued*)

Table 2. (*Continued*)

Variables	Healthy		Mixed		Western		*P*-trend
	2000	2005	2000	2005	2000	2005	
Plausible reporters	0.11	0.09	0.12	0.16	0.57*	0.77	<0.001[a]
	-0.01,0.24	-0.01,0.19	-0.19,0.44	0.08,0.23	0.46,0.67	0.68,0.87	<0.001[b]
Under-reporters	0.01	0.08	0.37	0.08	0.53	0.25	0.099 [a]
	-0.33,0.52	-0.02,0.17	0.08,0.66	0.007,0.15	0.24,0.82	0.17,0.33	0.008[b]
Carbohydrates, %							
All participants	45.1	42.8	41.6	40.8	39.1	40.0	<0.001[a]
	44.4, 45.7	42.4, 43.1	41.1, 42.1	40.5, 41.1	38.6, 39.7	39.7, 40.4	<0.001[b]
Plausible reporters	44.9	43.1	40.6	40.8	39.4	39.6	<0.001[a]
	44.3,45.5	42.7,43.6	39.2,42.1	40.5,41.1	38.9,39.9	39.2,40.0	<0.001[b]
Under-reporters	46.6	40.5	42.3	41.8	39.6	40.4	<0.001[a]
	45.1,48.1	39.7,41.3	41.3,43.3	41.2,42.4	38.6,40.6	39.7,41.0	0.799 [b]
Proteins, %							
All participants	18.5	18.7	17.9	17.5	17.2	16.9	<0.001[a]
	18.3, 18.8	18.5, 18.8	17.7, 18.1	17.4, 17.6	17.0, 17.4	16.7, 17.0	<0.001[b]
Plausible reporters	18.1	18.5	17.0	16.9	17.2	16.8	<0.001[a]
	17.9,18.3	18.3,18.6	16.5,17.5	16.8,17.0	17.0,17.4	16.7,17.0	<0.001[b]
Under-reporters	19.1	20.1	18.8	18.4	18.0	17.3	0.006[a]
	18.4,19.7	19.7,20.4	18.4,19.3	18.1,18.6	17.5,18.4	17.0,17.5	<0.001[b]
Lipids, %							
All participants	37.8	40.1	40.2	41.7	41.2	42.5	<0.001[a]
	37.2, 38.4	39.7, 40.4	39.8,40.7	41.5, 42.0	40.7, 41.7	42.2, 42.8	<0.001[b]
Plausible reporters	38.3	39.9	42.9	42.5	41.5	42.5	<0.001[a]
	37.8,38.9	39.6,40.3	41.5,44.2	42.2,42.8	41,41.9	42.2,42.9	<0.001[b]
Under-reporters	35.6	40.5	39.8	40.4	39.4	41.0	<0.001[a]
	34.2,37.0	39.7,41.2	38.9,40.8	39.9,40.9	38.4,40.3	40.4,41.6	0.252 [b]
Energy, MJ							
All participants	11.0	11.1	8.01*	8.26	11.9	12.0	<0.001[a]
	10.8, 11.2	11.0, 11.2	7.87, 8.14	8.18, 8.34	11.8,12.1	11.9, 12.1	<0.001[b]
Plausible reporters	10.9**	11.5	10.9**	9.87	11.2**	12.5	0.008 [a]
	10.7, 11.0	11.4,11.6	10.5, 11.3	9.78, 9.96	11.0, 11.3	12.4, 12.6	<0.001[b]
Under-reporters	7.68*	7.98	6.59	6.58	7.64	8.09	0.848 [a]
	7.38, 7.97	7.82, 8.14	6.39, 6.80	6.46, 6.69	7.43, 7.85	7.96, 8.22	0.324 [b]

Values are means and 95% C.I. or percentages and 95% C.I.

P for linear trend was obtained using polynomial contrasts.

[a]p-trend for the REGICOR 2000 survey.

[b]p-trend for the REGICOR 2005 survey.

*p-value ≤0.05 and **p-value<0.001 for the comparison of the REGICOR 2000 and 2005.

doi:10.1371/journal.pone.0127647.t002

under-reporters, only low-fat dairy in the REGICOR 2000 survey, and soft drinks and fast food in the REGICOR 2005 survey were increased.

Energy under-reporting and secular trends, between surveys

Excluding energy under-reporters had consequences for secular trends of food consumption and prevalence of participants in the same type of cluster between surveys (Fig 2). Secular

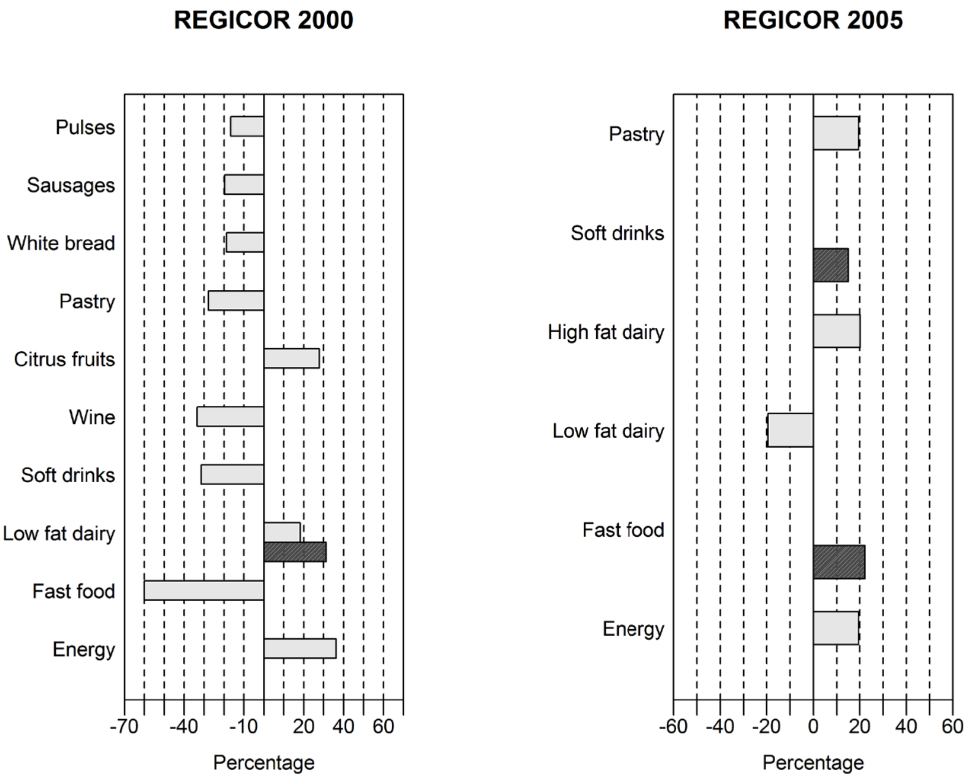


Fig 1. Meaningful* percentage changes in absolute intake of food group consumption in grams in plausible energy reporters after excluding energy under-reporters within 1) the REGICOR 2000 and 2) the REGICOR 2005 surveys. Only groups of food are included, which had meaningful changes. White—mixed dietary patter, black—western dietary pattern. *≥15% difference in food group consumption in plausible energy reporters compared with all reporters.

doi:10.1371/journal.pone.0127647.g001

trend of prevalence of the participants shifted towards higher prevalence of the "mixed" pattern with 40.9% increase in the REGICOR 2005 survey, and subsequent decreases of the "healthy" and "western" patterns, 13.1% and 27.8%, respectively.

Less healthy perceived food. In the "healthy" cluster, all food groups with meaningful changes of secular trends had the same direction in both all reporters and plausible energy reporters (Fig 2). Among them, pastry, soft drinks, and fast food consumption decreased. Main changes in the food consumption occurred in the "mixed" dietary pattern. The direction of meaningful secular trends changed to the opposite direction in pastry and fast food in this pattern (Fig 2). In the "mixed" cluster six food groups showed meaningful changes only in plausible energy reporters, where the unhealthy food groups, such as sausages, white bread and soft drink increased the consumption. In the same cluster high fat dairy only in all reporters and fried potatoes in both, all and plausible energy reporters decreased meaningfully (Fig 2). In the "western" cluster consumption of sausages, white bread, and pastry increased only in plausible energy reporters, and soft drinks in both all and plausible energy reporters (Fig 2).

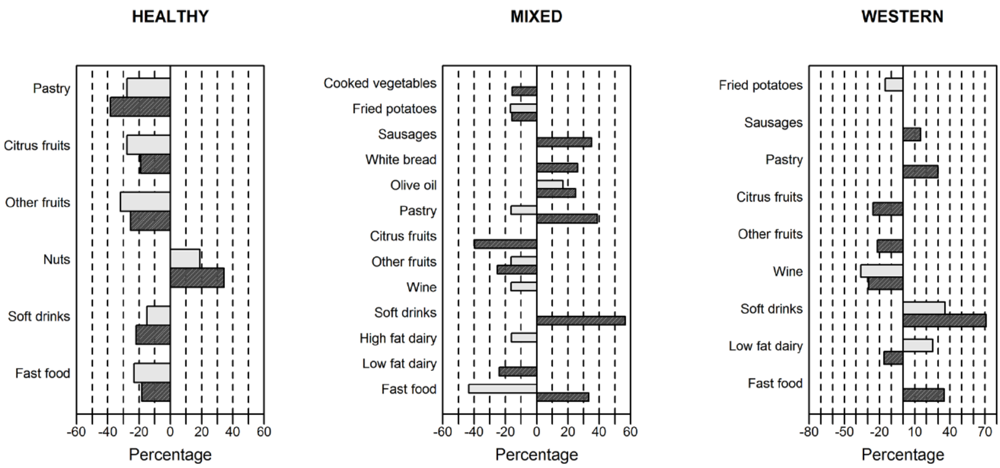


Fig 2. Meaningful* percentage temporal changes in absolute intake of food group consumption in grams of secular trends in all reporters and plausible energy reporters in the 1) "healthy", 2) "mixed" and 3) "western" patterns. Only groups of food are included, which had meaningful changes. The analysis was unadjusted for population differences between the two surveys in age, sex, smoking and physical activity. The unhealthy perceived food groups go first, the healthy food groups are in continuation. A positive value means an increase from the REGICOR 2000 to the REGICOR 2005 and a negative value means a decrease from the REGICOR 2000 to the REGICOR 2005. White—all reporters, black—plausible energy reporters. *≥15% change in food group consumption compared between the REGICOR 2000 and the REGICOR 2005 surveys.

doi:10.1371/journal.pone.0127647.g002

Healthy perceived foods. In the "healthy" cluster all fruits consumption decreased and nuts consumption increased over time (Fig 2). Such food groups of the "mixed" clusters, as cooked vegetables, citrus fruits and low fat dairy decreased only in plausible energy reporters. However, consumption of olive oil increased in both all and plausible energy reporters (Fig 2). In the "western" pattern the direction of meaningful secular trends changed to the opposite direction in low fat dairy, it decreased in plausible energy reporters. Also, fruits and wine consumption decreased in plausible energy reporters only and in both all and plausible energy reporters, respectively (Fig 2).

Pooled analysis. Cluster analysis of the data pooled from both surveys gave similar cluster solutions to the clusters analysed separately in every survey (not shown). Clusters with all participants and only with plausible energy reporters had three-cluster solutions, with "healthy", "mixed" and "western" patterns, similar to the cluster solutions with all participants and plausible energy reporters in each survey. In plausible energy reporters, we defined two sets of three-cluster solutions, which had similar clusters but different proportions. One set had similar proportions between clusters and another set had a very low proportion of participants in the "mixed" cluster (not shown).

Discussion

Using cluster analysis of both cross-sectional surveys, REGICOR 2000 and REGICOR 2005, we identified three dietary patterns: "healthy", "mixed" and "western". Similar dietary patterns were defined in all participants, plausible energy reporters, and energy under-reporters. Energy underreporting affected distribution of the participants between clusters, secular trends of food consumption and amounts of food consumed in the "mixed" and "western" dietary patterns.

Our study is the first to construct dietary patterns for energy under-reporters and analyse them separately from data for all participants and for plausible energy reporters.

The dietary patterns defined in our study population resembled those defined in other populations in Europe and the USA [24–34]. Most of these studies had analogues to our "healthy", "mixed" and "western" patterns regarding sociodemographic, lifestyle and dietary characteristics. The proportion of energy under-reporters in our population (26%-27%) was comparable with some studies [35–37] but lower than others [29,32,38]. The distribution of energy under-reporters among dietary patterns also differed between studies. In several studies, a "healthy" dietary pattern had the highest proportion of energy under-reporters [32,38]. In another study, energy under-reporters among women were distributed evenly [29], but Martikainen et al. [37] reported uneven distribution. In the present study, the gender distribution between different dietary patterns was uneven in all groups of participants. Also, the "mixed" pattern had the highest proportion of energy under-reporters and the highest number of participants, similar to the "convenience" dietary pattern in men reported by Pryer et al. [29]. Therefore, the "mixed" pattern in the REGICOR 2000 survey was most affected by energy under-reporting.

Effect of under-reporting on post-hoc dietary pattern analysis within surveys

Excluding energy under-reporters did not alter the general structure of the dietary patterns. At the same time, excluding energy under-reporters affected socio-demographic and lifestyle characteristics, food consumption, and distribution of participants between patterns. Bailey et al. [35] showed that seven of 25 food groups of dietary patterns changed consistently after excluding energy under-reporters, in the present study we found nine and five groups out of twenty with meaningful changes within the REGICOR 2000 and 2005 surveys respectively. The "healthy" pattern was not affected meaningfully by exclusion of energy under-reporters, probably, due to low prevalence of energy under-reporters in this dietary pattern and similarity of diet quality, according to the mMDS measurement between all participants and energy under-reporters of the "healthy" cluster. The strongest impact the exclusion of energy under-reporters had on the "mixed" pattern, especially in the REGICOR 2000 survey. The dramatic decrease in the proportion of participants in the "mixed" pattern of the REGICOR 2000 after exclusion of energy under-reporters (43.8% vs. 6.40%) underlined the importance of considering energy under-reporters in the analysis of nutritional surveys data and in the construction of dietary patterns. In the "western" cluster just few food groups were affected by excluding energy under-reporters. This difference in effect of energy under-reporters on the dietary patters partially could be due to different prevalence of energy under-reporters in the patterns. In a study using principal component analysis [36], nutrient intakes were slightly higher in the patterns with plausible energy reporters, but the association of nutrients with dietary patterns remained the same. In another study [32], the dietary patterns remained similar after exclusion of energy under-reporters and in one more study [37], 70% of plausible energy reporters fell into the same dietary patterns as in the analysis of all participants. This was comparable with the results obtained in the present study. It is of importance to note that the exclusion of energy under-reporters in the REGICOR 2000 caused meaningful changes both in healthy and unhealthy food groups and in different directions, with slight predominance of decreases in unhealthy food groups in the REGICOR 2000 and increases of those food groups in the REGICOR 2005 surveys. We did not reveal a constant pattern of changes, and we suppose, it was due to strong change in the proportion of participants between patterns after excluding the energy under-reporters in the REGICOR 2000.

Effect of under-reporting on secular trends between surveys

Secular trends, changes occurred between two different samples of the same population in a certain period of time, of dietary patterns found in the present study were stable in sociodemographic and lifestyle characteristics. We found increases only in two variables: physical activity, especially in the "healthy" (19.8%) and "mixed" (11.8%) patterns, and level of education in all patterns. Lifestyle characteristics were more stable than dietary characteristics. Several changes in quantity of food consumption occurred from 2000 to 2005, such as the increase of soft drinks consumption in the "western" pattern (36.0%). This increase paralleled a considerable decline in consumption of wine (35.8%).

Energy under-reporters affected secular trends in food consumption in several food groups mostly in the "mixed", in less proportion in the "western", but not in the "healthy" patterns. Secular trends in the "healthy" pattern maintained the same direction and similar meaningful changes in food consumption both in all and plausible energy reporters (≥15% change in comparison with the REGICOR 2000 survey). Excluding energy under-reporters, the "healthy" pattern kept similar food consumption characteristics as the "healthy" pattern in original data set. An explanation for this finding could be healthy dietary habits of energy under-reporters [35,39,40], similar diet quality between all and energy under-reporters in the "healthy" cluster, according to the mMDS measurement, and low prevalence of energy under-reporters in the "healthy" cluster of the original data. These results confirm the theory that energy under-reporters tend to report healthier dietary habits [35,39,40].The strongest changes in secular trends were found in the "mixed" pattern, what was expected, as the energy under-reporting provoked dramatic change in the percentage of the participants in this dietary pattern in the REGICOR 2000 survey. The changes after excluding energy under-reporters were characterized mainly by increases in consumption of unhealthy food groups and decreases of healthy food groups. In the "western" cluster the effect was the same, but less food groups were affected. This was slightly different from the effect the energy under-reporters had on the "mixed" and "western" patterns within the REGICOR 2000 survey, but similar to the REGICOR 2005. Some food groups in the "mixed" and "western" dietary patterns even had different directions of secular trends between all participants and plausible energy reporters. Since the large proportion of the energy under-reporters were excluded from the "mixed" pattern, the healthy food groups consumption in this pattern decreased and unhealthy food groups increased. However, it is difficult to draw any strong conclusions, as the proportion of the participants in the "mixed" pattern of the REGICOR 2000 survey decreased dramatically. Therefore, secular trends of prevalence of participants within the "mixed" pattern substantially increased and within "healthy" and "western" patterns decreased. These results highlight an impact of energy under-reporting on time trends in nutritional surveys. Energy under-reporting influenced consumption of both healthy and unhealthy food groups in different directions, therefore, it is difficult to predict how under-reporting can influence nutritional survey data analysis. Consequently, public health investigators should pay more careful attention every time they make conclusions without taking in account energy under-reporting.

To the best of our knowledge, only one previous study in an adult population has used the cluster approach to investigate secular trends of dietary patterns [5]. The study was performed in Brazil and two dietary patterns were revealed through surveys. The patterns were stable when analysed for sociodemographic and lifestyle characteristics, and for food consumption. The diet quality index remained constant, although the timeframe for manifestation of greater changes was very short (2007–2009). The increase in soft drink consumption was similar to our findings. Two explored patterns were similar to our "healthy" and "western" patterns, but the distribution of the individuals was uneven between the patterns (86.4–90.5% vs. 9.5–12.5%, respectively). A study from Korea also used the cluster approach in secular trends analysis but it was performed in adolescents [4] The authors defined three analogous dietary patterns and impairment of dietary habits over time. In another study in adolescents, this time in the USA [3], the authors found stable patterns with principal component analysis. The patterns changed only slightly between 1998–1999 and 2003–2004. The only difference of note was the emergence of a new "fast food" pattern in boys; the patterns in girls were almost identical at both time points.

Besides cluster analysis, a priori analysis of dietary patterns has also been used to define secular trends in population dietary habits. Two independent studies in the USA analysed secular trends of dietary patterns using Heart Disease Prevention Eating Index [41] and Revised Diet Quality Index [42]. The timeframes of both surveys were long (20 and 30 years, respectively), reasonably allowing for major changes in dietary habits. Both studies revealed an overall improvement in the diet. Another study analysed secular trends for the traditional diet in Italy [43], using the Mediterranean Adequacy Index. In contrast with the USA studies, the diet of one geographic area of the Italian study sample underwent dramatic changes in all age ranges. The Mediterranean Adequacy Index decreased from 8.2–10.6 in 1967 to 2.9–6.2 in 1999. None of the mentioned studies above took into account energy under-reporting.

To look more carefully at the effect of energy underreporting on the secular trends of food consumption, we performed an additional analysis with data pooled from both surveys. The clusters from the pooled data did not differ from the clusters of the separate surveys. In one cluster solution of the plausible energy reporters, the proportion of the "mixed" cluster was dramatically decreased, as well as in the REGICOR 2000 data. In this manuscript we decided to focus on the effect of the energy under-reporters on secular trends, and pooled analysis is a good additional analysis, but it cannot fully cover the topic. Therefore, we preferred to use separate analysis.

General characteristics of energy under-reporters

To the best of our knowledge the present study is the first to analyse dietary patterns of energy under-reporters separately from plausible energy reporters. Therefore, we briefly discuss the main features of energy under-reporters and dietary patterns associated with energy under-reporting. The characteristics of energy under-reporters in the present study were echoed in the earlier investigations. Bailey et al. [35] demonstrated that energy under-reporters had higher BMI and waist circumference, and lower education than plausible energy reporters, but unlike in our study they smoked more. Additionally, they reported lower lipids consumption, on average 400 kcal less than plausible energy reporters. Similar characteristics were found in another study [32], where energy under-reporters also had higher BMI and lower education. As in the present study, they were older and more active than plausible energy reporters. The reported dietary habits of energy under-reporters differed in comparison with all reporters in all dietary patterns, which highlights the importance of considering energy under-reporters in the analysis of dietary data in nutritional epidemiology.

Three separate dietary patterns were identified in the energy under-reporters. This demonstrates that energy under-reporters also reported different dietary habits, and not always trending toward the consumption of healthier foods. The "western" pattern, known as the least healthy pattern, was identified with a similar proportion of individuals in the dietary patterns of all participants, of plausible energy reporters and of energy under-reporters. It has been shown that healthy dietary patterns are more prevalent in energy under-reporters compared to plausible energy reporters [36,38]. Therefore, we expected the energy under-reporters to report a healthier diet than the plausible energy reporters [35,39,40]. However, energy-adjusted mMDS showed higher diet quality in energy under-reporters than in plausible energy reporters only in the "western" pattern in the REGICOR 2000 survey and in the "mixed" and "western" patterns in the REGICOR 2005 survey. On the other hand, these results are not surprising because energy under-reporters usually report lower amounts of all consumed foods along with higher proportion of intakes from foods perceived as healthy. Therefore, the diet score based on relative amounts, as in case of the mMDS, showed the reasonable values.

A limitation of the present study is the use of the FFQ, which could cause recall bias and provides an approximate amount of the consumed foods using an absolute measurement. Cluster analysis has its own weaknesses, such as arbitrary decisions made during the process of dietary patterns derivation, including number of clusters, type and standardization of variables, formation of food groups, etc. Furthermore, the Goldberg method is an indirect measure of energy misreporting, but is considered a reasonable approach in the face of the impossibility of applying a technique such as doubly labeled water in large-scale epidemiological studies to objectively measure energy underreporting. Assignation of a single PAL of 1.55 was based on the assumption of low activity levels among study participants, which results in a poor sensitivity to detect energy underreporters [44]. Additionally, the Schofield equations have been found to underestimate energy underreporters in obese individuals [45]. Therefore, we used the Mifflin equation to calculate BMR and applied individual PAL values, which improved sensitivity [44]. Finally, the results obtained in this study are population-specific and cannot be compared directly with the results in other populations.

Strengths of our study include the population-based design and the use of validated questionnaires. To our knowledge, no study has reported the results of separate cluster analysis comparing three groups: all participants, plausible energy reporters, and energy under-reporters.

This study contributes to the growing knowledge about the role of energy under-reporting in the analysis of dietary data and, particularly, in the exploration of dietary patterns defined by cluster analysis. In conclusion, energy under-reporting did not affect the structure of dietary patterns derived in 2000 and 2005, but did have an impact on the distribution of participants between dietary patterns and surveys, and influenced secular trends of food consumption and amounts of food consumed in the "mixed" and "western" dietary patterns. Analogous studies in other populations are needed to obtain a deeper understanding of the impact of energy under-reporting on the exploration of dietary data.

Acknowledgments

The authors appreciate the English revision by Elaine Lilly, PhD (Writers First Aid).

This research was supported by a grant (2FD097-0297-CO2-01) from Fondo Europeo de Desarrollo Regional (FEDER); by scholarship for PhD formation from national program of university professors formation, FPU, from Ministry of Education of Spain (AP2010-3198); by portions of grants from Spain's Ministry of Health (Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III: FEDER [PI11/01900], FEDER [CB06/02/0029] and Red Investigación Cardiovascular, Programa HERACLES [RD12/0042]), and by a joint contract (CP03/00115) between the Instituto de Salud Carlos III and the Health Department of the Catalan Government (Generalitat de Catalunya), and AGAUR (2014 SGR 240). The CIBERESP and CIBEROBN are an initiative of the Instituto de Salud Carlos III, Madrid, Spain.

Author Contributions

Conceived and designed the experiments: SFG MF RE AABA HS. Analyzed the data: ANF. Wrote the paper: ANF HS.

References

1. Diet, nutrition and the prevention of chronic diseases: scientific background papers of the joint WHO/FAO expert consultation, Geneva. Public Health Nutr. 2002; 7.
2. Wirfalt AK, Jeffery RW. Using cluster analysis to examine dietary patterns: nutrient intakes, gender, and weight status differ across food pattern clusters. J Am Diet Assoc. 1997; 97: 272–279. PMID: 9060944
3. Cutler GJ, Flood A, Hannan P, Neumark-Sztainer D. Major patterns of dietary intake in adolescents and their stability over time. J Nutr. 2009; 139: 323–328. jn.108.090928 [pii]; doi: 10.3945/jn.108.090928 PMID: 19091799
4. Song Y, Park MJ, Paik HY, Joung H. Secular trends in dietary patterns and obesity-related risk factors in Korean adolescents aged 10–19 years. Int J Obes (Lond). 2010; 34: 48–56. ijo2009203 [pii]; doi: 10.1038/ijo.2009.203 PMID: 19823182
5. Souza AM, Bezerra IN, Cunha DB, Sichieri R. Evaluation of food intake markers in the Brazilian surveillance system for chronic diseases—VIGITEL (2007–2009). Rev Bras Epidemiol. 2011; 14 Suppl 1: 44–52. S1415-790X2011000500005 [pii]. PMID: 22002141
6. Subar AF, Kipnis V, Troiano RP, Midthune D, Schoeller DA, Bingham S, et al. Using intake biomarkers to evaluate the extent of dietary misreporting in a large sample of adults: the OPEN study. Am J Epidemiol. 2003; 158: 1–13. PMID: 12835280
7. Nielsen BM, Nielsen MM, Toubro S, Pedersen O, Astrup A, Sorensen TI,et al. Past and current body size affect validity of reported energy intake among middle-aged Danish men. J Nutr, 2009; 139: 2337–2343. jn.109.112599 [pii]; doi: 10.3945/jn.109.112599 PMID: 19828683
8. Devlin UM, McNulty BA, Nugent AP, Gibney MJ. The use of cluster analysis to derive dietary patterns: methodological considerations, reproducibility, validity and the effect of energy mis-reporting. Proc Nutr Soc 71: 599–609. S0029665112000729 [pii]; doi: 10.1017/S0029665112000729 PMID: 22863306
9. Grau M, Subirana I, Elosua R, Solanas P, Ramos R, Masia R, et al. Trends in cardiovascular risk factor prevalence (1995-2000-2005) in northeastern Spain. Eur J Cardiovasc Prev Rehabil. 2007; 14: 653–659. doi: 10.1097/HJR.0b013e3281764429 00149831-200710000-00009 [pii]. PMID: 17925624
10. Willett W. Nutritional epidemiology. Oxford University Press, New York. 1998.
11. Mullaney L, O'Higgins AC, Cawley S, Doolan A, McCartney D, Turner MJ. An estimation of periconceptional under-reporting of dietary energy intake. J Public Health (Oxf). 2014. fdu086 [pii]; doi: 10.1093/pubmed/fdu086
12. Azizi F, Esmaillzadeh A, Mirmiran P. Correlates of under- and over-reporting of energy intake in Tehranians: body mass index and lifestyle-related factors. Asia Pac J Clin Nutr. 2005; 14: 54–59. PMID: 15734708
13. Livingstone MB, Robson PJ, Black AE, Coward WA, Wallace JM, McKinley MC, et al. An evaluation of the sensitivity and specificity of energy expenditure measured by heart rate and the Goldberg cut-off for energy intake: basal metabolic rate for identifying mis-reporting of energy intake by adults and children: a retrospective analysis. Eur J Clin Nutr. 2003; 57: 455–463. doi: 10.1038/sj.ejcn.1601563 1601563 [pii]. PMID: 12627183
14. Schroder H, Covas MI, Marrugat J, Vila J, Pena A, Alcantara M, et al. Use of a three-day estimated food record, a 72-hour recall and a food-frequency questionnaire for dietary assessment in a Mediterranean Spanish population. Clin Nutr. 2001; 20: 429–437. doi: 10.1054/clnu.2001.0460 S0261-5614(01) 90460-1 [pii]. PMID: 11534938
15. Benitez-Arciniega AA, Mendez MA, Baena-Diez JM, Rovira Martori MA, Soler C, Marrugat J, et al. Concurrent and construct validity of Mediterranean diet scores as assessed by an FFQ. Public Health Nutr. 2011; 14: 2015–2021. S1368980011001212 [pii]; doi: 10.1017/S1368980011001212 PMID: 21740628
16. Schroder H, Marrugat J, Vila J, Covas MI, Elosua R. Adherence to the traditional mediterranean diet is inversely associated with body mass index and obesity in a spanish population. J Nutr. 2004; 134: 3355–3361. 134/12/3355 [pii]. PMID: 15570037
17. Mendez MA, Popkin BM, Buckland G, Schroder H, Amiano P, Barricarte A, et al. Alternative methods of accounting for underreporting and overreporting when measuring dietary intake-obesity relations. Am J Epidemiol. 2011; 173: 448–458. kwq380 [pii]; doi: 10.1093/aje/kwq380 PMID: 21242302
18. Goldberg GR, Black AE, Jebb SA, Cole TJ, Murgatroyd PR, Coward WA, et al. Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording. Eur J Clin Nutr. 1991; 45: 569–581. PMID: 1810719
19. Mifflin MD, St Jeor ST, Hill LA, Scott BJ, Daugherty SA, Koh YO. A new predictive equation for resting energy expenditure in healthy individuals. Am J Clin Nutr. 1990; 51: 241–247. PMID: 2305711

20. Black AE. Critical evaluation of energy intake using the Goldberg cut-off for energy intake:basal metabolic rate. A practical guide to its calculation, use and limitations. Int J Obes Relat Metab Disord. 2000; 24: 1119–1130. PMID: 11033980

21. Elosua R, Marrugat J, Molina L, Pons S, Pujol E. Validation of the Minnesota Leisure Time Physical Activity Questionnaire in Spanish men. The MARATHOM Investigators. Am J Epidemiol. 1994; 139: 1197–1209. PMID: 8209878

22. Elosua R, Garcia M, Aguilar A, Molina L, Covas MI, Marrugat J. Validation of the Minnesota Leisure Time Physical Activity Questionnaire In Spanish Women. Investigators of the MARATDON Group. Med Sci Sports Exerc. 2000; 32: 1431–1437. PMID: 10949009

23. Ammerman AS, Lindquist CH, Lohr KN, Hersey J. The efficacy of behavioral interventions to modify dietary fat and fruit and vegetable intake: a review of the evidence. Prev Med. 2002; 35: 25–41. S0091743502910285 [pii]. PMID: 12079438

24. Berg CM, Lappas G, Strandhagen E, Wolk A, Toren K, Rosengren A, et al. Food patterns and cardiovascular disease risk factors: the Swedish INTERGENE research program. Am J Clin Nutr. 2008; 88: 289–297. 88/2/289 [pii]. PMID: 18689363

25. Engeset D, Alsaker E, Ciampi A, Lund E. Dietary patterns and lifestyle factors in the Norwegian EPIC cohort: the Norwegian Women and Cancer (NOWAC) study. Eur J Clin Nutr. 2005; 59: 675–684. 1602129 [pii]; doi: 10.1038/sj.ejcn.1602129 PMID: 15785773

26. Hearty AP, Gibney MJ. Comparison of cluster and principal component analysis techniques to derive dietary patterns in Irish adults. Br J Nutr. 2009; 101: 598–608. S0007114508014128 [pii]; doi: 10.1017/S0007114508014128 PMID: 18577300

27. Liu E, McKeown NM, Newby PK, Meigs JB, Vasan RS, Quatromoni PA, et al. Cross-sectional association of dietary patterns with insulin-resistant phenotypes among adults without diabetes in the Framingham Offspring Study. Br J Nutr. 2009; 102: 576–583. S0007114509220836 [pii]; doi: 10.1017/S0007114509220836 PMID: 19216828

28. Oliveira A, Rodriguez-Artalejo F, Gaio R, Santos AC, Ramos E, Lopes C. Major habitual dietary patterns are associated with acute myocardial infarction and cardiovascular risk markers in a southern European population. J Am Diet Assoc. 2001; 111: 241–250. S0002-8223(10)01823-7 [pii]; doi: 10.1016/j.jada.2010.10.042

29. Pryer JA, Nichols R, Elliott P, Thakrar B, Brunner E, Marmot M. Dietary patterns among a national random sample of British adults. J Epidemiol Community Health. 2001; 55: 29–37. PMID: 11112948

30. Quatromoni PA, Copenhafer DL, Demissie S, D'Agostino RB, O'Horo CE, Nam BH, et al. The internal validity of a dietary pattern analysis. The Framingham Nutrition Studies. J Epidemiol Community Health. 2002; 56: 381–388. PMID: 11964437

31. Villegas R, Salim A, Collins MM, Flynn A, Perry IJ. Dietary patterns in middle-aged Irish men and women defined by cluster analysis. Public Health Nutr. 2004; 7: 1017–1024. doi: 10.1079/PHN2004638 S1368980004001314 [pii]. PMID: 15548339

32. Winkvist A, Hornell A, Hallmans G, Lindahl B, Weinehall L, Johansson I. More distinct food intake patterns among women than men in northern Sweden: a population-based survey. Nutr J. 2009; 8: 12. 1475-2891-8-12 [pii]; doi: 10.1186/1475-2891-8-12 PMID: 19228378

33. Wirfalt E, Mattisson I, Gullberg B, Berglund G. Food patterns defined by cluster analysis and their utility as dietary exposure variables: a report from the Malmo Diet and Cancer Study. Public Health Nutr. 2000; 3: 159–173. PMID: 10948383

34. Wirfalt E, Hedblad B, Gullberg B, Mattisson I, Andren C, Rosander U, et al. Food patterns and components of the metabolic syndrome in men and women: a cross-sectional study within the Malmo Diet and Cancer cohort. Am J Epidemiol. 2001; 154: 1150–1159. PMID: 11744521

35. Bailey RL, Mitchell DC, Miller C, Smiciklas-Wright H. Assessing the effect of underreporting energy intake on dietary patterns and weight status. J Am Diet Assoc. 2007; 107: 64–71. S0002-8223(06)02283-8 [pii]; doi: 10.1016/j.jada.2006.10.009 PMID: 17197273

36. Krebs-Smith SM, Cleveland LE, Ballard-Barbash R, Cook DA, Kahle LL. Characterizing food intake patterns of American adults. Am J Clin Nutr. 1997; 65: 1264S–1268S. PMID: 9094931

37. Martikainen P, Brunner E, Marmot M. Socioeconomic differences in dietary patterns among middle-aged men and women. Soc Sci Med. 2003; 56: 1397–1410. S0277953602001375 [pii]. PMID: 12614692

38. Scagliusi FB, Ferriolli E, Pfrimer K, Laureano C, Cunha CS, Gualano B, et al. Under-reporting of energy intake is more prevalent in a healthy dietary pattern cluster. Br J Nutr. 2008; 100: 1060–1068. S0007114508971300 [pii]; doi: 10.1017/S0007114508971300 PMID: 18377688

39. Johnson RK, Goran MI, Poehlman ET. Correlates of over- and underreporting of energy intake in healthy older men and women. Am J Clin Nutr. 1994; 59: 1286–1290. PMID: 8198052

40. Lutomski JE, van den Broeck J, Harrington J, Shiely F, Perry IJ. Sociodemographic, lifestyle, mental health and dietary factors associated with direction of misreporting of energy intake. Public Health Nutr. 2011; 14: 532–541. S1368980010001801 [pii]; doi: 10.1017/S1368980010001801 PMID: 20707944

41. Lee S, Harnack L, Jacobs DR Jr., Steffen LM, Luepker RV, Arnett DK. Trends in diet quality for coronary heart disease prevention between 1980–1982 and 2000–2002: The Minnesota Heart Survey. J Am Diet Assoc. 2007; 107: 213–222. S0002-8223(06)02491-6 [pii]; doi: 10.1016/j.jada.2006.11.003 PMID: 17258957

42. Popkin BM, Zizza C, Siega-Riz AM. Who is leading the change?. U.S. dietary quality comparison between 1965 and 1996. Am J Prev Med. 2003; 25: 1–8. S0749379703000990 [pii]. PMID: 12818303

43. Alberti-Fidanza A, Fidanza F, Chiuchiu MP, Verducci G, Fruttini D. Dietary studies on two rural italian population groups of the Seven Countries Study. 3. Trend Of food and nutrient intake from 1960 to 1991. Eur J Clin Nutr. 1999; 53: 854–860. PMID: 10556997

44. Black AE. The sensitivity and specificity of the Goldberg cut-off for EI:BMR for identifying diet reports of poor validity. Eur J Clin Nutr. 2000; 54: 395–404. PMID: 10822286

45. Horgan GW, Stubbs J. Predicting basal metabolic rate in the obese is difficult. Eur J Clin Nutr. 2003; 57: 335–340. PMID: 12571669

4th paper

Soft drink consumption is positively associated with increased waist circumference and 10-year incidence of abdominal obesity in Spanish adults. Journal of Nutrition 2015; 145(2): 328-4.

The Journal of Nutrition. First published ahead of print December 17, 2014 as doi: 10.3945/jn.114.205229.

The Journal of Nutrition
Nutritional Epidemiology

Soft Drink Consumption Is Positively Associated with Increased Waist Circumference and 10-Year Incidence of Abdominal Obesity in Spanish Adults[1–3]

Anna N Funtikova,[4,6,8] Isaac Subirana,[5,6] Santiago F Gomez,[4,9] Montserrat Fitó,[4,7] Roberto Elosua,[5] Alejandra A Benítez-Arciniega,[10] and Helmut Schröder [4,6]*

[4]Cardiovascular Risk and Nutrition Research Group and [5]Cardiovascular Epidemiology and Genetics, Research Program in Epidemiology and Public Health, Hospital del Mar Research Institute, Barcelona, Spain; [6]CIBER Epidemiology and Public Health (CIBERESP) and [7]CIBER Physiopathology of Obesity and Nutrition (CIBEROBN), Carlos III Health Institute, Madrid, Spain; [8]Food and Nutrition PhD program, University of Barcelona, Barcelona, Spain; [9]Thao Foundation, Barcelona, Spain; and [10]School of Medicine, Autonomous University of Mexico State, Toluca, Mexico

Abstract

Background: The accumulation of abdominal fat increases risk of metabolic disorders and premature death. There is a dearth of prospective data on the association between caloric beverage consumption and surrogate markers of abdominal adiposity.
Objective: The aim of this study was to assess the relation between consumption of nonalcoholic caloric beverages, including soft drinks, fruit juice, whole milk, and skim and low-fat milk, and changes in waist circumference (WC) and odds of 10-y incidence of abdominal obesity.
Methods: We conducted a prospective, population-based study of 2181 Spanish men and women aged 25–74 y who were followed from 2000 to 2009. We measured weight, height, and WC, and recorded data on diet and leisure-time physical activity (LTPA) with the use of validated questionnaires. We fit multivariable linear and logistic regression models.
Results: A 100 kcal increase in soft drink consumption was associated with a 1.1 cm increase in WC ($P = 0.018$) after 10 y of follow-up. Substitution of 100 kcal of soft drinks with 100 kcal of whole milk or 100 kcal of juice was associated with a 1.3 cm (95% CI: 0.3, 2.4) and 1.1 cm (95% CI: 0.03, 2.2) decrease in WC, respectively. Increasing consumption of soft drinks from baseline to follow-up led to WC gain compared with maintaining nonconsumption. Greater soft drink consumption was positively associated ($P = 0.029$) with increased odds of 10-y incidence of abdominal obesity.
Conclusion: Adults' consumption of soft drinks was associated with increased WC and odds of 10-y incidence of abdominal obesity. This association was moderate but consistent in all statistical models. *J Nutr* doi: 10.3945/jn.114.205229.

Keywords: abdominal obesity, Mediterranean diet, soft drinks, beverages, diet quality, waist circumference

Introduction

In the past few decades, waist circumference (WC)[11], a surrogate marker for abdominal adiposity, has tended to increase in many populations (1–3). Furthermore, there is evidence that abdominal adiposity increases proportionally more than general adiposity, as measured by BMI (3–5). This is an important concern for health policy because abdominal adiposity is a strong predictor of metabolic disorders (6, 7) and cardiovascular events (8). Therefore, it is essential to identify predictors of change in abdominal adiposity.

Consumption of caloric beverages represents an important portion of total energy intake, and that of soft drinks has received particular attention during the past decade (9). Whereas the consumption of fruit juices and milk or milk products in Spain has decreased (10, 11), soft drink consumption has increased (12), and Spain now has the highest level of consumption per capita of all southern European countries. Although evidence from prospective studies and randomized controlled trails indicates that soft drink consumption promotes

[1]HS was funded by a contract from the Catalan Government and the Carlos III Health Institute European Fund for Regional Development (FEDER), (FIS CES09/030). This work was supported by grants from the Carlos III Health Institute FEDER (CB06/02/0029) (PI11/01900), and the Catalonian Agency for the Administration of University and Research Grants (2014 SGR 240). CIBERESP and CIBEROBN are an initiative of the Carlos III Health Institute, Madrid, Spain.
[2]Author disclosures: AN Funtikova, I Subirana, SF Gomez, M Fitó, R Elosua, AA Benítez-Arciniega, and H Schröder, no conflicts of interest.
[3]Supplemental Tables 1–3 and Supplemental Figure 1 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
[11]Abbreviations used: BMR, basal metabolic rate; LTPA, leisure-time physical activity; mMDS, modified Mediterranean diet score; PAL, physical activity level; rEI, reported energy intake; WC, waist circumference.
* To whom correspondence should be addressed: E-mail: hschroeder@imim.es.

Manuscript received October 7, 2014. Initial review completed October 23, 2014. Revision accepted November 24, 2014.
doi: 10.3945/jn.114.205229.

TABLE 1 Demographic and lifestyle characteristics of Spanish adults[1]

Variables	Soft drinks	Whole milk	Skim and low-fat milk	Juice
Women				
No consumption[2]	55.6	56.3	44.7	53.6
Weekly[3]	51.3	40.1	47.6	49.9
Daily[4]	37.6	48.7	66.8	59.1
P-trend	<0.001	<0.001	<0.001	0.014
Age, y				
No consumption[2]	53.6 (52.9, 54.2)	50.6 (49.9, 51.2)	48.2 (47.5, 49)	54.0 (53.1, 54.8)
Weekly[3]	43.4 (42.6, 44.3)	44.3 (42.9, 45.8)	46.6 (45.0, 48.2)	45.8 (45.1, 46.6)
Daily[4]	43.1 (41.3, 44.9)	47.7 (46.5, 48.9)	51.5 (50.6, 52.4)	47.8 (46.4, 49.2)
P-trend	<0.001	<0.001	<0.001	<0.001
Higher education[5]				
No consumption[2]	29.0	35.5	37.3	24.5
Weekly[3]	48.0	45.5	44.3	45.0
Daily[4]	41.8	35.0	33.2	40.9
P-trend	<0.001	0.004	0.006	<0.001
Current smokers[6]				
No consumption[2]	20.9	21.6	30.0	22.5
Weekly[3]	28.4	34.8	28.5	28.3
Daily[4]	49.4	34.0	18.5	26.6
P-trend	<0.001	<0.001	<0.001	0.019
LTPA, METs · min/d				
No consumption[2]	210 (98.6, 367)	201 (100, 342)	193 (97.3, 348)	201 (91.2, 367)
Weekly[3]	194 (102, 338)	208 (121, 392)	210 (108, 340)	191 (103, 333)
Daily[4]	158 (91.4, 291)	187 (92.6, 355)	203 (103, 349)	221 (113, 367)
P-trend	0.026	0.21	0.56	0.044
mMDS				
No consumption[2]	18.1 (17.9, 18.3)	17.5 (17.3, 17.6)	17.5 (17.3, 17.6)	17.0 (16.7, 17.2)
Weekly[3]	17.3 (17.1, 17.6)	17.7 (17.3, 18.1)	17.9 (17.5, 18.3)	17.6 (17.4, 17.8)
Daily[4]	16.2 (15.8, 16.4)	17.3 (17.0, 17.6)	17.3 (17.0, 17.6)	18.1 (17.8, 18.5)
P-trend	<0.001	0.21	0.30	<0.001
WC, cm				
No consumption[2]	90.6 (89.8, 91.4)	90.1 (89.4, 90.8)	89.7 (88.9, 90.5)	92.3 (91.3, 93.3)
Weekly[3]	88.0 (87.0, 89.0)	89.2 (87.6, 90.9)	89.8 (88.0, 91.5)	88.1 (87.3, 89.0)
Daily[4]	89.9 (87.8, 92.0)	88.2 (86.9, 89.5)	89.4 (88.3, 90.4)	87.4 (85.9, 89.0)
P-trend	0.56	0.013	0.58	<0.001
Abdominal obesity				
No consumption[2]	33.8	33.0	26.9	39.2
Weekly[3]	24.8	24.7	26.8	24.1
Daily[4]	25.3	23.5	35.8	25.3
P-trend	0.022	<0.001	<0.001	<0.001
Energy, MJ				
No consumption[2]	10.7 (10.4, 10.9)	10.7 (10.5, 10.9)	11.2 (10.9, 11.4)	10.1 (9.8, 10.4)
Weekly[3]	11.6 (11.3, 11.9)	11.8 (11.3, 12.3)	11.0 (10.5, 11.6)	11.5 (11.3, 11.8)
Daily[4]	12.9 (12.3, 13.6)	12.3 (11.9, 12.7)	11.2 (10.9, 11.5)	12.6 (12.1, 13.1)
P-trend	<0.001	<0.001	0.75	<0.001
Implausible energy intake reported				
No consumption[2]	37.3	36.0	38.0	41.6
Weekly[3]	32.7	33.3	31.3	30.7
Daily[4]	34.5	34.5	32.5	34.4
P-trend	0.20	0.58	0.016	0.025

[1] Values are means (95% CIs) for continues variables (age, mMDS, WC, and energy), medians (25th, 75th percentiles) for LTPA, or percentages. Reference: no consumption (soft drinks, *n* = 1202; whole milk, *n* = 1403; low-fat milk, *n* = 1136; and juice, *n* = 795). >0 and <200mL: no consumption (soft drinks, *n* = 739; whole milk, *n* = 279; low-fat milk, *n* = 246; and juice, *n* = 108). ≥200mL: no consumption (soft drinks, *n* = 170; whole milk, *n* = 428; low-fat milk, *n* = 729; and juice, *n* = 308). LTPA, leisure-time physical activity; MET, metabolic equivalent task; mMDS, modified Mediterranean diet score; WC, waist circumference.
[2] 0 mL/d.
[3] <1 serving (200mL)/d.
[4] ≥1 serving (200mL)/d.
[5] More than primary school education.
[6] Active smokers or ex-smokers <1 y.

weight gain (13, 14), its impact on abdominal adiposity remains unclear (15, 16). Moreover, prospective studies on the impact of milk and fruit juice consumption are also scarce and inconclusive (15–19). Therefore, this study sought to examine the association between consumption of caloric beverages (whole, skim, and low-fat milk, 100% juice, and soft drinks) and changes in WC, and to explore the effect of changes in consumption as a predictor of WC changes in a population-based sample of Spanish adults. Additionally, we determined the odds of 10-y incidence of abdominal obesity defined by WC cut-offs, according to caloric beverage consumption and changes in consumption patterns.

Methods

Study population. Data were obtained from 2 population-based surveys conducted in Girona, Spain, in 2000 and 2009. The baseline survey in 2000 examined a randomly selected population-based sample of 3058 men and women aged 25–74 y (participation rate: 71.0%).

In 2009, the 2715 noninstitutionalized participants still residing in the catchment area were invited for re-examination, and 2181 attended (follow-up participation: 80.3%). The final sample size was 2112 after excluding 37 and 32 participants with missing data for WC and smoking status, respectively. Participants gave signed informed consent to participate in the study, and the project was approved by the local clinical research ethics committee (Hospital del Mar Clinical Investigations Ethics Committee, Barcelona, Spain).

Anthropometrics. WC was measured midway between the lowest rib and the iliac crest, with the participant lying horizontally, and the measurement was rounded to the nearest 0.5 cm. Abdominal obesity was defined by sex-specific cut-offs: >102 cm for men and >88 cm for women (20). All anthropometric measurements were taken through use of the same protocol at both baseline and follow-up.

Dietary assessment. Energy intake and food consumption was determined with the use of a validated FFQ, administered by a trained interviewer at baseline and at follow-up (21, 22). In a 166-item food list including alcoholic and nonalcoholic beverages, participants indicated their usual consumption and chose from 10 frequency categories ranging from "never or less than once per month" to "6 or more times per day." Individual caloric beverages were aggregated into 4 groups: *1*) soft drinks, including carbonated sugar-sweetened beverages (1 item in the FFQ); 2) whole milk (4% fat; 1 item in the FFQ); *3*) skim and low-fat milk (2 items in the FFQ); and *4*) 100% juices, including commercial and natural fruit and vegetable juices (5 items in the FFQ: apple, peach, orange, grape, and tomato).

Adherence to the Mediterranean diet was evaluated with the use of the validated modified Mediterranean Diet Score (mMDS) (21). Briefly, we calculated the tertile distribution of food intake for each sex, coding the bottom, middle, and top tertiles as 1, 2, and 3, respectively, for cereals, fruits, vegetables, legumes, fish, olive oil, and nuts. For meat (including red meat, poultry, and sausages) and dairy products, this score was inverted, with the top tertile coded as 1 and bottom as 3. Moderate red wine consumption (≤20 g/d) was coded as 3, and more or less than this daily portion was coded as 1. The MDS was then calculated as the sum of all of these dietary components, with a range of 10–30.

Energy misreporting. Individuals with implausible reported energy intake (rEI) were identified by the revised Goldberg method, as described previously (23). The basal metabolic rate (BMR) was estimated with the use of the Mifflin equation (24). The ratio between rEI and BMR was calculated. The plausibility of rEI was estimated by comparing the ratio of rEI to BMR (rEI:BMR) with physical activity levels (PALs). The cut-off values to identify plausible reported energy intake were taken as the confidence limits of agreement between rEI:BMR and PAL. These cut-off values were created on the basis of the coefficient of variation of subjects' energy intake, the accuracy of the measurement of their BMR, and the total variation in PAL, as proposed by Black et al. (25).

Other variables. The validated Minnesota leisure-time physical activity (LTPA) questionnaire (26, 27) was administered by a trained interviewer. Data on smoking and demographic and socioeconomic variables were obtained from structured standard questionnaires administered by trained personnel. Participants were dichotomized into nonsmokers (never smokers and nonsmokers for >1 y) and current smokers. Maximum education level attained was dichotomized in "primary school" and "secondary school or university."

TABLE 2 Linear regression analysis of association between changes in WC and a 100 kcal increase in beverage consumption at baseline and changes in WC and a 100 kcal substitution of soft drinks with 100 kcal of juice and whole, skim, and low-fat milk in Spanish adults[1]

	Model 1[2]			Model 2[3]		
Variables	β	95% CI	P	β	95% CI	P
Separate analysis						
Soft drinks	1.03	(0.11, 1.95)	0.028	1.11	(0.19, 0.03)	0.018
Whole milk	−0.34	(−0.78, 0.11)	0.14	−0.26	(−0.72, 0.19)	0.29
Skim and low-fat milk	0.23	(−0.23, 0.76)	0.30	0.30	(−0.22, 0.80)	0.26
Juice	−0.23	(−0.94, 0.47)	0.52	−0.03	(−0.68, 0.74)	0.93
Mutually adjusted model						
Soft drinks	1.06	(0.14, 1.98)	0.024	1.10	(0.18, 2.03)	0.018
Whole milk	−0.32	(−0.77, 0.11)	0.18	−0.19	(−0.66, 0.28)	0.42
Skim and Low-fat milk	0.15	(−0.39, 0.69)	0.58	0.25	(−0.30, 0.79)	0.37
Juice	−0.31	(−1.01, 0.40)	0.39	−0.03	(−0.74, 0.68)	0.93
Substitution						
Whole milk	−1.37	(−2.39, −0.35)		−1.34	(−2.36, −0.31)	
Skim and low-fat milk	−0.80	(−1.86, 0.26)		−0.73	(−1.76, 0.29)	
Juice	−1.33	(−2.49, −0.17)		−1.12	(−2.21, −0.03)	

[1] β coefficients reflect changes in WCs per 100 kcal increase of beverage consumption. LTPA, leisure-time physical activity; mMDS, modified Mediterranean diet score; WC, waist circumference.

[2] Adjusted for sex (men/women, dichotomous), age (years, continuous), and baseline WC (centimeters; continuous).

[3] Includes additionally smoking (yes/no, dichotomous), energy intake (kilocalories, continuous), smoking (yes/no, dichotomous), educational level [more than primary school (yes/no), dichotomous], LTPA (metabolic equivalent tasks times minutes per day, continuous), mMDS (continuous), and energy under- and over-reporting (yes/no, dichotomous).

Statistical analysis. General linear modeling procedures were used to compare the baseline characteristics of participants for each beverage consumption category. We used ANOVA tests and polynomial contrasts to obtain overall *P*-values and *P*-values for linear trend for normally distributed continuous variables, respectively, and the Kruskal-Wallis test to obtain overall *P*-values for non-normally distributed variables. We used the Pearson chi-square and Mantel-Haenszel linear-by-linear association chi-square test to obtain overall *P*-values and *P*-values for linear trend for categoric variables, respectively.

Linear regression models were fitted to analyze the association between changes in WC and *1*) baseline consumption of soft drinks, juice, and whole milk, and skim and low-fat milk, and *2*) changes in consumption of these beverages. WC change (centimeters; continuous) was the dependent variable and beverage consumption was the explanatory variable. Two models were fitted. The first included 3 variables: sex (men/women, dichotomous), age (years, continuous), and baseline WC. The second added 7 additional variables: smoking (yes/no, dichotomous), energy intake (kilocalories, continuous), dieting (yes/no, dichotomous), educational level [more than primary school education (yes/no), dichotomous], LTPA (metabolic equivalent tasks times minutes per day, continuous) and under- and over-reporting of energy intake (yes/no, dichotomous). In the first linear regression analysis, beverages were included separately and mutually in both models. In the second linear regression analysis, 3 categories of beverage consumption were included as dummy variables: "maintained or shifted to no consumption," "maintained or shifted to weekly consumption of 1 serving," and "consumed at least 1 serving per day." Results are reported as regression coefficients with 95% CIs.

Cubic spline analysis was performed to investigate nonlinear associations between changes in WC and consumption of soft drinks, juice, whole milk, and skim, and low-fat milk, with the use of the "gam" package in R version 3.0.2. The assumption of normality in the regression models was assessed with the use of the normal probability plot.

Substitution models were fitted to analyze the effect of replacing soft drinks with juice, whole milk, or low-fat milk. For this purpose, soft drinks were included simultaneously with juice, whole milk, or skim and low-fat milk in multivariable linear regression models. We converted the consumption of beverages expressed in volume (milliliters) to their corresponding energy content (kilocalories). The difference in the coefficients from the substitution models was used to estimate the effect of substituting 100 kcal of soft drinks with 100 kcal of juice, whole milk, or skim and low-fat milk.

Participants with abdominal obesity at baseline were excluded from the logistic regression models assessing the association between beverage consumption and odds of 10-y incidence of abdominal obesity. Baseline levels of beverage consumption were categorized as no consumption, <1 serving/d, and ≥1 serving/d. Results are reported as ORs with 95% CIs.

Additionally, ORs were translated to RRs according to Zhang (28)

$$RR = \frac{\mathrm{OR}}{(1-Pref) + (Pref \times OR)}$$

where OR is the adjusted OR of the multivariable logistic regression of the association between beverage consumption and incidence of abdominal obesity, and *Pref* is the prevalence of abdominal obesity in the reference group.

TABLE 3 Linear regression analysis of association between changes in WC and changes in beverage consumption in Spanish adults[1]

		Model 1[2]			Model 2[3]		
Variables	*n*	β	95% CI	*P*	β	95% CI	*P*
Soft drinks							
No consumption	915	Reference			Reference		
Decrease in consumption[4]	388	0.32	(−0.78, 1.41)	0.57	0.49	(−0.61, 1.58)	0.38
Increase in consumption[5]	334	1.58	(0.44, 2.73)	0.007	1.50	(0.36, 2.64)	0.010
Maintained consumption[6]	475	1.09	(−0.01, 2.19)	0.05	1.22	(0.13, 2.31)	0.029
Whole milk							
No consumption	1232	Reference			Reference		
Decrease in consumption[4]	469	−0.15	(−1.12, 0.82)	0.76	−0.03	(−1.00, 0.94)	0.95
Increase in consumption[5]	219	−0.38	(−1.68, 0.92)	0.57	−0.26	(−1.56, 1.04)	0.69
Maintained consumption[6]	195	−0.93	(−2.29, 0.43)	0.18	−0.68	(−2.05, 0.70)	0.33
Skim and low-fat milk	n						
No consumption	778	Reference			Reference		
Decrease in consumption[4]	405	0.35	(−0.73, 1.43)	0.52	0.39	(−0.68, 1.47)	0.47
Increase in consumption[5]	445	0.43	(−0.61, 1.48)	0.42	0.40	(−0.64, 1.43)	0.45
Maintained consumption[6]	484	0.30	(−0.75, 1.34)	0.58	0.31	(−0.73, 1.35)	0.56
Juices							
No consumption	479	Reference			Reference		
Decrease in consumption[4]	500	0.00	(−0.91, 0.92)	0.99	0.25	(−0.67, 1.17)	0.59
Increase in consumption[5]	429	0.12	(−0.86, 1.10)	0.80	0.25	(−0.73, 1.22)	0.62
Maintained consumption[6]	697	−0.44	(−2.53, 1.65)	0.68	0.15	(−1.93, 2.24)	0.89

[1] β coefficients reflect changes in WCs. LTPA, leisure-time physical activity; mMDS, modified Mediterranean diet score; WC, waist circumference.

[2] Results of multivariable linear regression adjusted for sex (men/women, dichotomous), age (years, continuous), and baseline WC (centimeters; continuous).

[3] Results adjusted for variables in model 1, as well as smoking (yes/no, dichotomous), energy intake (kilocalories, continuous), dieting (yes/no, dichotomous), educational level [more than primary school (yes/no), dichotomous], LTPA (metabolic equivalent tasks times minutes per day, continuous), mMDS (continuous), and energy under- and over-reporting (yes/no, dichotomous).

[4] Decrease from at least 1 serving/d at baseline to <1 serving/d or no consumption at follow-up and <1 serving/d at baseline or no consumption at follow-up).

[5] Increase from no consumption at baseline to <1 serving/d or ≥1 serving/d at follow-up and from <1 serving/d at baseline to ≥1 serving/d at follow-up.

[6] <1 serving/d or ≥1 serving/d at baseline and follow-up.

To explore how the relation between WC and beverage consumption may be modulated by energy underreporting and sex, we modeled the interaction between these variables and each beverage. Differences were considered statistically significant if $P < 0.05$. Statistical analysis was performed with the use of SPSS version 18.0 (SPSS).

Results

The mean ± SD daily intake of soft drinks, whole, skim, and low-fat milk, and juice at baseline was 42 ± 109 mL, 63 ± 131 mL, 115 ± 178 mL, and 64 ± 114 mL, respectively. Consumption of whole milk (48%), juice (14%), and soft drinks (11%) decreased significantly during follow-up; consumption of skim and low-fat milk remained stable. At 10-y follow-up, the prevalence of general (BMI ≥ 30) and abdominal obesity increased from 23.6% to 24.2% and from 30.0% to 41.4%, respectively. One-third (35.3%) of participants were classified as having implausible energy intakes (11.8% energy over-reported and 23.5% energy under-reported). However, energy misreporting did not markedly affect the relation between exposure and outcome (**Tables 1–4**).

No significant interactions were observed for sex and soft drink ($P = 0.70$), sex and juice ($P = 0.96$), sex and whole milk ($P = 0.58$), sex and skim and low-fat milk ($P = 0.99$), energy under-reporting and soft drink ($P = 0.23$), energy under-reporting and juice ($P = 0.40$), energy under-reporting and whole milk ($P = 0.68$), and energy under-reporting and skim and low-fat milk ($P = 0.47$). The main findings were similar for men and women (**Supplemental Tables 1–3**).

As soft drink consumption increased (from none to daily), age, LTPA, adherence to the mMDS, the percentage of women with a prevalence of abdominal obesity decreased, whereas smoking and educational level increased (Table 1). These trends were similar for the categories of age, sex, abdominal obesity, smoking, and whole milk consumption. In addition, WC decreased with an increase in whole milk consumption. Women and individuals with plausible energy intake generally consumed more skim and low-fat milk and juice, and had lower rates of abdominal obesity and smoking. Additionally, LTPA and adherence to the mMDS increased and WC decreased with greater consumption of juice. Energy intake increased with the amount of beverage consumption, except in the case of skim and low-fat milk.

In the linear regression analysis, consumption of soft drinks was significantly associated with WC change, both for each beverage separately, and in mutually adjusted models ($P = 0.018$ and $P = 0.018$, respectively, in fully adjusted models) (Table 2). A 100 kcal increase in soft drink consumption predicts a 1.1 cm increase in WC after 10 y of follow-up in both models. All models were consistent with the assumption of normality. Sensitivity analysis, excluding nonconsumers in each beverage category, revealed similar results per 100 kcal increment (soft drinks $\beta = 0.969$, $P = 0.028$; whole milk $\beta = -0.371$, $P = 0.23$; skim and low-fat milk $\beta = 0.436$, $P = 0.22$; juice $\beta = -0.003$, $P = 0.99$).

We observed no evidence of nonlinearity in the associations ($P_{curvature}$-values for soft drinks, whole milk, skim and low-fat milk, and juice were 0.83, 0.80, 0.42, and 0.25, respectively (**Supplemental Figure 1**).

Substitution models revealed a 1.3 cm and 1.1 cm decrease in WC when 100 kcal of soft drinks were replaced by 100 kcal of whole milk and juice, respectively (Table 2).

We were also interested in determining whether changes in time trends of beverage consumption affect changes in WC. A 1.5 cm and 1.2 cm increase in WC was associated with increased and maintained consumption of soft drinks, respectively, compared with maintaining no consumption (Table 3).

Logistic regression models adjusted for sex, age, energy intake, dieting, LTPA, education, smoking, diet quality (measured by mMDS adherence), and over- and under-reporting of energy intake revealed an increased odds of 10-y incidence of abdominal obesity (336 incident cases; 22.7%), with an increase in soft drink consumption (Table 4). This association was stronger when all 4 beverages were mutually included in the model. The odds of 10-y incidence of abdominal obesity among individuals with a daily consumption of ≥200 mL of soft drinks was 76% higher than that among individuals who did not consume soft drinks. Estimates of RRs were somewhat lower than those of the ORs (Table 4).

Discussion

This prospective study showed that soft drink consumption is predictive of increased WC, regardless of whether the behavior is present at baseline or acquired over time. Furthermore, replacing soft drinks with whole milk and juice decreases WC. Finally, daily consumption of soft drinks increases the odds of 10-y incidence of abdominal obesity.

There is little evidence of a relation between nonalcoholic caloric beverage consumption and weight change (29). Soft drinks in particular are suspected of promoting weight gain and increased risk of obesity (13, 14). However, few studies examined the association between consumption of nonalcoholic caloric beverages and changes in WC (15, 16). This association is of particular interest because a proportionally greater increase in WC than in BMI was reported in children and adults (3–5, 30–33). In the present study, the prevalence of general obesity increased by 2.5% over 10 y. This moderate increase contrasts

TABLE 4 Association between caloric beverage consumption and odds of 10 y incidence of abdominal obesity in Spanish adults[1]

Variable	OR (95% CI)	RR (95% CI)
Soft drinks		
No consumption	1	1
>0 and <200 mL	1.22 (0.90, 1.66)	1.18 (0.94, 1.47)
≥200 mL	1.77 (1.07, 2.93)	1.48 (1.01, 2.05)
Whole milk		
No consumption	1	1
>0 and <200 mL	1.58 (1.07, 2.32)	1.38 (1.03, 1.81)
≥200 mL	1.00 (0.72, 1.40)	1.15 (0.89, 1.47)
Skim and low-fat milk		
No consumption	1	1
>0 and <200 mL	0.76 (0.49, 1.18)	0.81 (0.55, 1.13)
≥200 mL	0.94 (0.69, 1.27)	0.90 (0.72, 1.12)
Juice		
No consumption	1	1
>0 and <200 mL	0.98 (0.72, 1.31)	1.00 (0.80, 1.24)
≥200 mL	0.74 (0.49, 1.13)	0.82 (0.72, 1.12)

[1] Logistic regression models adjusted for sex (men/women, dichotomous), age (years, continuous), baseline WC (centimeters, continuous), smoking (yes/no, dichotomous), energy intake (kilocalories, continuous), educational level [more than primary school (yes/no), dichotomous], LTPA (metabolic equivalent tasks times minutes per day, continuous), mMDS (continuous), and energy under- and over-reporting (yes/no, dichotomous). LTPA, leisure-time physical activity; mMDS, modified Mediterranean diet score; WC, waist circumference.

with the 38% increase in prevalence of abdominal obesity during the same time frame. Increase in WC was associated with both baseline consumption of soft drinks and changes therein over time. The 1.1 cm increase in WC observed for each 100 kcal increment in soft drink consumption might be considered moderate, but has a detrimental impact on WC in soft drink consumers with high intakes. Our findings are consistent with previous results that showed a direct association between soft drink consumption and WC increase (15, 16). American adolescents who increased their consumption of soft drinks showed a significantly higher increase in WC compared with those who maintained low consumption (16). Romaguera et al. reported a significant increase in WC among men and women from the EPIC (European Prospective Investigation into Cancer and Nutrition) cohort, whereas the opposite was observed for milk and juice consumption (15). Additionally, replacing healthy beverage choices such as milk and fruit juice with soft drinks promoted moderate gains in WC, a finding that is confirmed by the present study.

Prospective data on the association between beverage consumption and incidence of abdominal obesity are scarce (18, 34, 35). Dhingra and collaborators (34) investigated the impact of soft drink consumption on the risk of developing cardiovascular disease risk factors, and observed a 30% higher risk of abdominal obesity among consumers of at least 1 serving of soft drinks per day compared with nonconsumption. In a prospective study in Spain, participants who increased their consumption of soft drinks had a significantly higher risk of developing abdominal obesity (35). In line with this finding, a US study showed that increased consumption of soft drinks was associated with a higher risk of abdominal obesity in adolescents (18). In the present study, daily consumption of soft drinks increased the risk of abdominal obesity by 76% compared with nonconsumption. Furthermore, replacing whole milk and juices with soft drinks significantly increased the incidence of abdominal obesity. These data add prospective evidence of the adverse impact of soft drink consumption on the incidence of abdominal obesity.

Among nonalcoholic caloric beverages, consumption of soft drinks showed the most consistent association with abdominal adiposity. Fructose derived from sucrose and high-fructose corn syrup was proposed to explain this link (36, 37), although there is no conclusive evidence on the effect of fructose on weight gain and obesity risk (38, 39). Beverage consumption is related to energy intake and diet quality (40–43), and soft drink consumption was associated with a less healthy lifestyle and higher caloric intakes (43–45). In the present study, soft drink consumers generally showed unfavorable lifestyle patterns characterized by low adherence to the Mediterranean diet, low LTPA, and a high prevalence of smoking. This is consistent with previous studies showing low diet quality, a high prevalence of smoking, and physical inactivity in individuals who consume a lot of soft drinks (46, 47). However, adjusting for these lifestyle variables did not markedly affect our results.

This study has both limitations and strengths. Because of the nature of observational studies, causal relations cannot be drawn. Furthermore, all the dietary instruments that measure past food intake are vulnerable to random and systematic measurement errors. This study has a 10-y loss to follow-up of 19.7%. This can be considered an acceptable follow-up rate. However, there was some evidence of selection bias among the participants who were followed up, in that they were generally younger, more likely to be women, and had generally lower WCs than subjects who were not followed up. In contrast, we observed no significant difference in soft drink consumption between these groups. The strengths of the present study include its population-based design, long-term follow-up, and the availability of WC measurements. Furthermore, lifestyle assessments were performed with the use of validated questionnaires at baseline and follow-up.

In conclusion, the prospective data in the present study highlight the moderate adverse effects of soft drinks on abdominal adiposity in adults. This finding is important for health policy in the effort to counteract the increasing prevalence of obesity, particularly abdominal obesity.

Acknowledgments

We thank Dr. Gavin Lucas, PhD (ThePaperMill), for the English revision. ANF and HS designed and conducted the research; SFG, MF, RE, and AAB-A provided essential data; IS and HS performed the statistical analyses; ANF and HS wrote the manuscript; IS, SFG, MF, RE, and AAB-A critically revised the manuscript; and HS takes responsibility for the integrity of the data and had primary responsibility for the final content. All authors read and approved the final manuscript.

References

1. Li C, Ford ES, McGuire LC, Mokdad AH. Increasing trends in waist circumference and abdominal obesity among US adults. Obesity (Silver Spring) 2007;15:216–24.
2. Howel D. Trends in the prevalence of abdominal obesity and overweight in English adults (1993–2008). Obesity (Silver Spring) 2012;20:1750–2.
3. Xi B, Liang Y, He T, Reilly KH, Hu Y, Wang Q, Yan Y, Mi J. Secular trends in the prevalence of general and abdominal obesity among Chinese adults, 1993–2009. Obes Rev 2012;13:287–96.
4. Lean ME, Katsarou C, McLoone P, Morrison DS. Changes in BMI and waist circumference in Scottish adults: use of repeated cross-sectional surveys to explore multiple age groups and birth-cohorts. Int J Obes (Lond) 2013;37:800–8.
5. Elobeid MA, Desmond RA, Thomas O, Keith SW, Allison DB. Waist circumference values are increasing beyond those expected from BMI increases. Obesity (Silver Spring) 2007;15:2380–3.
6. Ghandehari H, Le V, Kamal-Bahl S, Bassin SL, Wong ND. Abdominal obesity and the spectrum of global cardiometabolic risks in US adults. Int J Obes (Lond) 2009;33:239–48.
7. Després JP, Lemieux I, Bergeron J, Pibarot P, Mathieu P, Larose E. Rodés-CabauJ, Bertrand OF, Poirier P.Abdominal obesity and the metabolic syndrome: contribution to globalcardiometabolic risk. Arterioscler Thromb Vasc Biol 2008;28:1039–49.
8. de Koning L, Merchant AT, Pogue J, Anand SS. Waist circumference and waist-to-hip ratio as predictors of cardiovascular events: meta-regression analysis of prospective studies1. Eur Heart J 2007;28:850–6.
9. Bray GA, Popkin BM. Dietary sugar and body weight: have we reached a crisis in the epidemic of obesity and diabetes?: health be damned! Pour on the sugar. Diabetes Care 2014;37:950–6.
10. European Fruit Juice Association Market Report 2012 [cited 2012 Oct 31]. Available from: http://www.aijn.org/pages/main/file.handler?f=AIJNMarketReport2012.pdf.
11. de Agricultura M, Alimentación y Medio AE. 2013. Available from: http://www.magrama.gob.es/es/megustalaleche/estudios-e-informes/Ficha_de_Consumo_LECHE_TAM_Marzo_2013_tcm7–285250.pdf.
12. Euromonitor International 2014. Available from: http://www.slate.com/articles/health_and_science/map_of_the_week/2012/07/map_of_soda_consumption_americans_drink_more_than_anyone_else_.html.
13. Te Morenga L, Mallard S, Mann J. Dietary sugars and body weight: systematic review and meta-analyses of randomized controlled trials and cohort studies. BMJ. 2012 15;346:e7492.
14. Malik VS, Pan A, Willett WC, Hu FB. Sugar-sweetened beverages and weight gain in children and adults: a systematic review and meta-analysis. Am J Clin Nutr 2013;98:1084–102.

15. Romaguera D, Ängquist L, Du H, Jakobsen MU, Forouhi NG, Halkjær J, Feskens EJ. van der A DL, Masala G, Steffen A, Palli D, Wareham NJ, Overvad K, Tjønneland A, Boeing H, Riboli E, Sørensen TI.Food composition of the diet in relation to changes in waist circumference adjusted for body mass index. PLoS ONE 2011;6:e23384.
16. Ambrosini GL, Oddy WH, Huang RC, Mori TA, Beilin LJ, Jebb SA. Prospective associations between sugar-sweetened beverage intakes and cardiometabolic risk factors in adolescents. Am J Clin Nutr 2013;98:327–34.
17. Wang H, Troy LM, Rogers GT, Fox CS, McKeown NM, Meigs JB, Jacques PF. Longitudinal association between dairy consumption and changes of body weight andwaist circumference: the Framingham Heart Study. Int J Obes (Lond) 2014;38:299–305.
18. Duffey KJ, Gordon-Larsen P, Steffen LM, Jacobs DR, Jr., Popkin BM. Drinking caloric beverages increases the risk of adverse cardiometabolic outcomes in the Coronary Artery Risk Development in Young Adults (CARDIA) Study. Am J Clin Nutr 2010;92:954–9.
19. Vergnaud AC, Péneau S, Chat-Yung S, Kesse E, Czernichow S, Galan P, Hercberg S, Bertrais S. Dairy consumption and 6-y changes in body weight and waist circumference in middle-aged French adults. Am J Clin Nutr 2008;88:1248–55.
20. Lean ME, Han TS, Morrison CE. Waist circumference as a measure for indicating need for weight management. BMJ 1995;311:158–61.
21. Benítez-Arciniega AA, Mendez MA, Baena-Diez JM, Rovira Martori MA, Soler C, Marrugat J, Covas MI, Sanz H, Llopis A, Schroder H. Concurrent and construct validity of Mediterranean diet scores as assessed by an FFQ. Public Health Nutr 2011;14:2015–21.
22. Schröder H, Covas MI, Marrugat J, Vila J, Pena A, Alcantara M, Masia R. Use of a three-day estimated food record, a 72-hour recall and a food-frequency questionnaire for dietary assessment in a Mediterranean Spanish population. Clin Nutr 2001;20:429–37.
23. Mendez MA, Popkin BM, Buckland G, Schroder H, Amiano P, Barricarte A, Huerta JM, Quirós JR, Sánchez MJ, González CA. Alternative methods of accounting for underreporting and overreporting when measuring dietary intake-obesity relations. Am J Epidemiol 2011;173:448–58.
24. Mifflin MD, St Jeor ST, Hill LA, Scott BJ, Daugherty SA. Koh YO. A new predictive equation for resting energy expenditure in healthy individuals. Am J Clin Nutr 1990;51:241–7.
25. Black AE. Critical evaluation of energy intake using the Goldberg cut-off for energy intake:basal metabolic rate. A practical guide to its calculation, use and limitations. Int J Obes Relat Metab Disord 2000;24:1119–30.
26. Elosua R, Marrugat J, Molina L, Pons S, Pujol E. Validation of the Minnesota Leisure Time Physical Activity Questionnaire in Spanish men. The MARATHOM Investigators. Am J Epidemiol 1994;139:1197–209.
27. Elosua R, Garcia M, Aguilar A, Molina L, Covas MI, Marrugat J. Validation of the Minnesota Leisure Time Physical Activity Questionnaire in Spanish women. Investigators of the MARATDON Group. Med Sci Sports Exerc 2000;32:1431–7.
28. Zhang J. What's the relative risk? A method of correcting the odds ratio in cohort studies of common outcome. JAMA 1998;280:1690–1.
29. Mozaffarian D, Hao T, Rimm EB, Willett WC, Hu FB. Changes in diet and lifestyle and long-term weight gain in women and men. N Engl J Med 2011;364:2392–404.
30. Okosun IS, Boltri JM, Eriksen MP, Hepburn VA. Trends in abdominal obesity in young people: United States 1988–2002. Ethn Dis 2006;16:338–44.
31. McCarthy HD, Jarrett KV, Emmett PM, Rogers I. Trends in waist circumferences in young British children: a comparative study. Int J Obes (Lond) 2005;29:157–62.
32. McCarthy HD, Ellis SM, Cole TJ. Central overweight and obesity in British youth aged 11–16 years: cross sectional surveys of waist circumference. BMJ 2003;326:624.
33. Garnett SP, Baur LA, Cowell CT. The prevalence of increased central adiposity in Australian school children 1985 to 2007. Obes Rev 2011;12:887–96.
34. Dhingra R, Sullivan L, Jacques PF, Wang TJ, Fox CS, Meigs JB, D'Agostino RB, Gaziano JM, Vasan RS. Soft drink consumption and risk of developing cardiometabolic risk factors andt he metabolic syndrome in middle-aged adults in the community. Circulation 2007;116:480–8.
35. Barrio-Lopez MT, Martinez-Gonzalez MA, Fernandez-Montero A, Beunza JJ, Zazpe I, Bes-Rastrollo M. Prospective study of changes in sugar-sweetened beverage consumption and the incidence of the metabolic syndrome and its components: the SUN cohort. Br J Nutr 2013;110:1722–31.
36. Bray GA. Energy and fructose from beverages sweetened with sugar or high-fructose corn syrup pose a health risk for some people. Adv Nutr 2013;4:220–5.
37. Sievenpiper JL, de Souza RJ, Cozma AI, Chiavaroli L, Ha V, Mirrahimi A. Fructose vs. glucose and metabolism: do the metabolic differences matter? Curr Opin Lipidol 2014;25:8–19.
38. Tappy L, Lê KA, Tran C, Paquot N. Fructose and metabolic diseases: new findings, new questions. Nutrition 2010;26:1044–9.
39. Tappy L, Mittendorfer B. Fructose toxicity: is the science ready for public health actions? Curr Opin Clin Nutr Metab Care 2012;15:357–61.
40. Piernas C, Mendez MA, Ng SW, Gordon-Larsen P, Popkin BM. Low-calorie- and calorie-sweetened beverages: diet quality, food intake, and purchase patterns of US household consumers. Am J Clin Nutr 2014;99:567–77.
41. Stern D, Piernas C, Barquera S, Rivera JA, Popkin BM. Caloric beverages were major sources of energy among children and adults in Mexico, 1999–2012. J Nutr 2014;144:949–56.
42. O'Neil CE, Nicklas TA, Rampersaud GC, Fulgoni, 3rd VL. 100% orange juice consumption is associated with better diet quality, improved nutrient adequacy, decreased risk for obesity, and improved biomarkers of health in adults: National Health and Nutrition Examination Survey, 2003–2006. Nutr J 2012;11:107.
43. Schröder H, Mendez MA, Ribas L, Funtikova AN, Gomez SF, Fíto M, Aranceta J, Serra-Majem L. Caloric beverage drinking patterns are differentially associated with diet quality and adiposity among Spanish girls and boys. Eur J Pediatr 2014;173:1169–77.
44. Libuda L, Alexy U, Buyken AE, Sichert-Hellert W, Stehle P, Kersting M. Consumption of sugar-sweetened beverages and its association with nutrient intakes and diet quality in German children and adolescents. Br J Nutr 2009;101:1549–57.
45. Balcells E, Delgado-Noguera M, Pardo-Lozano R, Roig-González T, Renom A, González-Zobl G, Muñoz-Ortego J, Valiente-Hernández S, Pou-Chaubron M, Schröder H. Soft drinks consumption, diet quality and BMI in a Mediterranean population. Public Health Nutr 2011;14:778–84.
46. Ma J, Sloan M, Fox CS, Hoffmann U, Smith CE, Saltzman E, Rogers GT, Jacques PF, McKeown N. Sugar-sweetened beverage consumption is associated with abdominal fat partitioning in healthy adults. J Nutr 2014;144:1283–90.
47. Kvaavik E, Andersen LF, Klepp KI. The stability of soft drinks intake from adolescence to adult age and the association between long-term consumption of soft drinks and lifestyle factors and body weight. Public Health Nutr 2005;8:149–57.

5th paper

Mediterranean diet impact on changes in abdominal fat and 10-year incidence of abdominal obesity in a Spanish population. British Journal of Nutrition 2014; 111(8): 1481-7.

British Journal of Nutrition (2014), **111**, 1481–1487
doi:10.1017/S0007114513003966

Mediterranean diet impact on changes in abdominal fat and 10-year incidence of abdominal obesity in a Spanish population

Anna N. Funtikova[1,2]†, Alejandra A. Benítez-Arciniega[3]†, Santiago F. Gomez[1,4], Montserrat Fitó[1,5], Roberto Elosua[6] and Helmut Schröder[1,2]*

[1]Cardiovascular Risk and Nutrition Research Group (CARIN-ULEC), Research Program in Inflammatory and Cardiovascular Disorders (RICAD), Institut de Recerca Hospital del Mar (IMIM), Biomedical Research Park, c/Doctor Aiguader 88, Barcelona 08003, Spain
[2]CIBER Epidemiology and Public Health (CIBERESP), Instituto de Salud Carlos III, Madrid, Spain,
[3]Faculty of Medicine, Autonomous University of Mexico State, Toluca, Mexico
[4]Fundación THAO, Barcelona, Spain
[5]CIBER Physiopathology of Obesity and Nutrition (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain
[6]Cardiovascular Epidemiology and Genetics (EGEC-ULEC), Research Program in Inflammatory and Cardiovascular Disorders (RICAD), Institut de Recerca Hospital del Mar (IMIM), Barcelona, Spain

(Submitted 9 July 2013 – Final revision received 6 November 2013 – Accepted 12 November 2013 – First published online 2 January 2014)

Abstract

Abdominal obesity is a strong predictor of metabolic disorders. Prospective data on the association between the Mediterranean diet and surrogate markers of abdominal adiposity are scarce. The present study evaluated the relationship between adherence to the Mediterranean diet and (1) changes in waist circumference (WC) and (2) 10-year incidence of abdominal obesity. We conducted a prospective, population-based study in 3058 male and female Spaniards aged 25–74 years, followed from 2000 to 2009. Dietary intake and leisure-time physical activity levels were recorded using validated questionnaires. Weight, height and WC were measured. Adherence to the Mediterranean diet, determined using the previously validated REGICOR-Mediterranean diet score (R-MDS), based on the distribution of population food intake and on the dietary recommendations (MDS-rec), was negatively associated with WC gain ($P=0{\cdot}007$ and 0·024, respectively) in fully adjusted models. In the multivariate logistic analysis, the odds of abdominal obesity incidence decreased across the tertiles of the R-MDS, but the association was not significant. In conclusion, adherence to the Mediterranean diet was associated with lower abdominal fat gain, but not with 10-year incidence of abdominal obesity.

Key words: **Abdominal obesity: Mediterranean diet: Waist circumference**

Abdominal obesity is a strong predictor of metabolic disorders and premature mortality(1,2). Therefore, increasing secular trends of abdominal obesity in recent decades(3) and the recently reported high prevalence of abdominal obesity(3,4) are a matter of health policy concern.

Sedentary lifestyle and dietary patterns promoting energy overconsumption are considered to be the main drivers of the obesity epidemic(5). Epidemiological evidence indicates an inverse association between adherence to the Mediterranean diet and general obesity(6,7). However, little is known about the impact of this dietary pattern, which includes foods characteristic of the Mediterranean olive grove areas, on abdominal fat accumulation(8). An inverse association has been found in some(9) but not in all(10) cross-sectional studies, and long-term prospective data are lacking.

Most studies have measured adherence to the Mediterranean diet by scores based on consumption patterns and modified to reflect the particular study population. This approach makes it difficult to compare results between different populations(11). A Mediterranean diet score has also been created *a priori* by a few studies(12), using food intake recommendations.

The present study had three objectives: (1) to explore the prospective association of adherence to the Mediterranean diet with waist circumference (WC) changes; (2) to determine the 10-year incidence of abdominal obesity defined by WC cut-offs, according to adherence to the Mediterranean diet in

Abbreviations: MDS-rec, Mediterranean diet score based on the dietary recommendations; R-MDS, REGICOR-Mediterranean diet score based on the distribution of population food intake; LTPA, leisure-time physical activity; WC, waist circumference.

* **Corresponding author:** H. Schröder, fax +34 933 160 796, email hschroeder@imim.es

† A. N. F. and A. A. B.-A. contributed equally to the study, and each can be considered first authors of this article.

a population-based sample in Spain; (3) to assess whether the use of a different strategy to define the Mediterranean diet construct had an impact on the results obtained.

Materials and methods

Study population

Data were obtained from a population-based survey conducted in Girona (Spain) in 2000 and 2009. The baseline survey carried out in 2000 examined a randomly selected, population-based sample of 3058 men and women aged 25–74 years (participation rate: 71·0%). A two-stage sampling method was used in 2000: seventeen towns were randomly selected in the first stage. Half of the towns were urban (>10 000 inhabitants) and half were rural (500–10 000 inhabitants). The second sampling stage consisted of randomly recruiting the same number of female and male participants, stratifying by 10-year age groups from the closest census.

In 2009, the 2715 non-institutionalised participants still residing in the catchment area were invited for re-examination, and 2181 attended (follow-up participation rate: 80·3%). Participants following a special diet at re-examination (*n* 207 (9·8%)) were excluded. The participants were duly informed, and they signed their consent to participate in the study. The project was approved by the local Ethics Committee (CEIC-PSMAR, Barcelona, Spain).

Anthropometrics

A calibrated precision scale was used for measuring weight. Readings were rounded up to 200 g. Height was measured in the standing position and rounded up to the nearest 0·5 cm. Weight was divided by height squared (kg/m^2) to establish the BMI.

WC was measured midway between the lowest rib and the iliac crest in the horizontal position. Readings were rounded up to the nearest 0·5 cm. Abdominal obesity was defined by sex-specific cut-offs of more than 102 cm for men and more than 88 cm for women[13].

All the anthropometric parameters were measured both at baseline and at follow-up, using the same protocol.

Dietary intake assessment

Dietary intake was determined using a validated[14,15] FFQ, administered by a trained interviewer at baseline and at follow-up. In a 166-item food list including alcoholic and non-alcoholic beverages, the participants indicated their usual consumption frequency and chose from ten frequency categories ranging from never or less than once per month to six or more times per d.

Adherence to the Mediterranean diet was determined by the validated REGICOR-Mediterranean diet score (R-MDS)[14]. The published Pearson correlation for the energy-adjusted R-MDS *v.* multiple recalls was 0·48[13]. The R-MDS was calculated using two strategies: sex-specific tertile distribution of food intake[14] (R-MDS) and dietary recommendations established in Spain[16,17] (MDS-rec). The lowest tertile was coded as 1, medium as 2 and the highest as 3 for cereals, fruits, vegetables, legumes, fish, olive oil and nuts. For meat (including red meat, poultry and sausages) and dairy products, the score was inverted, with the highest tertile being coded as 1 and the lowest as 3. Moderate red wine consumption (up to 20 g/d) was coded as 3, and that more or less than this daily portion was coded as 1. Using the second strategy, consumption that met the recommendations for cereals, fruits, vegetables, legumes, fish, olive oil, nuts and dairy products was coded as 3, that at least weekly as 2 and that less than weekly levels as 1 for legumes, fish and nuts; consumption at least daily was coded as 2 and that less than daily levels as 1 for cereals, fruits, vegetables, olive oil and dairy products (see Supplementary Appendix 2, available online). For meat (including red meat, poultry and sausages) and dairy products, the score was partially inverted, with consumption more than weekly levels being coded as 1, that weekly as 2 and that meeting the recommended levels as 3. Red wine consumption was coded as described previously.

Energy misreporting

Implausible energy reporters were identified by the revised Goldberg method described previously[18]. BMR was calculated using the Mifflin equation[19]:

$$\text{BMR} = (\text{weight}_{\text{kg}} \times 9{\cdot}99) + (\text{height}_{\text{cm}} \times 6{\cdot}25) - (\text{age}_{\text{y}} \times 4{\cdot}92) + 5\,(\text{among men}),$$

$$\text{BMR} = (\text{weight}_{\text{kg}} \times 9{\cdot}99) + (\text{height}_{\text{cm}} \times 6{\cdot}25) - (\text{age}_{\text{y}} \times 4{\cdot}92) + 161\,(\text{among women}).$$

The index of variability (S) in the components of energy balance was determined. Subjects with BMR falling above or below the upper and lower 95% CI limits of 1·96 standard deviations for plausible energy intake were characterised as implausible energy reporters.

The following formula was used:

$$\text{Cut-off} = \text{PAL} \times \exp\left[\pm 1.96 \times \frac{S/100}{\sqrt{n}}\right],$$

where

$$S = \sqrt{\left[\frac{\text{CV}^2_{\text{wEI}}}{d} + \text{CV}^2_{\text{wBMR}} + \text{CV}^2_{\text{tP}}\right]},$$

where d is the number of recording days; CV^2_{wEI} and $\text{CV}^2_{\text{wBMR}}$ represent the intra-individual variations in the repeated measures of energy intake and BMR, respectively; and CV^2_{tP} represents the inter-individual variation in physical activity levels (PAL)[20].

Other variables

The validated Minnesota leisure-time physical activity (LTPA) questionnaire was administered by a trained interviewer[21,22]. LTPA is expressed as metabolic equivalents × min/d.

The measurements of smoking habits and demographic and socio-economic variables were obtained from structured standard questionnaires administered by trained personnel. The participants were dichotomously categorised into non-smokers (never smokers and ex-smokers with more than 1 year of smoking cessation) and current smokers. Maximum educational level attained was determined and dichotomously recorded for analysis as primary school *v.* secondary school or university.

All dietary and energy intake and expenditure measurements were performed both at baseline and at follow-up, using the same protocol.

Statistical analysis

General linear modelling procedures were used to compare the baseline characteristics of the participants by tertiles of the R-MDS and by categories of the MDS-rec. ANOVA test and polynomial contrasts were used to determine overall *P* and *P* for linear trend, respectively, for normally distributed continuous variables. The Kruskal–Wallis test was used to determine overall *P* for non-normal distribution. Overall *P* and *P* for linear trend for categorical variables were obtained using the Pearson χ^2 and Mantel–Haenszel linear-by-linear association χ^2 tests, respectively.

Linear regression models were fitted to analyse the association between changes in WC and adherence to the Mediterranean diet at baseline. Changes in WC (in cm; continuous variable) were included as the dependent variable and scores as explanatory variables. For analyses, three models were fitted. The first model included sex (men/women; dichotomous), age (years; continuous) and baseline WC. The second model included smoking (yes/no; dichotomous), energy intake (kcal; continuous), alcohol consumption (g, continuous), educational level (more than primary school yes/no; dichotomous), LTPA (metabolic equivalents × min/d; continuous), and energy under- and over-reporting (both yes/no; dichotomous). The third model included changes in BMI (BMI at follow-up − BMI at baseline; continuous) to determine whether changes in WC are independent of changes in BMI. This model was adjusted by sex (men/women; dichotomous), age (years; continuous), smoking (yes/no; dichotomous), energy intake (kcal; continuous), alcohol consumption (g; continuous), educational level (more than primary school yes/no; dichotomous), LTPA (metabolic equivalents × min/d; continuous), and energy

Table 1. General characteristics of the sample at baseline according to tertiles of the REGICOR-Mediterranean diet score (R-MDS)*

	R-MDS tertiles							
	1st (*n* 650)		2nd (*n* 618)		3rd (*n* 611)			
Variables	Proportions	95 % CI	Proportions	95 % CI	Proportions	95 % CI	*P*†	*P*‡
Women (%)	51·4	47·5, 55·2	53·1	49·1, 57·0	50·4	46·4, 54·4	0·639	0·741
Age (years)							<0·001	<0·001
Mean	47·4		49·9		49·8			
95 % CI	46·4, 48·4		48·9, 50·9		48·8, 50·8			
Education (%)§	37·5	33·8, 41·3	34·8	31·0, 38·6	38·1	34·3, 42·0	0·429	0·887
Smokers (%)‖	30·3	26·9, 33·7	24·1	20·7, 27·6	23·4	19·9, 26·9	0·008	0·005
LTPA (MET × min/d)							<0·001	–
Median	167		190		240			
25th–75th percentiles	87–321		89–338		130–396			
WC (cm)							0·387	0·387
Mean	89·6		89·5		89·0			
95 % CI	88·6, 90·7		88·4, 90·6		87·9, 90·1			
WC at follow-up (cm)							0·030	0·030
Mean	94·2		93·4		92·6			
95 % CI	93·2, 95·2		92·4, 94·4		91·6, 93·6			
Abdominal obesity (%)¶	30·6	27·1, 34·1	32·2	28·6, 35·8	24·9	21·3, 28·5	0·028	0·028
Abdominal obesity at follow-up (%)¶	44·2	40·4, 47·9	42·4	38·5, 46·3	35·7	31·8, 39·6	0·002	0·002
Alcohol consumption (g/d)							0·001	–
Mean	2·8		4·2		7·8			
95 % CI	0, 22·7		0, 13·7		2·1, 15·0			
Energy (MJ)							<0·001	<0·001
Mean	9·6		11·1		13·0			
95 % CI	9·2, 9·9		10·8, 11·5		12·7, 13·3			
Energy under-reporters (%)	35·2	32·1, 38·4	20·1	16·8, 23·3	12·8	9·5, 16·0	<0·001	<0·001
Energy over-reporters (%)	5·4	2·9, 7·8	11·7	9·1, 14·2	19·0	16·5, 21·5	<0·001	<0·001

LTPA, leisure-time physical activity; MET, metabolic equivalents; WC, waist circumference.
* Age, WC, WC at follow-up, alcohol consumption and energy are continuous variables; women, education, smokers, abdominal obesity, abdominal obesity at follow-up, energy under-reporters and energy over-reporters are categorical variables.
† *P* values were obtained using the ANOVA, Kruskal–Wallis and Pearson χ^2 tests for normal continuous, non-normal continuous and categorical variables, respectively.
‡ *P* values for linear trend were obtained using polynomial contrast for normal continuous variables and using the Mantel–Haenszel linear-by-linear association χ^2 test for categorical variables.
§ More than secondary school education.
‖ Active smokers or ex-smokers for less than 1 year.
¶ WC > 102 cm in men and > 88 cm in women.

British Journal of Nutrition

under- and over-reporting (both yes/no; dichotomous). Participants with baseline abdominal obesity were excluded from analysis in multiple logistic regression models assessing the association between R-MDS and MDS-rec adherence at baseline and 10-year incidence of abdominal obesity. Baseline R-MDS and MDS-rec values are represented by tertiles and categories, respectively.

To explore effect modification according to sex, R-MDS and MDS-rec, we modelled interaction terms for sex/R-MDS and sex/MDS-rec. Differences were considered significant if $P<0{\cdot}05$. Statistical analysis was carried out using SPSS version 18.0. (SPSS, Inc.).

Results

The prevalence of abdominal obesity at baseline was 29·3 % (men: 23·8 %; women: 34·4 %) and at follow-up was 40·8 % (men: 30·5 %; women: 50·5 %). Of the 1329 participants without abdominal obesity at baseline, 291 (21·9 %) had abdominal obesity at follow-up (men: 14·9 %; women: 29·6 %).

Participants with high adherence to the Mediterranean diet were less prone to smoke and had a lower prevalence of abdominal obesity than those with low dietary adherence (Tables 1 and 2). Age, LTPA, alcohol consumption and energy intake increased across the R-MDS tertiles and MDS-rec categories. The proportion of energy under-reporters decreased with greater adherence to the Mediterranean diet; with lesser adherence, energy over-reporting increased.

Linear regression analysis revealed a significant negative association of WC gain with the R-MDS and MDS-rec ($P=0{\cdot}007$ and 0·024, respectively) in the fully adjusted models (Table 3). A 10-point increase in the R-MDS and MDS-rec was associated with a decrease of 1·7 and 1·5 cm in WC, respectively. The association of changes in WC with adherence to the Mediterranean diet was independent of BMI changes.

In the multivariate logistic analysis, high adherence to the Mediterranean diet (top tertile) was associated with a reduced 10-year incidence of abdominal obesity, but this association did not reach significance (Table 4). The magnitude of risk reduction was similar for both scores.

Discussion

In the present prospective study, it was found that high adherence to the Mediterranean diet was associated with a

Table 2. General characteristics of the sample at baseline according to categories of the Mediterranean diet score based on dietary recommendations (MDS-rec)*

	MDS-rec categories							
	1st (*n* 693)		2nd (*n* 507)		3rd (*n* 679)			
Variables	Proportions	95 % CI	Proportions	95 % CI	Proportions	95 % CI	*P*†	*P*‡
Women (%)	50·1	46·3, 53·8	50·1	45·7, 54·5	54·3	50·6, 58·1	0·207	0·114
Age (years)							0·021	0·089
Mean	48·0		50·1		49·2			
95 % CI	47·1, 49·0		49·0, 51·2		48·2, 50·2			
Education (%)§	36·9	33·3, 40·5	34·5	30·3, 38·7	38·4	34·8, 42·1	0·382	0·570
Smokers (%)‖	31·2	27·9, 34·4	26·8	23·0, 30·6	20·2	16·9, 23·5	<0·001	<0·001
LTPA (MET × min/d)							<0·001	–
Median	171		190		232			
25th–75th percentiles	84–322		105–334		117–387			
WC (cm)							0·324	0·112
Mean	89·4		90·3		88·6			
95 % CI	88·4, 90·4		89·1, 91·5		87·6, 89·7			
WC at follow-up (cm)							0·026	0·005
Mean	93·8		94·6		92·2			
95 % CI	92·8, 94·7		93·5, 95·7		91·3, 93·2			
Abdominal obesity (%)¶	29·7	26·3, 33·1	32·7	28·8, 36·7	26·2	22·8, 29·6	0·156	0·048
Abdominal obesity at follow-up (%)¶	42·0	38·3, 45·6	44·6	40·3, 48·9	36·8	33·1, 40·5	0·053	0·020
Alcohol consumption (g/d)							0·001	–
Mean	2·8		4·9		6·5			
95 % CI	0, 25·0		0, 14·7		1·9, 14·7			
Energy (MJ)							<0·001	<0·001
Mean	9·4		11·2		13·1			
95 % CI	9·1, 9·7		10·8, 11·5		12·8, 13·4			
Energy under-reporters (%)	37·5	34·5, 40·5	20·5	17·0, 24·0	9·9	6·8, 12·9	<0·001	<0·001
Energy over-reporters (%)	5·2	2·8, 7·6	10·5	7·7, 13·2	19·7	17·3, 22·1	<0·001	<0·001

LTPA, leisure-time physical activity; MET, metabolic equivalents; WC, waist circumference.
* Age, WC, WC at follow-up, alcohol consumption and energy are continuous variables; women, education, smokers, abdominal obesity, abdominal obesity at follow-up, energy under-reporters and energy over-reporters are categorical variables.
† *P* values were obtained using the ANOVA, Kruskal–Wallis and Pearson χ^2 tests for normal continuous, non-normal continuous and categorical variables, respectively.
‡ *P* values for linear trend were obtained using polynomial contrast for normal continuous variables and using the Mantel–Haenszel linear-by-linear association χ^2 test for categorical variables.
§ More than secondary school education.
‖ Active smokers or ex-smokers for less than 1 year.
¶ WC > 102 cm in men and > 88 cm in women.

Table 3. Multiple linear regression analysis of the association between 10-point increase in the REGICOR-Mediterranean diet score (R-MDS) and the Mediterranean diet score based on dietary recommendations (MDS-rec) and 10-year changes in waist circumference (WC) (in cm)

(β-Coefficients and 95 % confidence intervals)

	β	95 % CI	P
R-MDS			
Model 1*	−1·50	−2·74, −0·25	0·019
Model 2†	−1·55	−2·88, −0·21	0·033
Model 3‡	−1·65	−2·84, −0·45	0·007
MDS-rec			
Model 1*	−1·53	−2·87, 0·19	0·259
Model 2†	−1·64	−3·16, −0·13	0·033
Model 3‡	−1·49	−2·85, −0·13	0·024

*Model 1 includes sex (men/women; dichotomous), age (years; continuous) and baseline WC (cm; continuous).
†Model 2 additionally includes smoking (yes/no; dichotomous), energy intake (kcal; continuous), alcohol consumption (g; continuous), educational level (more than primary school yes/no; dichotomous), leisure-time physical activity (metabolic equivalents × min/d; continuous), and energy under- and over-reporting (both yes/no; dichotomous).
‡Model 3 includes variables of model 2 and changes in BMI (BMI at follow-up − BMI at baseline; continuous).

lower gain in abdominal adiposity but not with the incidence of abdominal obesity within 10 years. In recent decades, increased WC has been reported for several populations[3]. In the present study, general obesity was about 7% less prevalent than abdominal obesity at baseline. Most importantly, 10-year incidence of abdominal obesity was 14·8 % higher than that of general obesity. This is a cause of concern due to the direct association between WC and cardiometabolic risk[2].

A recently published meta-analysis[23] of the impact of adherence to the Mediterranean diet on the metabolic syndrome and its components has revealed a positive effect on WC changes in eleven randomised controlled trials. It is worth noting that this was mainly due to one trial that demonstrated a mean WC difference of −4·20 cm (−7·99 to −0·41) between the Mediterranean and control diets[24].

Among the prospective observational studies, only three surveys[11,25,26] evaluated the association between the Mediterranean diet and WC changes. In the SUN prospective cohort (Seguimiento Universidad de Navarra; University of Navarra follow-up) the difference in WC between the highest and lowest tertiles of the Mediterranean diet score in sex- and age-adjusted models was almost 0[26]. Participants in the Framingham Heart Study Offspring Cohort who closely adhered to the Mediterranean diet had a significantly lower WC than their low-adhering peers[25]. Additionally, the SUpplementation en VItamines et Minéraux AntioXydants (SU.VI.MAX) study reported a negative association between WC gain and two of the three Mediterranean diet score indices calculated[11].

Our data confirm the findings of previous studies showing a protective association of the Mediterranean diet with abdominal adiposity gain. In the present study, a 10-point increase in dietary scores was associated with a 1·7 cm decrease in WC.

Cross-sectional studies[23,27] have reported mixed results, which might be due to differences in the surrogate markers of abdominal obesity used (WC, waist:hip ratio and waist: height ratio). In the present study, cross-sectional analysis revealed that the risk of abdominal obesity decreased with high adherence to the Mediterranean diet.

Some[6,28,29] but not all[30,31] prospective studies provide evidence for the protective effects of the Mediterranean diet on the incidence of general obesity. To our knowledge, no previous study has addressed the association between the Mediterranean diet and incidence of abdominal obesity. We defined abdominal obesity by WC, a surrogate marker of abdominal adiposity. We observed an inverse, but non-significant, association between adherence to the Mediterranean diet and incidence of abdominal obesity. One explanation for these results is the moderate magnitude of the association of WC change with the R-MDS. On the other hand, a substantial increase in the prevalence of abdominal obesity at follow-up and low mean change in the R-MDS (−0·5 points) during 10 years cancelled the effect of the Mediterranean diet on the incidence of abdominal obesity. This outcome is of interest at the public health level because it is also important to know which diets do not promote abdominal obesity.

Higher adherence to the Mediterranean diet was associated with high consumption of MUFA and PUFA, high dietary fibre intake and low energy density (data not shown). High consumption of fibre might partially explain the impact of the Mediterranean diet on abdominal adiposity through various protective mechanisms, with the increase of satiety and decrease of energy consumption[32,33], modification of hepatic cholesterol metabolism[30], and increased production of SCFA being among them[34]. Du *et al.*[35] found that a 1 kcal/g (4 kJ/g) increase in energy density was associated with a 0·09 cm gain in WC per year. It has been hypothesised that energy density could be a mediator between depressive symptoms and abdominal adiposity gain[36]. However, the

Table 4. OR of 10-year incidence of abdominal obesity according to tertile distribution of the REGICOR-Mediterranean diet score (R-MDS) and categories of the Mediterranean diet score based on dietary recommendations (MDS-rec)*

(Odds ratios and 95 % confidence intervals)

	R-MDS tertiles and MDS-rec categories					
		2nd		3rd		
	1st	OR	95 % CI	OR	95 % CI	P
R-MDS						
Model 1†	Ref.	0·70	0·50, 0·97	0·75	0·55, 1·04	0·085
Model 2‡	Ref.	0·73	0·52, 1·04	0·79	0·55, 1·12	0·185
MDS-rec						
Model 1†	Ref.	0·99	0·70, 1·34	0·81	0·59, 1·11	0·186
Model 2‡	Ref.	1·08	0·76, 1·55	0·90	0·64, 1·29	0·571

Ref., reference.
*Total number of participants = 1329; distribution of the participants according to tertiles of the R-MDS: 1st tertile = 451; 2nd tertile = 419; 3rd tertile = 459; distribution of the participants according to categories of the MDS-rec: 1st category = 487; 2nd category = 341; 3rd category = 501.
†Model 1 includes sex (men/women; dichotomous), age (years; continuous) and baseline waist circumference (cm; continuous).
‡Model 2 additionally includes smoking (yes/no; dichotomous), energy intake (kcal; continuous), alcohol consumption (g; continuous), educational level (more than primary school yes/no; dichotomous), leisure-time physical activity (metabolic equivalents × min/d; continuous), and energy under- and over-reporting (both yes/no; dichotomous).

mechanisms linking WC changes and energy density are not understood. Fatty acids can activate the *PPARγ* gene[37], which is involved in lipid metabolism and particularly in the process of adipocyte differentiation. It has been demonstrated that the WC gain caused by the 12Ala allele carriers of the *PPARγ* gene was depressed by adherence to the Mediterranean diet[38].

Assessing the adherence to the Mediterranean diet by a score based on population-based food consumption distribution is specific to a particular population, making it difficult to compare results between studies. This construct has mainly been used by studies reporting on the association between adherence to the Mediterranean diet and incidence of obesity and/or changes in anthropometric markers of adiposity[11,27]. To overcome the limitation on comparability of results, we calculated two scores, one based on food distribution and the other based on food intake recommendations. We observed no meaningful impact of the type of construct used on the association between the score obtained and WC or incidence of abdominal obesity.

The strengths of the present study are the population-based design, the high participation rate during follow-up, and the measurement of anthropometric variables at baseline and at 10-year follow-up. Furthermore, dietary intake and LTPA data were recorded using validated questionnaires and all the analyses were controlled for misreporting. A benefit of using the MDS-rec is that it allows generalisability to other populations. However, all the dietary instruments measuring past food intake are vulnerable to random and systematic measurement errors.

In conclusion, adherence to the Mediterranean diet was negatively associated with changes in abdominal adiposity measured by WC. Participants with high adherence to the Mediterranean diet were less likely to develop abdominal obesity according to R-MDS tertile distribution and MDS-rec categories. However, the association was not significant. The type of R-MDS calculation (population distribution *v.* dietary recommendations) did not have a strong impact on these associations. Evidence of the present study underlines the usefulness of the Mediterranean diet for the prevention of abdominal weight gain.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S0007114513003966

Acknowledgements

The authors thank Elaine Lilly, PhD (Writers First Aid), for revising the manuscript for English language.

The present study was supported by a grant (2FD097-0297-CO2-01) from Fondo Europeo de Desarrollo Regional (FEDER); by portions of grants from Spain's Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III FEDER (PI11/01900), Red HERACLES (RD06/0009), by scholarship for PhD formation from the National Program of University Professors Formation (FPU), from the Ministry of Education of Spain (AP2010-3198), and by a joint Miguel Servet contract (CP03/00115) from the Instituto de Salud Carlos III and the Health Department of the Catalan Government (Generalitat de Catalunya). The CIBERESP is an initiative of the Instituto de Salud Carlos III, Madrid, Spain.

The authors' contributions are as follows: A. N. F. and A. A. B.-A. prepared the manuscript, with significant input and feedback from all the co-authors; S. F. G., M. F. and R. E. participated in the design and execution of the study and contributed to the critical revision of the manuscript for important intellectual content; H. S. conducted the analyses, with results being interpreted by all the co-authors. All the authors approved the final version of the manuscript.

None of the authors has any conflicts of interest to declare.

References

1. Cameron AJ, Dunstan DW, Owen N, *et al.* (2009) Health and mortality consequences of abdominal obesity: evidence from the AusDiab study. *Med J Aust* **191**, 202–208.
2. Fox KA, Despres JP, Richard AJ, *et al.* (2009) Does abdominal obesity have a similar impact on cardiovascular disease and diabetes? A study of 91,246 ambulant patients in 27 European countries. *Eur Heart J* **30**, 3055–3063.
3. Ford ES, Mokdad AH & Giles WH (2003) Trends in waist circumference among U.S. adults. *Obes Res* **11**, 1223–1231.
4. Gutierrez-Fisac JL, Guallar-Castillon P, Leon-Munoz LM, *et al.* (2012) Prevalence of general and abdominal obesity in the adult population of Spain, 2008–2010: the ENRICA study. *Obes Rev* **13**, 388–392.
5. Apovian CM (2010) The causes, prevalence, and treatment of obesity revisited in 2009: what have we learned so far? *Am J Clin Nutr* **91**, 277S–279S.
6. Romaguera D, Norat T, Vergnaud AC, *et al.* (2010) Mediterranean dietary patterns and prospective weight change in participants of the EPIC-PANACEA project. *Am J Clin Nutr* **92**, 912–921.
7. Buckland G, Bach A & Serra-Majem L (2008) Obesity and the Mediterranean diet: a systematic review of observational and intervention studies. *Obes Rev* **9**, 582–593.
8. Rallidis LS, Lekakis J, Kolomvotsou A, *et al.* (2009) Close adherence to a Mediterranean diet improves endothelial function in subjects with abdominal obesity. *Am J Clin Nutr* **90**, 263–268.
9. Romaguera D, Norat T, Mouw T, *et al.* (2009) Adherence to the Mediterranean diet is associated with lower abdominal adiposity in European men and women. *J Nutr* **139**, 1728–1737.
10. Asghari G, Mirmiran P, Rashidkhani B, *et al.* (2012) The association between diet quality indices and obesity: Tehran Lipid and Glucose Study. *Arch Iran Med* **15**, 599–605.
11. Kesse-Guyot E, Ahluwalia N, Lassale C, *et al.* (2012) Adherence to Mediterranean diet reduces the risk of metabolic syndrome: a 6-year prospective study. *Nutr Metab Cardiovasc Dis* **3**, 677–683.
12. Rumawas ME, Dwyer JT, McKeown NM, *et al.* (2009) The development of the Mediterranean-style dietary pattern score and its application to the American diet in the Framingham Offspring Cohort. *J Nutr* **139**, 1150–1156.
13. Lean ME, Han TS & Morrison CE (1995) Waist circumference as a measure for indicating need for weight management. *BMJ* **311**, 158–161.
14. Benitez-Arciniega AA, Mendez MA, Baena-Diez JM, *et al.* (2011) Concurrent and construct validity of Mediterranean diet scores as assessed by an FFQ. *Public Health Nutr* **14**, 2015–2021.
15. Schroder H, Covas MI, Marrugat J, *et al.* (2001) Use of a three-day estimated food record, a 72-hour recall and a food-frequency questionnaire for dietary assessment in a Mediterranean Spanish population. *Clin Nutr* **20**, 429–437.

16. Agencia Española de Seguridad Alimentaria y Nutrición Ministerio de Sanidad y Consumo (2008) Come sano y muévete. 12 decisiones saludables (Eat healthy and move. 12 healthy decisions). AESAN.
17. Sociedad Española de Dietética y Ciencias de Alimentación (2009) Recomendaciones SEDCA 2009 para mantener una alimentación saludable (SEDCA 2009 Recommendations to maintain a healthy diet). SEDCA.
18. Mendez MA, Popkin BM, Buckland G, *et al.* (2011) Alternative methods of accounting for underreporting and overreporting when measuring dietary intake–obesity relations. *Am J Epidemiol* **173**, 448–458.
19. Mifflin MD, St Jeor ST, Hill LA, *et al.* (1990) A new predictive equation for resting energy expenditure in healthy individuals. *Am J Clin Nutr* **51**, 241–247.
20. Black AE (2000) Critical evaluation of energy intake using the Goldberg cut-off for energy intake: basal metabolic rate. A practical guide to its calculation, use and limitations. *Int J Obes Relat Metab Disord* **24**, 1119–1130.
21. Elosua R, Marrugat J, Molina L, *et al.* (1994) Validation of the Minnesota Leisure Time Physical Activity Questionnaire in Spanish men. The MARATHOM Investigators. *Am J Epidemiol* **139**, 1197–1209.
22. Elosua R, Garcia M, Aguilar A, *et al.* (2000) Validation of the Minnesota Leisure Time Physical Activity Questionnaire in Spanish Women. Investigators of the MARATDON Group. *Med Sci Sports Exerc* **32**, 1431–1437.
23. Kastorini CM, Milionis HJ, Esposito K, *et al.* (2011) The effect of Mediterranean diet on metabolic syndrome and its components: a meta-analysis of 50 studies and 534,906 individuals. *J Am Coll Cardiol* **57**, 1299–1313.
24. McManus K, Antinoro L & Sacks F (2001) A randomized controlled trial of a moderate-fat, low-energy diet compared with a low fat, low-energy diet for weight loss in overweight adults. *Int J Obes Relat Metab Disord* **25**, 1503–1511.
25. Rumawas ME, Meigs JB, Dwyer JT, *et al.* (2009) Mediterranean-style dietary pattern, reduced risk of metabolic syndrome traits, and incidence in the Framingham Offspring Cohort. *Am J Clin Nutr* **90**, 1608–1614.
26. Tortosa A, Bes-Rastrollo M, Sanchez-Villegas A, *et al.* (2007) Mediterranean diet inversely associated with the incidence of metabolic syndrome: the SUN prospective cohort. *Diabetes Care* **30**, 2957–2959.
27. Martinez-Gonzalez MA, Garcia-Arellano A, Toledo E, *et al.* (2012) A 14-item Mediterranean diet assessment tool and obesity indexes among high-risk subjects: the PREDIMED trial. *PLOS ONE* **7**, e43134.
28. Mendez MA, Popkin BM, Jakszyn P, *et al.* (2006) Adherence to a Mediterranean diet is associated with reduced 3-year incidence of obesity. *J Nutr* **136**, 2934–2938.
29. May AM, Romaguera D, Travier N, *et al.* (2012) Combined impact of lifestyle factors on prospective change in body weight and waist circumference in participants of the EPIC-PANACEA study. *PLOS ONE* **7**, e50712.
30. Beunza JJ, Toledo E, Hu FB, *et al.* (2010) Adherence to the Mediterranean diet, long-term weight change, and incident overweight or obesity: the Seguimiento Universidad de Navarra (SUN) cohort. *Am J Clin Nutr* **92**, 1484–1493.
31. Yannakoulia M, Panagiotakos D, Pitsavos C, *et al.* (2009) Five-year incidence of obesity and its determinants: the ATTICA study. *Public Health Nutr* **12**, 36–43.
32. Galisteo M, Duarte J & Zarzuelo A (2008) Effects of dietary fibers on disturbances clustered in the metabolic syndrome. *J Nutr Biochem* **19**, 71–84.
33. Schroder H (2007) Protective mechanisms of the Mediterranean diet in obesity and type 2 diabetes. *J Nutr Biochem* **18**, 149–160.
34. Hu GX, Chen GR, Xu H, *et al.* (2010) Activation of the AMP activated protein kinase by short-chain fatty acids is the main mechanism underlying the beneficial effect of a high fiber diet on the metabolic syndrome. *Med Hypotheses* **74**, 123–126.
35. Du H, van der AD, Ginder V, *et al.* (2009) Dietary energy density in relation to subsequent changes of weight and waist circumference in European men and women. *PLOS ONE* **4**, e5339.
36. Grossniklaus DA, Dunbar SB, Gary R, *et al.* (2012) Dietary energy density: a mediator of depressive symptoms and abdominal obesity or independent predictor of abdominal obesity? *Eur J Cardiovasc Nurs* **11**, 423–431.
37. Xu HE, Lambert MH, Montana VG, *et al.* (1999) Molecular recognition of fatty acids by peroxisome proliferator-activated receptors. *Mol Cell* **3**, 397–403.
38. Razquin C, Alfredo MJ, Martinez-Gonzalez MA, *et al.* (2009) The Mediterranean diet protects against waist circumference enlargement in 12Ala carriers for the PPARγ gene: 2 years' follow-up of 774 subjects at high cardiovascular risk. *Br J Nutr* **102**, 672–679.

6th paper

Yogurt consumption is negatively associated with cardiovascular disease events in Spanish adults. American Journal of Clinical Nutrition, under review.

Yogurt consumption is negatively associated with cardiovascular disease events in Spanish adults[1]

Anna N. Funtikova,[2,3] ,[4] Isaac Subirana,[3,5] Robert Elosua,[5] Montserrat Fitó,[2,6] Rowaedh Ahmed Bawaked,[2] and Helmut Schröder[2,3*]

[1] This research was supported by a grant (2FD097-0297-CO2-01) from Fondo Europeo de Desarrollo Regional (FEDER); by portions of grants from Spain's Ministry of Health (Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III: FEDER [PI11/01900], FEDER [CB06/02/0029] and Red Investigación Cardiovascular: HERACLES [RD12/0042]), and by a joint contract (CP03/00115) between the Instituto de Salud Carlos III and the Health Department of the Catalan Government (Generalitat de Catalunya), and AGAUR (2014 SGR 240). The CIBERESP and CIBEROBN are an initiative of the Instituto de Salud Carlos III, Madrid, Spain.

* To whom correspondence should be addressed: hschroeder@imim.es

[2] Cardiovascular Risk and Nutrition Research Group (CARIN), IMIM (Hospital del Mar Medical Research Institute), Barcelona, Spain; [3]CIBER Epidemiology and Public Health (CIBERESP), Instituto de Salud Carlos III, Madrid, Spain; [4]PhD program "Food and Nutrition", University of Barcelona, Spain; [5]Cardiovascular Epidemiology and Genetics Research Group (EGEC), IMIM (Hospital del Mar Medical Research Institute), Barcelona, Spain; [6]CIBER Physiopathology of Obesity and Nutrition (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain (MF);

[8]Abbreviations used: BMR, CVD, cardiovascular disease; CI, confidence interval; FFQ, food frequency questionnaire; HDL, high density lipoprotein; HR, hazard ratio; LTPA, leisure-time physical activity.

Word count: 2704

Figures: 1

Tables: 4

Supplemental online material: None

Running title: Yogurt consumption and CVD

Key words: cardiovascular disease, dairy products, yogurt, stroke

ABSTRACT

Background: Yogurt consumption is negatively associated with cardiovascular risk factors. However, little is known about the relationship between yogurt consumption and incidence of cardiovascular disease (CVD).

Objective: We assessed the association between yogurt consumption and incidence of myocardial infarction, angina, stroke, and cardiovascular death.

Design: Data were obtained from two population-based cohorts of the REGICOR study, including 9056 men and women with a medium follow-up of 6.7 years. Food consumption was estimated by a validated food frequency questionnaire and cardiovascular risk factors were measured. Cox regression models were used to estimate hazard ratios of cardiovascular events according to yogurt consumption.

Results: A total of 336 participants had at least one confirmed cardiovascular event. Yogurt consumption was reported by 78.4% of the participants, with a median intake of 62.5g among yogurt eaters. Those who consumed at least 1 daily serving (125g) of yogurt reduced CVD incidence by 33% compared to non-consumers [hazard ratio (HR) 0.67; 95% confidence interval (CI), 0.47-0.94] independently of lifestyle and sociodemographic and cardiovascular risk factors. The inverse linear trend across yogurt consumption categories was significant (p =0.010). The association was stronger for low-fat (p=0.018) compared to full-fat yogurt (p=0.043). In contrast, neither total dairy nor milk or cheese consumption were significantly associated with CVD incidence [HR 0.89, CI 0.67,1.18; HR 0.97, CI 0.63,1.50; HR 0.95, CI 0.63,1.43; respectively].

Conclusions: In this population-based study of Spanish men and women, yogurt consumption was negatively associated with risk of CVD events.

INTRODUCTION

Yogurt is one of 3500 types of fermented dairy products produced throughout the world. It has been part of the traditional diet in many different regions for thousands of years (1), and always has been considered part of a healthy diet (2). However, scientific interest in the benefits of this product in promoting cardiovascular health emerged much more recently. Until now, the debate focused on the beneficial properties of dairy products in general. For a significant period of time, excessive consumption of dairy products was considered harmful for the cardiovascular system because of a high level of saturated fats (3). Surprisingly, numerous studies then showed a protective effect of dairy products on cardiovascular risk factors and metabolic syndrome (4, 5). Furthermore, a protective association between both low-fat and high-fat dairy products and cardiovascular disease has been suggested (6). Dairy products are very diverse and, depending on the production process, the properties differ drastically. Yogurt also has its own specific characteristics. In addition to its properties in common with other dairy products, such as high content of calcium and proteins, yogurt differs because of its probiotic content and related aspects that make it especially attractive to study separately from other dairy products. Only a few studies have explored the association between yogurt consumption and CVD[8], with inconsistent findings (7-10).

We hypothesized a protective association between yogurt consumption and CVD events. Therefore, the objective of the study was to analyze the relationship between yogurt consumption and CVD events in Spanish men and women.

METHODS

Study population

Data were obtained from two population-based cross-sectional surveys conducted in Girona (Spain) in 2000 and 2005. The first survey included 3,058 randomly selected free-living men and women aged 25 to 74 years. The second survey included 6,352 men and women between 35 and 80 years old. The responds rate was 71.0% and 71.5% respectively. Participants who reported history of CVD (n=145), who participated in both surveys (n=28), and those with missing dietary data (n=181) were excluded from analysis. The final study was comprised of 9056 participants.

The studies were approved by the institutional ethics committee (CEIC-IMAS, Barcelona, Spain), participants signed an informed consent, and results of the examination were sent to all participants.

Outcomes

Multiple sources were used to identify potential CVD cases, including self-report, re-examination, and data links to primary care records, hospital admissions, and regional and national mortality data. All non-fatal diagnoses were verified by examining the corresponding electronic medical record. Unstable angina during follow-up was diagnosed by the presence of angina symptoms without an abnormal increase in the cardiac enzymes or troponin and with the relevant changes in serial electrocardiograms. Alternatively, with or without electrocardiographic changes, a diagnosis was made when suggestive symptoms were recorded during the event and confirmed either by a positive coronary angiography (stenosis >70%)

or by a positive stress test with or without isotopic stress gammagraphy. Non-fatal stroke events were included regardless of their hemorrhagic or ischemic origin, data which were not universally collected. Fatal events were assessed in the same manner as non-fatal cases for in-hospital deaths and by reviewing death certificates and autopsy results (available for approximately 15% of fatal events) for out-of-hospital deaths. Analyses considered the first cardiovascular event identified.

Dietary assessment

Dietary intakes were measured by a validated food frequency questionnaire (FFQ) (11) (12) administered by a trained interviewer. We collected usual intakes over the past year by a self-administered FFQ; participants indicated their usual consumption from a 165-item food and beverage list and chose one of 10 frequency categories that ranged from “never or <1 time/month” to “≥6 times/day”. Intakes were converted to mean g/day using standard reference portion sizes. The consumption of full fat and low fat yogurt was recorded. One serving per day of yogurt consumption corresponds to 125g of yogurt.

Measurement of non-dietary variables

Information on demographic and socioeconomic variables, medical history, and smoking was obtained through standard structured questionnaires. Leisure-time physical activity was measured by the Minnesota leisure-time physical activity questionnaire, also administered by a trained interviewer. This questionnaire has been validated for Spanish men and women (13, 14).

Anthropometric data

Measurements were performed by a team of trained nurses and interviewers who used the same standard methods in both surveys. A precision scale of easy calibration was used for weight measurement, with participants in underwear. Misreporting of energy intake was calculated by the revised Goldberg method as previously reported (15).

Laboratory measurements

Blood was drawn after 10 to 14 hours fasting. Serum-sample aliquots were stored at -80ºC. Glycemia was measured in an aliquot of serum. Total cholesterol and triglyceride concentrations were determined enzymatically (Roche Diagnostics, Basel, Switzerland). High-density lipoprotein (HDL) cholesterol was measured after precipitation of apoprotein B-containing lipoproteins with phosphotungstic-Mg++ (Boehringher, Mannheim, Germany). Analyses were performed in a Cobas Mira Plus autoanalyzer (Roche Diagnostics, Basel, Switzerland) and quality was monitored with External Quality Assessment-WHO Lipid Program (World Health Organization, Prague, Czech Republic) and Monitrol-Quality Control Program (Baxter Diagnostics, Dudingen, Switzerland). Low-density lipoprotein (LDL) cholesterol was calculated by the Friedewald equation whenever triglycerides were <300 mg/dL.

Blood pressure measurement

Blood pressure was measured with a periodically calibrated mercury sphygmomanometer. A cuff adapted to upper arm perimeter (young, adult, obese) was selected for each participant. Measurements were performed after a 5-minute rest. Two measurements were taken and the lower value was recorded for the study.

Statistical analysis

To compare baseline characteristics of participants according to categories of yogurt consumption (none or rare consumption, weekly, daily), ANOVA, Kruskal-Wallis test or Chi-squared test was performed, depending on normal or non-normal distributed or categorical distribution, respectively. To assess linear trend, polynomial contrasts or chi squared test were used for continuous and categorical variables, respectively.

Multiple-adjusted Cox proportional hazards regression models were fitted to assess the association between yogurt consumption and the incidence of CVD, adjusting for potential confounding variables. Linear trend was tested by introducing a continuous variable as the independent variable of interest, assigning to each individual the median level of food consumption of their respective category. Secondary analysis was performed for the associations between incidence of CVD and milk, cheese, and all dairy products.

Effect modification between yogurt consumption and sex, age, and cohort was tested using the likelihood ratio test for product-terms (interactions). Proportional hazard assumption was assessed by the R package function {survival} cox.7ph. Cubic spline analysis [ref:gam] was performed to test non-linear associations between log-transformed consumption of yogurt and incidence of CVD, using the R package 'gam'.

To deal with missing cardiovascular risk variables (total cholesterol n= 269; LDL-C n= 426; HDL-C n= 290; glycemia n= 209; systolic blood pressure n= 22; diastolic blood pressure n=24, and smoking n= 132), we performed multiple imputation with all explanatory variables considered in the model as well as the response variable, using the R package 'mice'. Multivariate models were then fitted to each of the 20 imputed data points computed from the multiple imputation procedure and the results, i.e Hazard Ratios, were calculated by the R package ‘mitools’ [ref:mitools].

The assumption of normality in the regression models was assessed using the normal probability plot. All p values were 2-tailed. Differences were considered significant if $p<0.05$. Statistical analysis was performed using SPSS version 18.0. (SPSS Inc. Chicago, Ill., USA) and R version 3.0.2 [ref:R].

RESULTS

During 6.7 years of median follow-up totaling 63841 person-years, 336 confirmed fatal and non-fatal CVD events (112 strokes, 156 myocardial infarctions and angina) were recorded.

No significant interactions were observed for sex/yogurt (p=0.62), age/yogurt (p=0.26), and cohort/yogurt (p= 0.67).

Yogurt consumption was reported by 78.4% (n=7100) of the population. Median yogurt intake of yogurt eaters was 62.5g/d (interquartile range 26.7-125).

Women were more prone to yogurt consumption than were men (Table 1). Participants with higher consumption of yogurts were less likely to smoke, weighed slightly more, were higher educated, and had lower levels of triglycerides, fasting glucose, blood pressure and cardiovascular events, along with higher levels of HDL-cholesterol, compared to their peers who did not consume yogurts (Table 1).

Furthermore, participants who consumed more yogurt also had higher consumption of vegetable, fruits, all dairy products, milk, cheese, fish and seafood, carbohydrates, proteins, calcium, dietary fiber, and total energy (Table 2). In contrast, they consumed less alcohol, cereals, meat, lipids, and mono- and polyunsaturated fatty acids (Table 2).

Total dairy, milk and cheese consumption levels were not significantly associated with CVD incidence (Figure 1). Daily consumption of yogurts decreased the risk of CVD up to 37% (HR 0.62; 95% CI 0.47, 0.84) in a model adjusted for sex and age (Table 3). Further adjustment for energy, fiber, calcium, saturated fatty acids, vegetable, red meat and derivatives (e.g., sausages), fish and seafood, energy over- and under-reporting, leisure-time physical activity, education, smoking, weight, triglycerides, total cholesterol, LDL cholesterol, HDL cholesterol, fasting glucose, and systolic and diastolic blood pressure slightly attenuated this association (HR 0.67; CI 0.47,0.94). The association was stronger for low-fat (p=0.018) compared to full-fat yogurt (p=0.043). The association was somewhat stronger for low-fat vs full-fat yogurt (Table 3). Risk reduction by yogurt consumption was stronger for stroke, compared to myocardial infarction and angina (Table 4).

We observed no evidence of non-linearity in the association between log-transformed yogurt intake and CVD incidence ($P_{curvature}$ value = 0.42); (Figure 1).

DISCUSSION

In the present study, we found that daily yogurt consumption significantly decreased CVD incidence. Furthermore, this association was similar for different types of CVD events and was independent of lifestyle, cardiovascular risk factors, and other dietary variables related to CVD. In contrast, milk and cheese consumption were not significantly associated with CVD risk reduction.

Results from previous studies on the association between yogurt consumption and CVD are inconsistent. Three of four studies showed protective associations, but these were much weaker than in the present study. The 1999 analysis by Iso et al. (8) of the Nurses'

Health Study was the first to analyze the association between yogurt consumption, among other dairy products, and risk of stroke in women. They reported a 31% lower risk, albeit not significant, in incidence of ischemic stroke in participants consuming yogurt more than 4 times per week, compared to those who almost never ate yogurts. In the Netherlands Cohort Study (7), each 100ml increase in yogurt consumption per day was associated with a significant decrease of 32% and 30% of stroke mortality in men and women, respectively. Furthermore, yogurt consumption showed a weak but significant inverse association with the risk of all-cause mortality in men. Results of the Whitehall II study showed an inverse association of yogurt consumption and all-cause mortality. Additionally, the risk of coronary heart disease decreased with increasing cheese consumption. However, the significance of these associations disappeared in fully adjusted models (10). In contrast, Larsson and colleagues (9) reported a positive association between yogurt consumption and subarachnoid hemorrhage. A case-control study (16) showed that patients with ischemic stroke had consumed less probiotic yogurt with lactobacillus species (OR 0.88, p=0.001) than controls; this trend persisted in all ischemic stroke subtypes. Fermented dairy consumption was associated with lower risk of myocardial infarction (17), stroke (18), coronary events (18) and all-cause mortality (19). In the present study, yogurt consumption was protectively associated with stroke, myocardial infarction, and angina. Furthermore, the association of yogurt consumption and CVD was independent of the fat content of the yogurt.

Many studies explain the beneficial properties of dairy products for cardiovascular health by their high calcium content (20). In the present study, yogurt consumption was associated with a healthy food and nutrient intake pattern, including calcium, and also with cardiometabolic health. However, adjustment for calcium, other foods and nutrients related to CVD, and baseline cardiovascular risk factors did not affect the association between yogurt consumption and CVD events. The role of gut microbiota in the development of CVD is currently under discussion (21). Yogurt contains a high content of live and active probiotics, which offers a probable explanation of the beneficial effects observed because probiotics are what most distinguishes it from other dairy products (22). Both animal and human studies have shown that probiotics protect against various cardiometabolic risk factors such as obesity, type 2 diabetes, and dyslipidemia (23). They affect the human organism in at least three ways: alteration of gut microbiota, improvement of gut mucosa, and modification of the immune system (24). These mechanisms produce an anti-inflammatory effect that has a major impact on the development of various cardiovascular risk factors (25). Experimental evidence indicates that some probiotics interact with the nuclear receptor PPRA-γ (26), which has an important role in lipid and cholesterol metabolism. Probiotics also affect lipid metabolism and decrease the concentration of cholesterol and blood lipids through modification of bile acids recirculation (27), degradation of bile acids (28), and decreased synthesis of cholesterol in the liver (29). In addition, probiotics decrease blood pressure (30), presumably by the production of nitric oxide (31). Furthermore, several clinical studies demonstrated that daily consumption of 200-300 gr of yogurt or other dairy products fermented with probiotics, such as cheese, has substantial effect on reduction of cholesterol, abdominal obesity, fasting glucose, and other cardiovascular risk factors (32-35).

Several studies have investigated the association between fermented dairy products (yogurt and cheese) and CVD (7, 10, 19). The inverse association found between consumption of fermented dairy products and CVD was mainly explained by yogurt intake (7, 10). Results of the present study agree with that finding, which seems somewhat surprising if the association between yogurt consumption and CVD is mediated by the probiotic effect on gut microbiota. The explanation could be the different amounts of probiotics consumed from yogurts and from cheese. The median weekly yogurt consumption was four times that of cheese and only 1% consumed 120g or more of cheese in the present study. Furthermore, one study demonstrated that cheese was less efficient than yogurt in successful delivery of probiotics to human intestine (36).

The present study has limitations. Due to the nature of observational studies, causal relationships cannot be drawn. Furthermore, all dietary instruments that measure past food intake are vulnerable to random and systematic measurement errors. The study's strengths are the population-based design, the use of validated assessment tools, and defining detailed, confirmed, clinical CVD end-points as the specific outcome.

The present study adds further evidence for the protective association between yogurt consumption and CVD. The results demonstrate a promising potential of yogurt consumption as an important food item in healthy diets for CVD prevention. Clinical intervention studies are needed to confirm the beneficial properties of yogurt for cardiovascular health.

ACKNOWLEDGMENTS

The authors appreciate the English revision by Elaine Lilly, PhD (Writers First Aid).

The authors' responsibilities were as follows: ANF and HS designed the research; ANF, IS, RE, MF, RAB, and HS conducted the research; IS and HS analyzed the data; ANF wrote the manuscript and ANF and HS had primary responsibility for the final content. All

authors read and approved the final manuscript. None of the authors report any conflicts of interest.

REFERENCES

1. Donovan SM, Shamir R. Introduction to the yogurt in nutrition initiative and the First Global Summit on the health effects of yogurt. Am J Clin Nutr 2014;99(5 Suppl):1209S-11S. doi: 10.3945/ajcn.113.073429ajcn.113.073429 [pii].

2. Cormier H, Thifault E, Garneau V, Tremblay A, Drapeau V, Perusse L, Vohl MC. Association between yogurt consumption, dietary patterns, and cardio-metabolic risk factors. Eur J Nutr 2015. doi: 10.1007/s00394-015-0878-1.

3. Astrup A, Dyerberg J, Elwood P, Hermansen K, Hu FB, Jakobsen MU, Kok FJ, Krauss RM, Lecerf JM, LeGrand P, et al. The role of reducing intakes of saturated fat in the prevention of cardiovascular disease: where does the evidence stand in 2010? Am J Clin Nutr 2011;93:684-8. doi: 10.3945/ajcn.110.004622 ajcn.110.004622 [pii].

4. Qin LQ, Xu JY, Han SF, Zhang ZL, Zhao YY, Szeto IM. Dairy consumption and risk of cardiovascular disease: an updated meta-analysis of prospective cohort studies. Asia Pac J Clin Nutr 2015;24:90-100. doi: 10.6133/apjcn.2015.24.1.09.

5. Abedini M, Falahi E, Roosta S. Dairy product consumption and the metabolic syndrome. Diabetes Metab Syndr 2015;9:34-7. doi: 10.1016/j.dsx.2014.04.027

6. Kratz M, Baars T, Guyenet S. The relationship between high-fat dairy consumption and obesity, cardiovascular, and metabolic disease. Eur J Nutr 2013;52:1-24. doi: 10.1007/s00394-012-0418-1.

7. Goldbohm RA, Chorus AM, Galindo Garre F, Schouten LJ, van den Brandt PA. Dairy consumption and 10-y total and cardiovascular mortality: a prospective cohort study in the Netherlands. Am J Clin Nutr 2011;93:615-27. doi: 10.3945/ajcn.110.000430ajcn.110.000430 [pii].

8. Iso H, Stampfer MJ, Manson JE, Rexrode K, Hennekens CH, Colditz GA, Speizer FE, Willett WC. Prospective study of calcium, potassium, and magnesium intake and risk of stroke in women. Stroke 1999;30:1772-9.

9. Larsson SC, Mannisto S, Virtanen MJ, Kontto J, Albanes D, Virtamo J. Dairy foods and risk of stroke. Epidemiology 2009;20:355-60. doi: 10.1097/EDE.0b013e3181935dd5.

10. Soedamah-Muthu SS, Masset G, Verberne L, Geleijnse JM, Brunner EJ. Consumption of dairy products and associations with incident diabetes, CHD and mortality in the Whitehall II study. Br J Nutr 2013;109:718-26. doi: 10.1017/S0007114512001845S0007114512001845 [pii].

11. Benitez-Arciniega AA, Mendez MA, Baena-Diez JM, Rovira Martori MA, Soler C, Marrugat J, Covas MI, Sanz H, Llopis A, Schroder H. Concurrent and construct validity of Mediterranean diet scores as assessed by an FFQ. Public health nutrition 2011;14:2015-21. doi: 10.1017/S1368980011001212.

12. Schroder H, Covas MI, Marrugat J, Vila J, Pena A, Alcantara M, Masia R. Use of a three-day estimated food record, a 72-hour recall and a food-frequency questionnaire for dietary assessment in a Mediterranean Spanish population. Clinical nutrition 2001;20:429-37.

13. Elosua R, Garcia M, Aguilar A, Molina L, Covas MI, Marrugat J. Validation of the Minnesota Leisure Time Physical Activity Questionnaire In Spanish Women. Investigators of the MARATDON Group. Medicine and science in sports and exercise 2000;32:1431-7.

14. Elosua R, Marrugat J, Molina L, Pons S, Pujol E. Validation of the Minnesota Leisure Time Physical Activity Questionnaire in Spanish men. The MARATHOM Investigators. American journal of epidemiology1994;139:1197-209.

15. Mendez MA, Popkin BM, Buckland G, Schroder H, Amiano P, Barricarte A, Huerta JM, Quiros JR, Sanchez MJ, Gonzalez CA. Alternative methods of accounting for underreporting and overreporting when measuring dietary intake-obesity relations. American journal of epidemiology 2011;173:448-58. doi: 10.1093/aje/kwq380.

16. Rodriguez-Campello A, Jimenez-Conde J, Ois A, Cuadrado-Godia E, Giralt-Steinhauer E, Schroeder H, Romeral G, Llop M, Soriano-Tarraga C, Garralda-Anaya M, et al. Dietary habits in patients with ischemic stroke: a case-control study. PLoS One 2014;9:e114716. doi: 10.1371/journal.pone.0114716 PONE-D-14-24941 [pii].

17. Warensjo E, Jansson JH, Cederholm T, Boman K, Eliasson M, Hallmans G, Johansson I, Sjogren P. Biomarkers of milk fat and the risk of myocardial infarction in men and women: a prospective, matched case-control study. Am J Clin Nutr 2010;92:194-202. doi: 10.3945/ajcn.2009.29054ajcn.2009.29054 [pii].

18. Sonestedt E, Wirfalt E, Wallstrom P, Gullberg B, Orho-Melander M, Hedblad B. Dairy products and its association with incidence of cardiovascular disease: the Malmo diet and cancer cohort. Eur J Epidemiol 2011;26:609-18. doi: 10.1007/s10654-011-9589-y.

19. van Aerde MA, Soedamah-Muthu SS, Geleijnse JM, Snijder MB, Nijpels G, Stehouwer CD, Dekker JM. Dairy intake in relation to cardiovascular disease mortality and all-cause mortality: the Hoorn Study. Eur J Nutr 2013;52:609-16. doi: 10.1007/s00394-012-0363-z.

20. Lorenzen JK, Nielsen S, Holst JJ, Tetens I, Rehfeld JF, Astrup A. Effect of dairy calcium or supplementary calcium intake on

postprandial fat metabolism, appetite, and subsequent energy intake. Am J Clin Nutr 2007;85:678-87. doi: 85/3/678 [pii].

21. Tang WH, Hazen SL. The contributory role of gut microbiota in cardiovascular disease. The Journal of clinical investigation 2014;124:4204-11. doi: 10.1172/jci72331.

22. Lourens-Hattingh A VB. Yogurt as probiotic carrier food. Int Dairy J 2001;11:1-17.

23. Ebel B, Lemetais G, Beney L, Cachon R, Sokol H, Langella P, Gervais P. Impact of probiotics on risk factors for cardiovascular diseases. A review. Crit Rev Food Sci Nutr 2014;54:175-89. doi: 10.1080/10408398.2011.579361.

24. Butel MJ. Probiotics, gut microbiota and health. Med Mal Infect 2014;44:1-8. doi: 10.1016/j.medmal.2013.10.002 S0399-077X(13)00307-7 [pii].

25. Korhonen R, Korpela R, Saxelin M, Maki M, Kankaanranta H, Moilanen E. Induction of nitric oxide synthesis by probiotic Lactobacillus rhamnosus GG in J774 macrophages and human T84 intestinal epithelial cells. Inflammation 2001;25:223-32.

26. Kelly D, Campbell JI, King TP, Grant G, Jansson EA, Coutts AG, Pettersson S, Conway S. Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. Nat Immunol 2004;5:104-12. doi: 10.1038/ni1018ni1018 [pii].

27. Martin FP, Wang Y, Sprenger N, Yap IK, Lundstedt T, Lek P, Rezzi S, Ramadan Z, van Bladeren P, Fay LB, et al. Probiotic modulation of symbiotic gut microbial-host metabolic interactions in a humanized microbiome mouse model. Mol Syst Biol 2008;4:157. doi: 10.1038/msb4100190 msb4100190 [pii].

28. Gilliland SE, Walker DK. Factors to consider when selecting a culture of Lactobacillus acidophilus as a dietary adjunct to produce a hypocholesterolemic effect in humans. J Dairy Sci 1990;73:905-11.

doi: S0022-0302(90)78747-4 [pii] 10.3168/jds.S0022-0302(90)78747-4.

29. Chiu CH, Lu TY, Tseng YY, Pan TM. The effects of Lactobacillus-fermented milk on lipid metabolism in hamsters fed on high-cholesterol diet. Appl Microbiol Biotechnol 2006;71:238-45. doi: 10.1007/s00253-005-0145-0.

30. Khalesi S, Sun J, Buys N, Jayasinghe R. Effect of probiotics on blood pressure: a systematic review and meta-analysis of randomized, controlled trials. Hypertension 2014;64:897-903. doi: 10.1161/HYPERTENSIONAHA.114.03469HYPERTENSIONAHA.114.03469 [pii].

31. Ait-Belgnaoui A, Han W, Lamine F, Eutamene H, Fioramonti J, Bueno L, Theodorou V. Lactobacillus farciminis treatment suppresses stress induced visceral hypersensitivity: a possible action through interaction with epithelial cell cytoskeleton contraction. Gut 2006;55:1090-4. doi: gut.2005.084194 [pii]10.1136/gut.2005.084194.

32. Ejtahed HS, Mohtadi-Nia J, Homayouni-Rad A, Niafar M, Asghari-Jafarabadi M, Mofid V. Probiotic yogurt improves antioxidant status in type 2 diabetic patients. Nutrition 2012;28:539-43. doi: 10.1016/j.nut.2011.08.013S0899-9007(11)00310-8 [pii].

33. Ejtahed HS, Mohtadi-Nia J, Homayouni-Rad A, Niafar M, Asghari-Jafarabadi M, Mofid V, Akbarian-Moghari A. Effect of probiotic yogurt containing Lactobacillus acidophilus and Bifidobacterium lactis on lipid profile in individuals with type 2 diabetes mellitus. J Dairy Sci 2011;94:3288-94. doi: 10.3168/jds.2010-4128S0022-0302(11)00321-3 [pii].

34. Anderson JW, Gilliland SE. Effect of fermented milk (yogurt) containing Lactobacillus acidophilus L1 on serum cholesterol in hypercholesterolemic humans. J Am Coll Nutr 1999;18:43-50.

35. Kadooka Y, Sato M, Imaizumi K, Ogawa A, Ikuyama K, Akai Y, Okano M, Kagoshima M, Tsuchida T. Regulation of abdominal

adiposity by probiotics (Lactobacillus gasseri SBT2055) in adults with obese tendencies in a randomized controlled trial. Eur J Clin Nutr 2010;64:636-43. doi: 10.1038/ejcn.2010.19ejcn201019 [pii].

36. Saxelin M, Lassig A, Karjalainen H, Tynkkynen S, Surakka A, Vapaatalo H, Jarvenpaa S, Korpela R, Mutanen M, Hatakka K. Persistence of probiotic strains in the gastrointestinal tract when administered as capsules, yoghurt, or cheese. Int J Food Microbiol 2010;144:293-300. doi: 10.1016/j.ijfoodmicro. 2010.10.009S0168-1605(10)00561-1 [pii].

TABLE 1 General characteristics of the study population[1]

	Yogurt consumption			p
	None or rare (n= 2871)	Weekly (n= 3751)	Daily (n= 2434)	
Women, %	40.2%	54.7	64.1	<0.001
Age, years	56.4±13.2	52.2 ±13.0	55.6 ±13.3	<0.001
Smoking,[2] %	28.1	24.4	17.7	<0.001
Education,[3] %	38.9	51.4	42.3	0.002
LTPA, METs·min/d	221 (104;414)	217 (114;367)	226 (119;389)	0.344
Weight, kg	74.1±14.3	73.3±14.4	71.2±13.5	<0.001
Total cholesterol, mg/dL	214±42.7	214±41.3	215±45.2	0.247
Triglycerides, mg/dL	97.0 (71.0;135)	93.0 (69.0;129)	91.0 (69.0;125)	<0.001
LDL cholesterol, mg/dL	139±38.5	139±37.9	139±40.2	0.858
HDL cholesterol, mg/dL	51.1±14.0	52.2±13.8	53.7±14.5	<0.001
Fasting glucose, mg/dL	102±27.2	99.7±25.0	99.6±25.9	<0.001

Table continues

Table 1 (continued)

	Yogurt consumption			p
	None or rare (n= 2871)	Weekly (n= 3751)	Daily (n= 2434)	
Systolic blood pressure, mmHg	130±23.5	126±25.9	127±22.4	<0.001
Diastolic blood pressure, mmHg	79.2±12.3	78.4±19.1	77.3±11.8	<0.001
All cardiovascular events (acute myocardial infarction, angina, stroke), %	5.5	2.9	2.8	<0.001
Acute myocardial infarction or angina, %	3.6	2.1	1.8	<0.001
Stroke, %	2.1	0.8	1.0	<0.001

LTPA, leisure-time physical activity; METs, metabolic equivalents; BMI, body mass index; CI, confidence interval; LTPA, leisure-time physical activity; HDL, high-density lipoprotein; LDL, low-density lipoprotein

[1]Means and 95% confidence intervals were calculated according to categories of yogurt consumption for continuous variables (age, BMI, weight, total, LDL cholesterol, HDL cholesterol, fasting glucose, systolic blood pressure, diastolic blood pressure), number and proportions for categorical variables (women, smoking, education, smokers, acute myocardial infarction or angina, all cardiovascular events, and stroke)..

Table continues

Table1 continued

and median and 25th and 75th percentiles for LTPA. *p* values were obtained by ANOVA, Kruskal Wallis, and Pearson chi-square for normal continuous, non-normal continuous, and categorical variables, respectively.

[2]Active smokers or ex-smokers less than 1 year. [3]More than secondary school education.

1 serving of yogurt = 125 g. Interquartile range of yogurt consumption categories: None or rare= 0g-8.4g; Weekly= 26.7g-62.5g; Daily= 125g-250g.

TABLE 2 Consumption of food groups and nutrients of the study population[1]

Characteristics	Yogurt consumption			p
	None (n= 2871)	Weekly (n= 3751)	Daily (n= 2434)	
Vegetables, g/4.18 MJ	144 (87.6;216)	151 (98.7;220)	167 (110;243)	<0.001
Fruits, g/4.18 MJ	153 (75.5;263)	152 (90.3;250)	186 (113;288)	<0.001
Cereals, g/4.18 MJ	74.0 (53.7;97.0)	72.7 (53.8;92.8)	65.7 (48.2;85.9)	<0.001
Fish and seafood, g/4.18 MJ	29.5 (19.9;42.1)	30.9 (21.6;42.6)	30.8 (20.5;43.0)	0.027
Red meat and derivatives, g/4.18 MJ	45.8 (30.9;61.7)	46.3 (32.9;61.4)	38.1 (24.5;52.9)	<0.001
Olive oil, g/4.18 MJ	10.5 (6.08;16.2)	10.2 (5.75;15.4)	10.3 (5.99;15.1)	0.032
Dairy products, g/4.18 MJ	74.8 (13.4;127)	105 (54.8;157)	164 (110;233)	<0.001
Milk, g/4.18 MJ	60.3 (0.00;106)	71.3 (10.9;109)	76.1 (12.3;125)	<0.001
Cheese, g/4.18 MJ	1.7 (0.0;5.1)	3.1 (0.0;6.6)	2.2 (0.0;6.6)	<0.001

Table continues

Table 2 (continued)

Characteristics	Yogurt consumption			p
	None (n= 2871)	Weekly (n= 3751)	Daily (n= 2434)	
Alcohol, g/4.18 MJ	5.0 (0.0;18.4)	3.9 (0.7;11.9)	2.1 (0.0;10.0)	<0.001
Energy, MJ	9.8±	10.2± (8.4;12.4)	10.8± (8.7;13.3)	<0.001
Energy underreporting, %	28.5	24.1	19.7	<0.001
Energy overreporting, %	9.1	9.2	14.1	<0.001
Carbohydrates, %	41.1±7.97	41.0±6.99	42.3±7.32	<0.001
Proteins, %	16.8±3.01	17.6±2.72	18.5±2.98	<0.001
Lipids, %	41.4±7.24	41.6±6.52	40.2±6.92	<0.001
Saturated fatty acids, %	11.9±2.60	12.3±2.37	11.9±2.65	0.019
Monounsaturated fatty acids, %	20.6±4.81	20.6±4.48	19.8±4.44	<0.001
Polyunsaturated fatty acids, %	6.3±1.8	6.1±1.5	5.9±1.7	<0.001
Calcium, mg/4.18 MJ	434±154	491±155	616±175	<0.001
Fiber, g/4.18 MJ	11.5±4.56	11.5±4.18	12.4±4.52	<0.001

Table continuous

Table 2 continued

[1]Means and 95% confidence intervals for continuous variables (energy, calcium, fiber) and median and 25th and 75th percentiles for the rest of the variables were calculated according to categories of yogurt consumption. [2]*p* values were obtained by ANOVA, Kruskal Wallis, and Pearson chi-square for normal continuous, non-normal continuous and categorical variables, respectively. Interquartile range of yogurt consumption categories: None or rare= 0g-8.4g; Weekly= 26.7g-62.5g; Daily= 125g-250g.

TABLE 3 Hazard ratios of yogurt consumption and risk of all cardiovascular events (acute myocardial infarction, angina, stroke)[1]

Yogurt consumption	Number	Person/year	CV events	Model 1	Model 2	Model 3
				HR (95%CI)	HR (95%CI)	HR (95%CI)
All yogurts[2]						
None or rare consumption	2871	20303	159	1	1	1
Weekly consumption	3751	26419	108	0.75 (0.56; 0.96)	0.76 (0.59; 0.98)	0.78 (0.58, 1.04)
Daily consumption	2434	17119	69	0.63 (0.47; 0.84)	0.63 (0.46; 0.87)	0.67 (0.47, 0.94)
p for trend				0.001	0.003	0.010
Low-fat yogurts[2,3]						
None or rare consumption	5830	41340	241	1	1	1
Weekly consumption	1962	13595	59	0.83 (0.62; 1.11)	0.83 (0.62; 1.12)	0.85 (0.63; 1.15)
Daily consumption	1264	8906	36	0.60 (0.42; 0.87)	0.60 (0.40; 0.91)	0.62 (0.42; 0.92)
p for trend				0.006	0.010	0.018

Table continues

Table 3 (continued)

Yogurt consumption	Number	Person/year	CV events	Model 1	Model 2	Model 3
				HR (95%CI)	HR (95%CI)	HR (95%CI)
Full-fat yogurts[2,3]						
Non or rare consumption	5365	37679	244	1	1	1
Weekly consumption	2510	17833	59	0.68 (0.51; 0.92)	0.70 (0.52; 0.94)	0.72 (0.54; 0.98)
Daily consumption	1181	8329	33	0.67 (0.47; 0.98)	0.69 (0.46; 1.02)	0.76 (0.51; 1.13)
p for trend				0.005	0.011	0.043

[1]Cox proportional hazards models were constructed to estimate HR and 95% CIs of association between yogurt consumption and cardiovascular events. Model 1: adjusted for sex and age. Model 2: adjusted for age, sex, energy intake, fiber, calcium, saturated fatty acids, vegetable consumption, fruit consumption, read meat and derivatives consumption, fish and seafood consumption, energy over- and under-reporting. Model 3: additionally to the model 2 adjusted for continues variables of triglycerides, total cholesterol, systolic and diastolic blood pressure, LDL-cholesterol, HDL-cholesterol, fasting glucose, LTPA, education, smoking and weight. [2]1 serving of yogurt = 125 g. [3]Mutually adjusted. CI, confidence interval; HR, hazard ratio; CV, cardiovascular; LTPA, leisure-time physical activity; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Interquartile range of yogurt consumption categories: None or rare= 0g-8.4g; Weekly= 26.7g-62.5g; Daily= 125g-250g.

TABLE 4 Hazard ratios of yogurt consumption and risk of cardiovascular events (acute myocardial infarction/angina, stroke)[1]

Yogurt consumption	Number	Person/year	CV events	Model 1	Model 2	Model 3
				HR (95%CI)	HR (95%CI)	HR (95%CI)
Myocardial infarction/angina						
None or rare consumption	2871	20303	103	1	1	1
Weekly consumption	3751	26419	79	0.84 (0.62; 1.13)	0.85 (0.63; 1.15)	0.87 (0.64; 1.18)
Daily consumption	2434	17119	44	0.65 (0.45; 0.93)	0.66 (0.44; 0.98)	0.73 (0.49; 1.09)
p for trend				0.017	0.037	0.113
Stroke						
None or rare consumption	2871	20303	60	1	1	1
Weekly consumption	3751	26419	31	0.94 (0.56, 1.58)	0.95 (0.56, 1.60)	0.61 (0.39; 0.96)
Daily consumption	2434	17119	25	0.58 (0.35, 0.95)	0.58 (0.35, 0.96)	0.54 (0.32; 0.92)
p for trend				0.029	0.024	0.012

Table continues

Table 4 (continued)

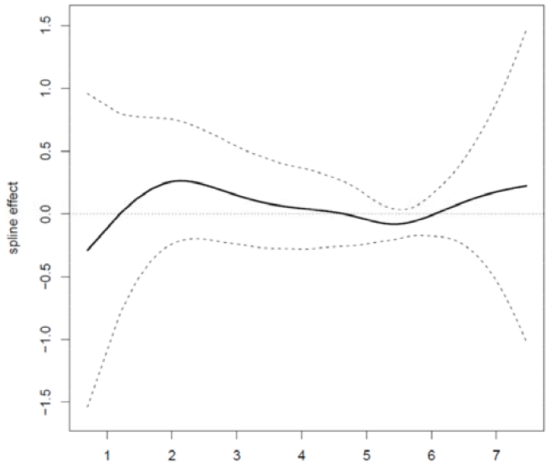
[1]Cox proportional hazards models were constructed to estimate hazard ratios and 95% CIs of association between yogurt consumption and cardiovascular events. Model 1: adjusted for sex and age. Model 2: adjusted for age, sex, energy intake, fiber, calcium, saturated fatty acids, vegetable consumption, fruit consumption, read meat and derivatives consumption, fish and seafood consumption, energy over- and under-reporting. Model 3: additionally to the model 2 adjusted for continues variables of triglycerides, total cholesterol, systolic and diastolic blood pressure, LDL-cholesterol, HDL-cholesterol, fasting glucose, LTPA, education, smoking and weight. 1 serving of yogurt = 125 g.

CI, confidence interval; HR, hazard ratio; CV, cardiovascular; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LTPA, leisure-time physical activity. Interquartile range of yogurt consumption categories: None or rare= 0g-8.4g; Weekly= 26.7g-62.5g; Daily= 125g-250g.

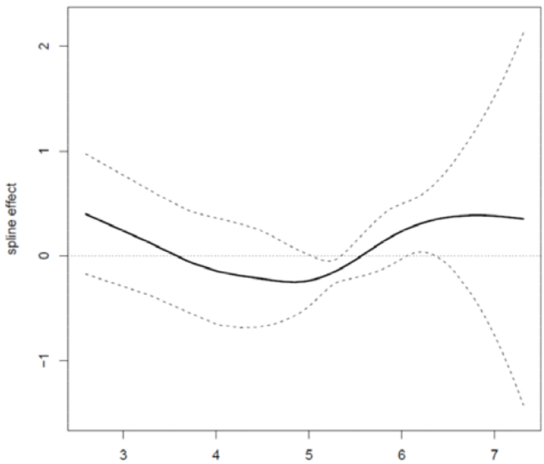
FIGURE 1

Model 1.

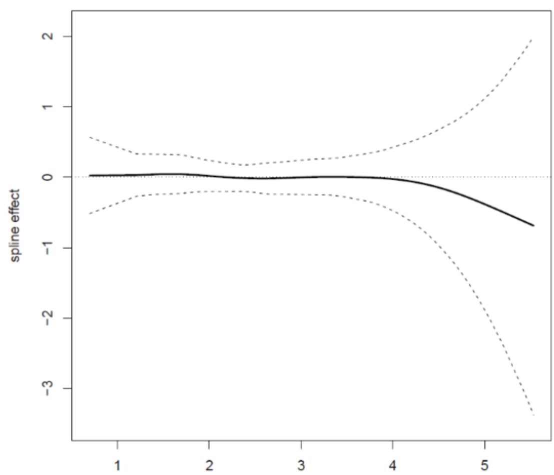
a) lineal p value = 0.39
non lineal *p value* = 0.42



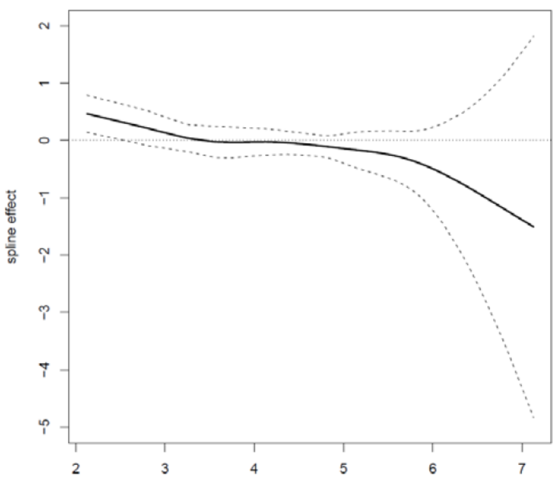
b) lineal p value = 0.81
non lineal *p value* = 0.016



c) lineal p value = 0.81
non lineal *p value* = 0.96

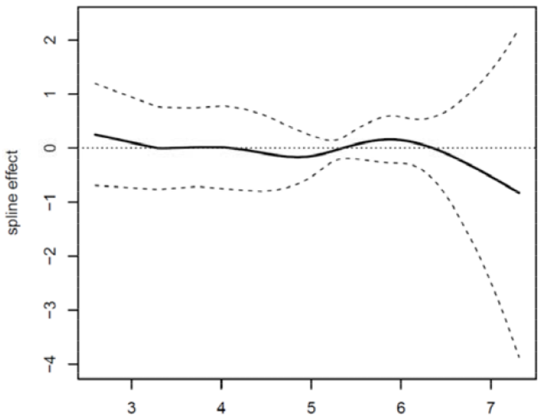


d) lineal p value = 0.007
non lineal *p value* = 0.42

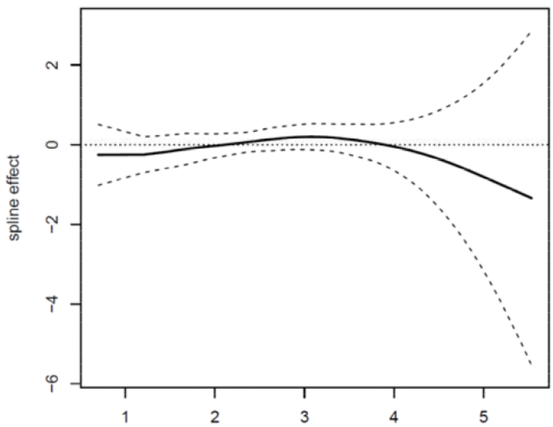


Model 2.

a) lineal *p value* = 0.83
non lineal *p value* = 0.70

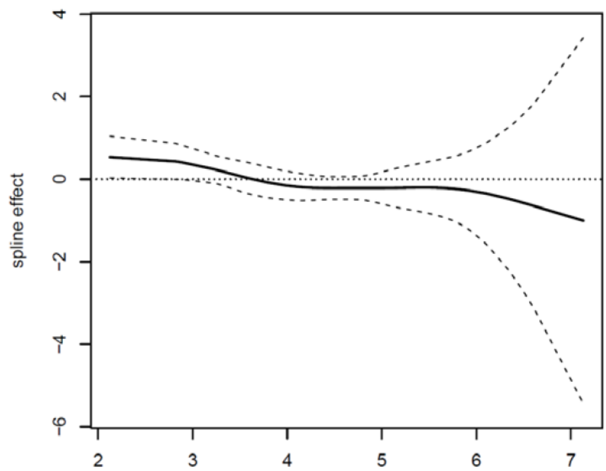


b) lineal *p value* = 0.37
non lineal *p value* = 0.63



c) lineal *p value* = 0.014
non lineal *p value* = 0.64

b



Estimated log transformed dairy (a model 1), milk (b model 1, a model 2), cheese (c model 1, b model 2), and yogurt (d model 1, c model 2) consumption based on spline regression (95% confidence interval = dotted lines) according to incidence of CVD in Spanish adults. Model 1 was adjusted for age, sex, energy intake, fiber, calcium, saturated fatty acids, vegetable consumption, fruit consumption, read meat and derivatives consumption, fish and seafood consumption, energy over- and under-reporting, LTPA, education, and weight. Model 2 Milk, cheese, and yogurt were in addition mutually adjusted.

7. Discussion

7.1. Main results

This thesis research presents studies about dietary pattern analysis, and the association between CVD risk factors and events and dietary patterns, and two of their components, soft drinks and yogurt. This research was based on data from 2 population-based surveys carried out within the REGICOR study. In this discussion section, we will consider the results of the papers included in the thesis in more general terms, provide an update on current research in this area, and present a broader interpretation of the main findings.

In this thesis research, we started with an exploration of methodological issues related to the dietary pattern approach. First, we demonstrated modest to good validity of *a priori* and *a posteriori* dietary patterns; second, we showed that energy under-reporting affected dietary patterns defined by cluster analysis, and secular trends in those dietary patterns; third, we showed that the incidence of abdominal obesity increased with higher consumption of soft drinks, and abdominal adiposity decreased with greater adherence to the Mediterranean dietary pattern; finally, we provided evidence of a beneficial relationship between yogurt consumption and CVD risk.

7.2 Update on recent research in this area

The sDQS is one of the few existing short assessment tools for estimating diet quality in adults. The simulation of answers on this screener in our study showed the good predictive validity of this tool. Most short dietary assessment tools, which to date have been developed in different populations, present good validity and correlation with biomarkers and food diaries [112], and can therefore identify people with nutritional risk. These tools were designed for use in time-limited settings. To date, most short dietary assessment tools have been developed and validated in the US population [112], and are very convenient for both clinical settings and nutritional epidemiology. Thus, other countries have gradually developed and validated their own tools, adapted to needs and dietary habits of their population [45, 113, 114].

The validity of dietary patterns derived using cluster analysis is less well established than that of dietary indexes. All studies, including ours, have demonstrated modest to fair validity, although only one study used two different methods of dietary data collection to perform relative validity analysis[114], while the others analyzed internal validity [12, 115]. The validity of dietary patterns defined using cluster analysis has received even less attention than their reproducibility [14]. The results of several studies have shown good reproducibility of the dietary clusters [12, 115, 116], and we also showed fair reproducibility in our study. Thus, to date there is insufficient evidence to draw a clear general conclusion about the validity and reproducibility of dietary clusters, as only a few studies have assessed this issue. Further research is required to analyze the validity and reproducibility of dietary clusters using different methods for collecting dietary data and different algorithms for cluster analysis.

Energy misreporting is a major concern for the analysis of dietary data [117]. There is little evidence on the effect of energy misreporting on the construction of dietary patterns, and secular trends therein [14]. Energy under-reporting is the most prevalent problem for energy misreporting, in that it affects not only consumption of nutrients, foods and food groups [118], but, as shown in our study, dietary patterns and secular trends in dietary habits.

Therefore, it also affects the diet-disease association, which makes it a serious challenge for nutritional epidemiology. This is especially problematic when analyzing the association between diet and obesity [119]. In our study we showed that conclusions about secular trends in dietary habits can be biased by energy under-reporting, which may lead to the development of misguided dietary guidelines and nutrition policies. Several studies have shown the effect of energy under-reporting on the association between diet and obesity, the exclusion of energy under-reporters changes the magnitude and even the direction of the association [120, 121]. Assessment methods should be improved in order to minimize the problem of implausible energy reporting. Modern computerized technologies will play an important role in reducing this problem, and in enhancing dietary assessment methods in general [117]. Considering the importance of implausible energy reporting, all further analyses carried out within this PhD research were controlled for this factor. In our study of the relationship between the Mediterranean diet and abdominal adiposity, the proportion of implausible energy reporters decreased with increased adherence to the Mediterranean dietary pattern. In the study of the association between soft drink consumption and abdominal obesity, one third of participants were categorized as implausible energy reporters, although this did not significantly affect the association between the exposure and the health outcome.

High consumption of soft drinks and their important contribution to total energy intake in the usual human diet is a quite recent dietary trend [122]. Work in ours and other groups highlights the important impact of soft drinks consumption in the development of general and abdominal obesity. A recent prospective study in children and adolescents confirmed our findings in the adult population, demonstrating that soft drinks consumption is associated with increased waist circumference during three years of follow-up, independently of general obesity [123]. The same analysis in plausible energy reporters revealed an even stronger association. Another new study performed a similar analysis to ours, namely the impact of substituting soft drinks with milk, juice or water on surrogate markers of general and central adiposity during 6 years of follow-up [124]. The investigators found a similar but slightly smaller magnitude of association for substitution with water and milk than in our studies, and no association with juice. Furthermore, in a recent review from the same group, Zheng et al. [125] summarized the findings of several prospective studies and RCTs on the effect of substituting soft drinks with other beverages. They confirmed that consumption of milk, artificially sweetened beverages, tea, coffee, juice or water instead of soft drinks was associated with lower general and central obesity, and risk of type 2 diabetes and stroke in cohort studies, and with reduced general obesity, high blood pressure and fasting glucose in RCTs. There are various reasons for the adverse health effects of soft drinks consumption, from damaging metabolic processes such as induced de novo hepatic lipogenesis or insulin resistance [126], to an association with unhealthy lifestyles, such as increased sodium consumption and generally unhealthy dietary patterns [127]. In our study, higher consumption of soft drinks was significantly associated with lower adherence to the healthy Mediterranean dietary pattern. The opposite trend was observed for juice consumption, with higher juice intake associated with greater adherence to the Mediterranean diet.

These results confirm that people with greater adherence to the Mediterranean diet are more likely to have a better lifestyle, and socioeconomic and anthropometric profile [128]. While healthy lifestyle habits are also a part of the traditional Mediterranean diet [129], the Mediterranean diet has been shown in many cohort studies and RCTs to be independently very effective in improving CVD risk factor profile and risk of CVD events [130]. New evidence has recently been published regarding a major risk factor of CVD, abdominal obesity, and the Mediterranean diet. In the PREDIMED RCT, Eguaras et al. [98] showed that even participants with high abdominal adiposity in the Mediterranean diet intervention group did not show increased risk of major CVD events. In contrast, the control diet did not protect participants with increased abdominal adiposity from CVD events, indicating that the Mediterranean diet is not only associated with reduced abdominal adiposity and risk of abdominal obesity, as shown in our study, but also protects the body from the adverse effects of the most harmful type of obesity. Abdominal obesity is associated with a low-grade inflammatory state that increases risk of developing CVD risk factors and thereby CVD events. The Mediterranean diet improves this low-grade inflammatory state by decreasing circulating levels of inflammatory bio-markers [131]. In addition to all of the beneficial properties of the Mediterranean diet, it is also a highly palatable dietary pattern with a long history and strong tradition without any adverse secondary effects in comparison with modern pharmaceutical remedies. An additional advantage of this dietary pattern is its popular acceptance and easy adoption in large populations. Thus, the Mediterranean diet is a relatively cheap, non-pharmaceutical approach to the prevention of CVD and other metabolic diseases in public health.

The traditional Mediterranean diet consists of many typical healthy foods consumed in Crete, including yogurt, which has been consumed for thousands years, but with little consideration of its beneficial health properties. The First Global Summit on the Health Effects of Yogurts was held in April 2013 with the aim of reviewing the existing scientific evidence on the health benefits of yogurt, promoting scientific research in this area, and disseminating information on the positive impact of yogurt on health in the general public. Until recently, all dairy products have been considered together, without separation of fermented and not-fermented products. Despite their relatively high saturated fat content, dairy products have been shown to have a positive impact on CVD risk factors such as insulin resistance, type 2 diabetes, dyslipidemia, and high blood pressure, and on CVD itself [132, 133]. Dairy products provide very valuable nutrients and are an excellent source of calcium and protein. However, the probiotic properties of fermented dairy products have a very specific impact on human health, especially on human microbiota [101], and more research is required to determine the health benefits of yogurt. There is a need for studies in healthy and diseased individuals in all age groups to discover the mechanisms of action of yogurt on gut health and the microbiome, the relationship between health and consumption of yogurt and its components.

7.3. Strengths and limitations of this research

The main strength of this research is its design, a population-based prospective cohort design with relatively long follow-up and a high participation rate at reexamination. All questionnaires used for collecting dietary and LTPA data were previously validated in the same population.

This thesis research has several limitations. First, dietary data was collected using the FFQ; while this is a very convenient method for collecting dietary data in large surveys, it collects data retrospectively and provides only very general information about diet, with approximate amounts of foods consumed by the individual. The FFQ can suffer from recall bias, implausible energy reporting, and a lack of information about certain foods or food types that are not included in the list. Second, the absence of intermediate measures of dietary habits during the study limited our analysis options. Third, the cross-sectional and observational design of our studies also prevents us from establishing a causal relationship between diet and health. Fourth, the cluster analysis method also has intrinsic limitations, particularly the arbitrary decisions that the investigator has to make when deriving dietary patterns, including forming food group variables and naming the final dietary patterns.

7.4. The dietary pattern approach and its application in public health

The utility of investigating the association between health and specific nutrients or foods is widely recognized and has been verified by thousands of studies in this field. However, analysis of dietary patterns and their components is a relatively new approach in nutritional epidemiology, and it has several of its own advantages. In reality, people eat a great variety of foods every day, which delivers different combinations of nutrients that act at various levels of the organism in a synergistic or antagonistic way. Thus, it is difficult to separate out the effect of particular foods or nutrients on our health. For example, diets that are rich in vitamin C are also usually rich in carotenoids, polyphenols, potassium and fiber; similarly, diets that are rich in fruit are also usually rich in vegetables, whole grain cereals and plant oils. Thus, the apparent beneficial association between CVD risk and vitamin C and fruit could also be due to other components of the diet.

While investigators and health policy makers focus on the effects of specific nutrients on cardiovascular health, health care practitioners and the general public are likely to be confused by dietary guidelines and nutrient intake targets for preventing and managing their health. Thus, the dietary pattern approach is especially valuable for analysis of its association with the development of diseases such as CVD. Furthermore, the etiology of CVD is very complicated, and it is unlikely that a single nutrient would be responsible for its development [134]. The dietary pattern approach might improve understanding of diet-disease relationships and help to explore the complexity of diet, whereas analysis of a specific nutrient deals with only a limited aspect of this broad area of knowledge. Dietary pattern analysis accounts for the complex relationships between nutrients and foods, and intervention studies have confirmed that analysis of dietary patterns gives more reliable findings than the single nutrient approach, where the association with health outcome is significantly weaker [8, 22].

Research into dietary patterns is becoming increasingly important for public health, as it is very easy to apply in the general population because of its simple interpretation and translation into dietary guidelines. Also, the dietary pattern approach, and hence the dietary recommendations based on dietary pattern analysis, is sensitive to ethnicity and can therefore be readily and naturally accepted by the general public. Dietary guidelines start to emphasize the importance of dietary patterns in preventing CVD.

The benefits of the dietary pattern approach in assessing diet-disease relationships are especially interesting for health care practitioners. Unfortunately, dietitians and nutrition specialists are not fully integrated within public health care systems, and family doctors are the only widely accessible specialists who can advises patients on healthy nutrition for the prevention and management of disease.

Moreover, patients usually consider their family doctor as the most trustworthy source of health information, including that on nutrition and diet. However, evidence suggests that even very supportive family doctors do not provide nutritional advice to their patients [135]. Among the barriers that prevent them from providing nutrition advice, family doctors mainly cite lack of time, counseling skills, information sources, the cultural adaptability of dietary guidelines, and the complexity and contrariety of dietary recommendations [136]. Thus, the dietary pattern approach provides an easy and convenient tool for family doctors to give useful advice about healthy nutrition to their patients.

7.5. Future perspectives

CVD mortality remains the most important health problem worldwide. Even with the decreasing prevalence of CVD in developed countries, the prevalence of CVD risk factors such as general and abdominal obesity and type 2 diabetes continues to rise. In light of intensive research in this area, it is now known that diet is a fundamental modifiable risk factor for CVD prevention. To date, abundant scientific evidence has been gathered from cohort and intervention studies regarding the strong association between diet and health, and the notable benefits of a healthy diet for risk of non-communicable diseases such as CVD.

There are now a number of important research priorities in this area:

First, a greater effort is required to deal with methodological issues. The dietary pattern approach is becoming increasingly popular, although there are no clear methodological standards for constructing dietary patterns. In particular, *a posteriori* dietary patterns lack standard and formalized procedures for every process action. Data measurement errors and variables associated with dietary patterns may affect the reliability of the analysis, and should be explored in more detail; for example, implausible energy reporting, data collection methods, specificity for ethnicity, health status, and lifestyle and social status. Public health authorities should encourage research in this area, as these issues are currently receiving little attention. Without rigorous research in this area, it will be difficult to obtain scientifically rigorous results from observational and intervention studies on dietary patterns.

Second, interventions based on dietary patterns and their components should use approaches that are subsequently easy to implement in a free-living population. These interventions are not only needed to extend knowledge on the mechanisms of action of nutrients, foods and dietary pattern on health, but also to reinforce the development of public health policies for healthy eating, which should shift focus from nutrient-based dietary recommendations to a holistic dietary pattern approach. Well-designed interventions with dietary pattern have two major difficulties, their significant cost and the complexity of implementing lifestyle changes in free-living populations. To obtain accurate results, interventions must overcome resistance to life-style modifications and inter-individual variability in dietary consumption. Furthermore, disease end-points should ideally be the primary outcome, and the intervention should include the whole dietary pattern. An excellent example of this kind of interventions is the PREDIMED study [3].

8. Conclusions

- The sDQS-derived DQI showed good predictive validity. Therefore, it is a useful tool for identifying individuals who benefit most from dietary intervention.
- Dietary patterns derived using cluster analysis from a previously validated FFQ show modest validity and fair reproducibility.
- Energy under-reporting affects post-hoc dietary patterns derived using cluster analysis, as well secular trends based on those dietary patterns
    - Energy under-reporting did not affect the structure of the dietary patterns, but did have an impact on the distribution of participants and consumption between clusters within the same survey
    - Excluding energy under-reporters from the analysis of secular trends increases the apparent level of consumption of unhealthy foods
- Greater adherence to the Mediterranean diet was associated with decreased abdominal adiposity, but not with the incidence of abdominal obesity over 10 years
    - There were no significant differences in results between the two types of Mediterranean diet index, that based on the tertile distribution of food group consumption in the study population, and that based on dietary recommendations
- Consumption of soft drinks was directly associated with the development of abdominal adiposity and the incidence of abdominal obesity over 10-years
    - Substitution of soft drinks with whole milk or juice was associated with a decrease in waist circumference
- Among all dairy products, only frequent consumption of any type of yogurt was associated with decreased incidence of cardiovascular events. This association was somewhat stronger for stroke than for coronary artery disease.

Appendix 1

Supplementary information for papers

Online supporting material for Paper II

Modest validity and fair reproducibility of dietary patterns derived by cluster analysis.

Supplemental Table 4a. FFQ1 x FFQ2 (2x2) tables of participants. Reproducibility analysis

		FFQ 2		Total
		Fruit & Vegetables	Meat	
FFQ 1	Fruit & Vegetables	52	14	66
	Meat	20	16	36
Total		72	30	102

Supplemental Table 4b. 24-HDR x FFQ2 (2x2) tables of participants. Validation analysis

		FFQ 2		Total
		Fruit & Vegetables	Meat	
24-HDR	Fruit & Vegetables	54	16	70
	Meat	18	15	33
Total		72	31	103

Supplemental Table 4c. 24-HDR x FFQ1 (2x2) tables of participants. Descriptive analysis

		FFQ 1		Total
		Fruit & Vegetables	Meat	
24-HDR	Fruit & Vegetables	53	19	72
	Meat	13	21	34
Total		66	40	106

Online supporting material for Paper IV

Soft drink consumption is positively associated with increased waist circumference and 10-year incidence of abdominal obesity in Spanish adults.

Supplemental Table 1. Stratified by gender linear regression analysis of association between changes in waist circumference (cm) and beverage consumption (100 mL units)[1]

Variables	Men			Women		
	β	95% C.I.	p	β	95% C.I.	p
Separate analysis						
Juices	-0.13	(-1.13; 0.87)	0.8	0.06	(-0.95; 1.07)	0.9
Whole milk	-0.44	(-1.06; 0.18)	0.17	-0.03	(-0.69; 0.63)	0.92
Skimmed/Low-fat milk	0.37	(-0.43; 1.16)	0.37	0.27	(-0.45; 0.98)	0.46
Soft drinks	0.96	(-0.14; 2.06)	0.09	1.31	(-0.23; 2.86)	0.1
Mutually adjusted model						
Juices	-0.25	(-1.26; 0.76)	0.63	0.06	(-0.95; 1.07)	0.91
Whole milk	-0.38	(-1.02; 0.26)	0.25	0.06	(-0.63; 0.74)	0.87
Skimmed/Low-fat milk	0.27	(-0.55; 1.08)	0.52	0.3	(-0.45; 1.04)	0.44
Soft drinks	0.97	(-0.14; 2.08)	0.09	1.32	(-0.23; 2.86)	0.1

[1]Adjusted for age (years; continuous), baseline waist circumference (cm; continuous), smoking (yes/no; dichotomous), energy intake (kcal; continuous), alcohol consumption (g; continuous), educational level (more than primary school yes/no; dichotomous), LTPA (METs·min/d; continuous) and energy under and over-reporting (both yes/no; dichotomous)

Supplemental Table 2. Linear regression analysis of association between changes in waist circumference (cm) and changes in beverages consumption in Spanish men and women.1,2

Variables	Men (n= 1000)			Women (n=1112)		
	β	95% CI	p	β	95% CI	p
Soft drinks						
No consumption[3]		Reference			Reference	
Decrease of consumption[4]	0.9	(-0.58;2.37)	0.23	0.17	(-1.42;1.76)	0.83
Increase of consumption[5]	1.52	(0.01;3.02)	0.049	1.49	(-0.21;3.18)	0.09
Maintain of consumption[6]	0.03	(-1.41;146)	0.97	2.44	(0.81;4.06)	0.003
Whole milk						
No consumption[3]		Reference			Reference	
Decrease of consumption[4]	-1.13	(-2.39;0.13)	0.08	1.05	(-0.41;2.51)	0.16
Increase of consumption[5]	-1.69	(-3.27;-0.12)	0.036	1.46	(-0.64;3.57)	0.17
Maintain of consumption[6]	-1.56	(-3.22;0.09)	0.06	0.35	(-1.92;2.61)	0.77
Skimmed/Low-fat milk						
No consumption[3]		Reference			Reference	
Decrease of consumption[4]	0.49	(-0.92;1.90)	0.52	0.4	(-1.21;2.01)	0.47
Increase of consumption[5]	0.12	(-1.18;1.42)	0.42	0.74	(-0.89;2.37)	0.45
Maintain of consumption[6]	0.33	(-1.18;1.83)	0.58	0.42	(-1.07;1.91)	0.56

Table continues

Table continued

Variables	Men (n= 1000)			Women (n=1112)		
	β	95% CI	p	β	95% CI	p
Juices						
No consumption[3]		Reference			Reference	
Decrease of consumption[4]	0.5	(-0.71;1.72)	0.42	0.1	(-1.35;1.56)	0.69
Increase of consumption[5]	-0.81	(-2.10;0.48)	0.22	1.05	(-0.40;2.51)	0.16
Maintain of consumption[6]	0.3	(-2.05;2.56)	0.6	0.38	(-1.80;2.55)	0.74

[1]β coefficients reflect changes in waist circumferences.

[2]Linear regression analysis adjusted for age (years; continuous), and baseline waist circumference (cm; continuous) smoking (yes/no; dichotomous), energy intake (kcal; continuous), dieting (yes/no; dichotomous), educational level (more than primary school yes/no; dichotomous), LTPA (METs·min/d; continuous), modified Mediterranean diet score (continuous), and energy under and over-reporting (both yes/no; dichotomous).

[3]Reference= Maintains of no consumption of corresponding beverage.

[4]Decrease of consumption = from at least 1 serving/d at baseline to less than 1 serving per day or no consumption at follow up and less than 1 serving per day at baseline to no consumption at follow up)

[5]Increase of consumption = from no consumption at baseline to less than 1 serving per day or at least 1 serving per day at follow-up and from less than 1 serving per day at baseline to at least 1 serving per day at follow-up.

[6]Maintains of less than 1 serving per day or at least 1 serving per day at baseline and follow-up

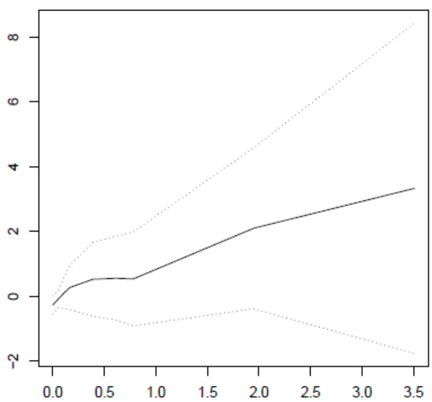
Supplemental Table 3. Association between caloric beverage consumption and odds of 10 year incidence of abdominal obesity in men and women.[1]

	Men (n=756)	Women (n= 723)
	OR (95% CI)	OR (95% CI)
Soft drinks		
No consumption	1	1
> 0 < 200mL	1.03 (0.63;1.69)	1.39 (0.94;2.05)
≥ 200mL	1.62 (0.83;3.14)	1.64 (0.74;3.64)
P for trend	0.31	0.07
Whole milk		
No consumption	1	1
> 0 < 200mL	1.32 (0.74;2.38)	1.90 (1.13;3.20)
≥ 200mL	1.25 (0.75;2.08)	1.21 (0.78;1.88)
P for trend	0.34	0.2
Skimmed/ Low-fat milk		
No consumption	1	1
> 0 < 200mL	0.67 (0.35;1.31)	0.85 (0.47;1.54)
≥ 200mL	0.77 (0.46;1.28)	0.95 (0.66;1.35)
P for trend	0.18	0.69
Juice		
No consumption	1	1
> 0 < 200mL	1.15 (0.72;1.82)	0.87 (0.60;1.30)
≥ 200mL	1.23 (0.64;2.36)	0.53 (0.35;1.00)
P for trend	0.62	0.027

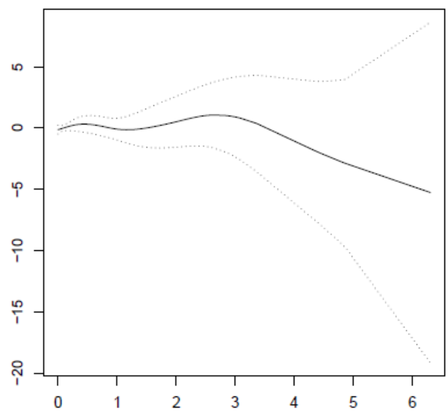
[1]Logistic regression models adjusted for sex (men/women; dichotomous), age (years; continuous), and baseline waist circumference (cm; continuous), smoking (yes/no; dichotomous), energy intake (kcal; continuous), educational level (more than primary school yes/no; dichotomous), LTPA (METs·min/d; continuous), modified Mediterranean diet score (continuous) and energy under and over-reporting (both yes/no; dichotomous).

Supplemental Figure 1. Estimated changes in waist circumferences based on spline regression (95% confidence interval = dotted lines) according to beverage consumption in Spanish adults. Models were adjusted for age, sex, baseline waist circumferences, energy intake, educational level, leisure-time physical activity, modified Mediterranean diet score, and energy under and over-reporting. A = Soft drinks, B= Juice, C = Whole milk, D= Skimmed/Low-fat milk.

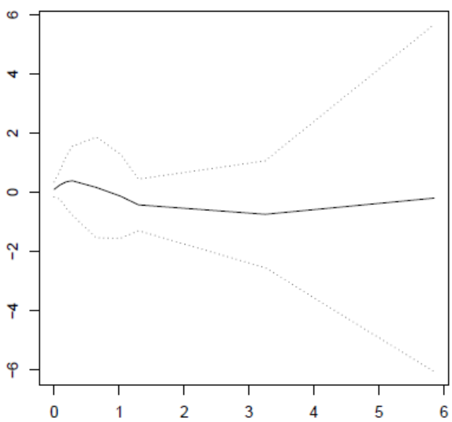
A. Non linear p value = 0.83



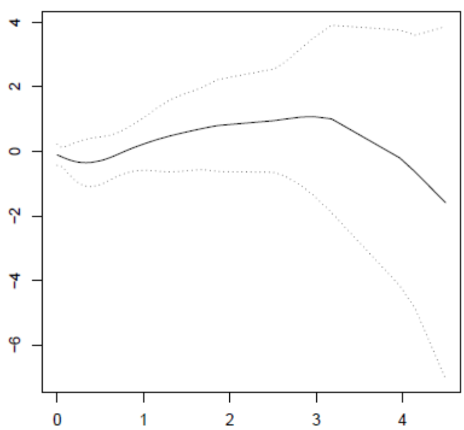
B. Non linear p value = 0.25



C. Non linear p value = 0.80



D. Non linear p value = 0.42



Appendix 2

About the Author

Summary

Anna N. Funtikova graduated in Biology from Nizhegorodsky State University in Russia in 2009. She obtained a Masters Degree in "Nutrition and Metabolism" from the University of Barcelona in 2011. After obtaining the FPU pre-doctoral fellowship (AP2010-3198) from the Spanish Ministry for Education in 2011, Anna continued her research career by joining the PhD program in "Food and Nutrition" at the university of Barcelona and IMIM (Hospital del Mar Medical Research Institute). As part of her PhD studies, Anna did two 3-months research stays outside Spain in 2014. The first, financed by a Eurolife grant for foreign research stays, was with Dr. Renée de Mutsert at Leiden University Medical Centre and focused on Metabolically Healthy Obesity and diet. The second, financed by and additional scholarship from FPU, was with Dr. Joan Sabaté at the Loma Linda University School of Public Health and focused on vegetarian diet in adolescents, obesity and puberty.

Additional publications related to the thesis

- Navarro E, Funtikova AN, Fito M, Schroder H: Long-term consequences of early life nutrition on the risk to adult obesity: nutrition effects on epigenetic mechanisms regulating gene expression. Submitted to Journal of Nutritional Biochemistry.
- Funtikova AN, Navarro E, Bawaked RA, Fíto M, Schröder H. Impact of diet on cardiometabolic health in children and adolescents. Submitted to Nutrition.
- Navarro E*, Funtikova AN*, Fito M, Schroder H: Can metabolically healthy obesity be explained by diet,

genetics, and inflammation? Mol Nutr Food Res 2015, 59(1):75-93. [*These authors contributed equally to the study, and both can be considered first authors of this article.]

- Schroder H, Mendez MA, Ribas L, Funtikova AN, Gomez SF, Fito M, Aranceta J, Serra-Majem L: Caloric beverage drinking patterns are differentially associated with diet quality and adiposity among Spanish girls and boys. Eur J Pediatr 2014, 173(9):1169-1177.
- Schroder H, Ribas L, Koebnick C, Funtikova A, Gomez SF, Fito M, Perez-Rodrigo C, Serra-Majem L: Prevalence of abdominal obesity in spanish children and adolescents. Do we need waist circumference measurements in pediatric practice? PLoS One 2014;9:e87549.

Conferences and meetings

- Meeting of Excellence in Investigation in Public Health, XXIV Summer School of Public Health of Menorca, Menorca, Spain, 2013
- Participation as an assistant in a scientific day of XXXV Anniversary of REGICOR, Reus, Spain 2013
- Funtikova A, Benítez-Arciniega AA, Shcröder H. Adherence to the Mediterranean Diet, prospective abdominal fat change, and 10-year incidence of abdominal obesity. Ann Nutr Metab, 2013, 62, p. 11.

World forum for nutrition research conference. Oral communication.

- Participation as an assistant in the “IX International Congress of Barcelona about Mediterranean diet as a healthy lifestyle for prevention of obesity”, Barcelona, Spain 2012.

References

1. Spurlock, M., Super Size Me. 2004, Samuel Goldwyn Films; Roadside Attractions: USA. p. 98 minutes.
2. Bauer, U.E., et al., Prevention of chronic disease in the 21st century: elimination of the leading preventable causes of premature death and disability in the USA. Lancet, 2014. 384(9937): p. 45-52.
3. Estruch, R., et al., Primary prevention of cardiovascular disease with a Mediterranean diet. N Engl J Med, 2013. 368(14): p. 1279-90.
4. Sacks, F.M., et al., Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. DASH-Sodium Collaborative Research Group. N Engl J Med, 2001. 344(1): p. 3-10.
5. Nascimento, B.R., et al., Global health and cardiovascular disease. Heart, 2014. 100(22): p. 1743-9.
6. Deckert, A., et al., Time trends in cardiovascular disease mortality in Russia and Germany from 1980 to 2007 - are there migration effects? BMC Public Health, 2010. 10: p. 488.
7. de Fatima Marinho de Souza, M., et al., Cardiovascular disease mortality in the Americas: current trends and disparities. Heart, 2012. 98(16): p. 1207-12.
8. Appel, L.J., et al., A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. N Engl J Med, 1997. 336(16): p. 1117-24.
9. Boyd, J.C., et al., Relationship of potassium and magnesium concentrations in serum to cardiac arrhythmias. Clin Chem, 1984. 30(5): p. 754-7.
10. Schroder, H., et al., Validity of two short screeners for diet quality in time-limited settings. Public Health Nutr, 2012. 15(4): p. 618-26.

11. Hearty, A.P. and M.J. Gibney, Comparison of cluster and principal component analysis techniques to derive dietary patterns in Irish adults. Br J Nutr, 2009. 101(4): p. 598-608.

12. Quatromoni, P.A., et al., The internal validity of a dietary pattern analysis. The Framingham Nutrition Studies. J Epidemiol Community Health, 2002. 56(5): p. 381-8.

13. Wirfalt, A.K. and R.W. Jeffery, Using cluster analysis to examine dietary patterns: nutrient intakes, gender, and weight status differ across food pattern clusters. J Am Diet Assoc, 1997. 97(3): p. 272-9.

14. Devlin, U.M., et al., The use of cluster analysis to derive dietary patterns: methodological considerations, reproducibility, validity and the effect of energy mis-reporting. Proc Nutr Soc, 2012. 71(4): p. 599-609.

15. Moeller, S.M., et al., Dietary patterns: challenges and opportunities in dietary patterns research an Experimental Biology workshop, April 1, 2006. J Am Diet Assoc, 2007. 107(7): p. 1233-9.

16. Roman-Vinas, B., et al., Validity of dietary patterns to assess nutrient intake adequacy. Br J Nutr, 2009. 101 Suppl 2: p. S12-20.

17. Collins, C.E., et al., The comparative validity and reproducibility of a diet quality index for adults: the Australian Recommended Food Score. Nutrients, 2015. 7(2): p. 785-98.

18. Dekker, L.H., et al., Dietary patterns within a population are more reproducible than those of individuals. J Nutr, 2013. 143(11): p. 1728-35.

19. Arvaniti, F. and D.B. Panagiotakos, Healthy indexes in public health practice and research: a review. Crit Rev Food Sci Nutr, 2008. 48(4): p. 317-27.

20. Kourlaba, G. and D.B. Panagiotakos, Dietary quality indices and human health: a review. Maturitas, 2009. 62(1): p. 1-8.

21. Menotti, A., et al., Seven Countries Study. First 20-year mortality data in 12 cohorts of six countries. Ann Med, 1989. 21(3): p. 175-9.

22. Trichopoulou, A., et al., Diet and overall survival in elderly people. BMJ, 1995. 311(7018): p. 1457-60.

23. Sofi, F., et al., Accruing evidence on benefits of adherence to the Mediterranean diet on health: an updated systematic review and meta-analysis. Am J Clin Nutr, 2010. 92(5): p. 1189-96.

24. Dilis, V., et al., Mediterranean diet and CHD: the Greek European Prospective Investigation into Cancer and Nutrition cohort. Br J Nutr, 2012. 108(4): p. 699-709.

25. Nordmann, A.J., et al., Meta-analysis comparing Mediterranean to low-fat diets for modification of cardiovascular risk factors. Am J Med, 2011. 124(9): p. 841-51 e2.

26. Sacks, F.M., et al., Rationale and design of the Dietary Approaches to Stop Hypertension trial (DASH). A multicenter controlled-feeding study of dietary patterns to lower blood pressure. Ann Epidemiol, 1995. 5(2): p. 108-18.

27. Chen, S.T., N.M. Maruthur, and L.J. Appel, The effect of dietary patterns on estimated coronary heart disease risk: results from the Dietary Approaches to Stop Hypertension (DASH) trial. Circ Cardiovasc Qual Outcomes, 2010. 3(5): p. 484-9.

28. Fung, T.T., et al., Adherence to a DASH-style diet and risk of coronary heart disease and stroke in women. Arch Intern Med, 2008. 168(7): p. 713-20.

29. Salehi-Abargouei, A., et al., Effects of Dietary Approaches to Stop Hypertension (DASH)-style diet on fatal or nonfatal cardiovascular diseases--incidence: a systematic review and meta-analysis on observational prospective studies. Nutrition, 2013. 29(4): p. 611-8.

30. Liese, A.D., et al., The Dietary Patterns Methods Project: synthesis of findings across cohorts and relevance to dietary guidance. J Nutr, 2015. 145(3): p. 393-402.

31. Harmon, B.E., et al., Associations of key diet-quality indexes with mortality in the Multiethnic Cohort: the Dietary Patterns Methods Project. Am J Clin Nutr, 2015. 101(3): p. 587-97.

32. Schwingshackl, L. and G. Hoffmann, Diet quality as assessed by the healthy eating index, the alternate healthy eating index, the dietary approaches to stop hypertension score, and health outcomes: a systematic review and meta-analysis of cohort studies. J Acad Nutr Diet, 2015. 115(5): p. 780-800 e5.

33. Kennedy, E.T., et al., The Healthy Eating Index: design and applications. J Am Diet Assoc, 1995. 95(10): p. 1103-8.

34. Guenther, P.M., et al., Update of the Healthy Eating Index: HEI-2010. J Acad Nutr Diet, 2013. 113(4): p. 569-80.

35. Guenther, P.M., J. Reedy, and S.M. Krebs-Smith, Development of the Healthy Eating Index-2005. J Am Diet Assoc, 2008. 108(11): p. 1896-901.

36. McCullough, M.L., et al., Diet quality and major chronic disease risk in men and women: moving toward improved dietary guidance. Am J Clin Nutr, 2002. 76(6): p. 1261-71.

37. Chiuve, S.E., et al., Alternative dietary indices both strongly predict risk of chronic disease. J Nutr, 2012. 142(6): p. 1009-18.

38. Hou, L., et al., Association between dietary patterns and coronary heart disease: a meta-analysis of prospective cohort studies. Int J Clin Exp Med, 2015. 8(1): p. 781-90.

39. Li, F., et al., Associations of dietary patterns with the risk of all-cause, CVD and stroke mortality: a meta-analysis of prospective cohort studies. Br J Nutr, 2014: p. 1-9.

40. Martinez-Gonzalez, M.A., et al., Empirically-derived food patterns and the risk of total mortality and cardiovascular events in the PREDIMED study. Clin Nutr, 2014.

41. Millen, B.E., et al., Dietary patterns, smoking, and subclinical heart disease in women: opportunities for primary prevention from the Framingham Nutrition Studies. J Am Diet Assoc, 2004. 104(2): p. 208-14.

42. Millen, B.E., et al., Dietary patterns and the odds of carotid atherosclerosis in women: the Framingham Nutrition Studies. Prev Med, 2002. 35(6): p. 540-7.

43. Oliveira, A., et al., Major habitual dietary patterns are associated with acute myocardial infarction and cardiovascular risk markers in a southern European population. J Am Diet Assoc, 2011. 111(2): p. 241-50.

44. Brunner, E.J., et al., Dietary patterns and 15-y risks of major coronary events, diabetes, and mortality. Am J Clin Nutr, 2008. 87(5): p. 1414-21.

45. Malik, V.S., et al., Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes: a meta-analysis. Diabetes Care, 2010. 33(11): p. 2477-83.

46. Te Morenga, L., S. Mallard, and J. Mann, Dietary sugars and body weight: systematic review and meta-analyses of randomised controlled trials and cohort studies. BMJ, 2013. 346: p. e7492.

47. Malik, V.S., et al., Sugar-sweetened beverages and weight gain in children and adults: a systematic review and meta-analysis. Am J Clin Nutr, 2013. 98(4): p. 1084-102.

48. Xi, B., et al., Sugar-sweetened beverages and risk of hypertension and CVD: a dose-response meta-analysis. Br J Nutr, 2015. 113(5): p. 709-17.

49. Keller, A., B.L. Heitmann, and N. Olsen, Sugar-sweetened beverages, vascular risk factors and events: a systematic literature review. Public Health Nutr, 2015. 18(7): p. 1145-54.

50. Pan, A., et al., Red meat consumption and mortality: results from 2 prospective cohort studies. Arch Intern Med, 2012. 172(7): p. 555-63.

51. Rohrmann, S., et al., Meat consumption and mortality--results from the European Prospective Investigation into Cancer and Nutrition. BMC Med, 2013. 11: p. 63.

52. Pan, A., et al., Changes in red meat consumption and subsequent risk of type 2 diabetes mellitus: three cohorts of US men and women. JAMA Intern Med, 2013. 173(14): p. 1328-35.

53. Michas, G., R. Micha, and A. Zampelas, Dietary fats and cardiovascular disease: putting together the pieces of a complicated puzzle. Atherosclerosis, 2014. 234(2): p. 320-8.

54. He, K., et al., Accumulated evidence on fish consumption and coronary heart disease mortality: a meta-analysis of cohort studies. Circulation, 2004. 109(22): p. 2705-11.

55. Strom, M., et al., Fish, n-3 fatty acids, and cardiovascular diseases in women of reproductive age: a prospective study in a large national cohort. Hypertension, 2012. 59(1): p. 36-43.

56. Chen, Q., et al., Effects of omega-3 fatty acid for sudden cardiac death prevention in patients with cardiovascular disease: a contemporary meta-analysis of randomized, controlled trials. Cardiovasc Drugs Ther, 2011. 25(3): p. 259-65.

57. Astrup, A., Yogurt and dairy product consumption to prevent cardiometabolic diseases: epidemiologic and experimental studies. Am J Clin Nutr, 2014. 99(5 Suppl): p. 1235S-42S.

58. Soedamah-Muthu, S.S., et al., Milk and dairy consumption and incidence of cardiovascular diseases and all-cause mortality: dose-

response meta-analysis of prospective cohort studies. Am J Clin Nutr, 2011. 93(1): p. 158-71.

59. Patterson, E., et al., Association between dairy food consumption and risk of myocardial infarction in women differs by type of dairy food. J Nutr, 2013. 143(1): p. 74-9.

60. Kondo, I., et al., Consumption of dairy products and death from cardiovascular disease in the Japanese general population: the NIPPON DATA80. J Epidemiol, 2013. 23(1): p. 47-54.

61. Aune, D., et al., Dairy products and the risk of type 2 diabetes: a systematic review and dose-response meta-analysis of cohort studies. Am J Clin Nutr, 2013. 98(4): p. 1066-83.

62. Schwingshackl, L. and G. Hoffmann, Monounsaturated fatty acids, olive oil and health status: a systematic review and meta-analysis of cohort studies. Lipids Health Dis, 2014. 13: p. 154.

63. Martinez-Gonzalez, M.A., L.J. Dominguez, and M. Delgado-Rodriguez, Olive oil consumption and risk of CHD and/or stroke: a meta-analysis of case-control, cohort and intervention studies. Br J Nutr, 2014. 112(2): p. 248-59.

64. Afshin, A., et al., Consumption of nuts and legumes and risk of incident ischemic heart disease, stroke, and diabetes: a systematic review and meta-analysis. Am J Clin Nutr, 2014. 100(1): p. 278-88.

65. Kelly, J.H., Jr. and J. Sabate, Nuts and coronary heart disease: an epidemiological perspective. Br J Nutr, 2006. 96 Suppl 2: p. S61-7.

66. Graham, I., et al., European guidelines on cardiovascular disease prevention in clinical practice: executive summary: Fourth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (Constituted by representatives of nine societies and by invited experts). Eur Heart J, 2007. 28(19): p. 2375-414.

67. Lichtenstein, A.H., et al., Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. Circulation, 2006. 114(1): p. 82-96.

68. Dauchet, L., et al., Fruit and vegetable consumption and risk of coronary heart disease: a meta-analysis of cohort studies. J Nutr, 2006. 136(10): p. 2588-93.

69. He, F.J., et al., Increased consumption of fruit and vegetables is related to a reduced risk of coronary heart disease: meta-analysis of cohort studies. J Hum Hypertens, 2007. 21(9): p. 717-28.

70. Crowe, F.L., et al., Fruit and vegetable intake and mortality from ischaemic heart disease: results from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Heart study. Eur Heart J, 2011. 32(10): p. 1235-43.

71. Widmer, R.J., et al., The Mediterranean diet, its components, and cardiovascular disease. Am J Med, 2015. 128(3): p. 229-38.

72. Atkins, J.L., et al., High diet quality is associated with a lower risk of cardiovascular disease and all-cause mortality in older men. J Nutr, 2014. 144(5): p. 673-80.

73. Schroder, H., et al., Baseline adherence to the Mediterranean diet and major cardiovascular events: Prevencion con Dieta Mediterranea trial. JAMA Intern Med, 2014. 174(10): p. 1690-2.

74. Cutler, G.J., et al., Major patterns of dietary intake in adolescents and their stability over time. J Nutr, 2009. 139(2): p. 323-8.

75. Song, Y., et al., Secular trends in dietary patterns and obesity-related risk factors in Korean adolescents aged 10-19 years. Int J Obes (Lond), 2010. 34(1): p. 48-56.

76. Lim, H., et al., Preservation of a traditional Korean dietary pattern and emergence of a fruit and dairy dietary pattern among adults in South Korea: secular transitions in dietary patterns of a

prospective study from 1998 to 2010. Nutr Res, 2014. 34(9): p. 760-70.

77. Souza Ade, M., et al., Evaluation of food intake markers in the Brazilian surveillance system for chronic diseases--VIGITEL (2007-2009). Rev Bras Epidemiol, 2011. 14 Suppl 1: p. 44-52.

78. Barzin, M., et al., Rising trends of obesity and abdominal obesity in 10 years of follow-up among Tehranian adults: Tehran Lipid and Glucose Study (TLGS). Public Health Nutr, 2015: p. 1-9.

79. Ford, E.S., L.M. Maynard, and C. Li, Trends in mean waist circumference and abdominal obesity among US adults, 1999-2012. JAMA, 2014. 312(11): p. 1151-3.

80. Howel, D., Trends in the prevalence of abdominal obesity and overweight in English adults (1993-2008). Obesity (Silver Spring), 2012. 20(8): p. 1750-2.

81. Stern, D., et al., Changes in waist circumference relative to body mass index in Chinese adults, 1993-2009. Int J Obes (Lond), 2014. 38(12): p. 1503-10.

82. Ogden, C.L., et al., Prevalence of childhood and adult obesity in the United States, 2011-2012. JAMA, 2014. 311(8): p. 806-14.

83. Xi, B., et al., Trends in abdominal obesity among U.S. children and adolescents. Pediatrics, 2014. 134(2): p. e334-9.

84. Schroder, H., et al., Prevalence of abdominal obesity in Spanish children and adolescents. Do we need waist circumference measurements in pediatric practice? PLoS One, 2014. 9(1): p. e87549.

85. Albrecht, S.S., et al., Is waist circumference per body mass index rising differentially across the United States, England, China and Mexico? Eur J Clin Nutr, 2015.

86. Freedman, D.S. and E.S. Ford, Are the recent secular increases in the waist circumference of adults independent of changes in BMI? Am J Clin Nutr, 2015. 101(3): p. 425-31.

87. de Koning, L., et al., Waist circumference and waist-to-hip ratio as predictors of cardiovascular events: meta-regression analysis of prospective studies. Eur Heart J, 2007. 28(7): p. 850-6.

88. Siren, R., J.G. Eriksson, and H. Vanhanen, Waist circumference a good indicator of future risk for type 2 diabetes and cardiovascular disease. BMC Public Health, 2012. 12: p. 631.

89. Uusitupa, M. and U. Schwab, Diet, inflammation and prediabetes-impact of quality of diet. Can J Diabetes, 2013. 37(5): p. 327-31.

90. Bray, G.A. and B.M. Popkin, Dietary sugar and body weight: have we reached a crisis in the epidemic of obesity and diabetes?: health be damned! Pour on the sugar. Diabetes Care, 2014. 37(4): p. 950-6.

91. Huang, C., et al., Sugar sweetened beverages consumption and risk of coronary heart disease: a meta-analysis of prospective studies. Atherosclerosis, 2014. 234(1): p. 11-6.

92. Wang, M., et al., Association between sugar-sweetened beverages and type 2 diabetes: A meta-analysis. J Diabetes Investig, 2015. 6(3): p. 360-6.

93. Maersk, M., et al., Sucrose-sweetened beverages increase fat storage in the liver, muscle, and visceral fat depot: a 6-mo randomized intervention study. Am J Clin Nutr, 2012. 95(2): p. 283-9.

94. Ministerio de Agricultura, A.y.M.A. Datos e información sobre el consumo de leche y lácteos en España. 2013; Available from: http://aijn.org/files/default/aijn2014-full.pdf.

95. AIJN European Fruit Juice Association. AIJN European Fruit Juice Association Market report 2014. 2014; Available from: http://aijn.org/files/default/aijn2014-full.pdf.

96. Euromonitor International. Americans Drink More Soda Than Anyone Else: A map of soda consumption in 80 countries. 2011; Available from:

http://www.slate.com/articles/health_and_science/map_of_the_week/2012/07/map_of_soda_consumption_americans_drink_more_than_anyone_else_.html.

97. Babio, N., et al., Mediterranean diets and metabolic syndrome status in the PREDIMED randomized trial. CMAJ, 2014. 186(17): p. E649-57.

98. Eguaras, S., et al., Does the Mediterranean diet counteract the adverse effects of abdominal adiposity? Nutr Metab Cardiovasc Dis, 2015.

99. Romaguera, D., et al., Adherence to the Mediterranean diet is associated with lower abdominal adiposity in European men and women. J Nutr, 2009. 139(9): p. 1728-37.

100. Asghari, G., et al., The association between diet quality indices and obesity: Tehran Lipid and Glucose Study. Arch Iran Med, 2012. 15(10): p. 599-605.

101. Ebel, B., et al., Impact of probiotics on risk factors for cardiovascular diseases. A review. Crit Rev Food Sci Nutr, 2014. 54(2): p. 175-89.

102. Ataie-Jafari, A., et al., Cholesterol-lowering effect of probiotic yogurt in comparison with ordinary yogurt in mildly to moderately hypercholesterolemic subjects. Ann Nutr Metab, 2009. 54(1): p. 22-7.

103. Ejtahed, H.S., et al., Effect of probiotic yogurt containing Lactobacillus acidophilus and Bifidobacterium lactis on lipid profile in individuals with type 2 diabetes mellitus. J Dairy Sci, 2011. 94(7): p. 3288-94.

104. Goldbohm, R.A., et al., Dairy consumption and 10-y total and cardiovascular mortality: a prospective cohort study in the Netherlands. Am J Clin Nutr, 2011. 93(3): p. 615-27.

105. Iso, H., et al., Prospective study of calcium, potassium, and magnesium intake and risk of stroke in women. Stroke, 1999. 30(9): p. 1772-9.

106. Larsson, S.C., et al., Dairy foods and risk of stroke. Epidemiology, 2009. 20(3): p. 355-60.

107. Soedamah-Muthu, S.S., et al., Consumption of dairy products and associations with incident diabetes, CHD and mortality in the Whitehall II study. Br J Nutr, 2013. 109(4): p. 718-26.

108. Elosua, R., et al., Validation of the Minnesota Leisure Time Physical Activity Questionnaire In Spanish Women. Investigators of the MARATDON Group. Med Sci Sports Exerc, 2000. 32(8): p. 1431-7.

109. Elosua, R., et al., Validation of the Minnesota Leisure Time Physical Activity Questionnaire in Spanish men. The MARATHOM Investigators. Am J Epidemiol, 1994. 139(12): p. 1197-209.

110. Benitez-Arciniega, A.A., et al., Concurrent and construct validity of Mediterranean diet scores as assessed by an FFQ. Public Health Nutr, 2011. 14(11): p. 2015-21.

111. Schroder, H., et al., Use of a three-day estimated food record, a 72-hour recall and a food-frequency questionnaire for dietary assessment in a Mediterranean Spanish population. Clin Nutr, 2001. 20(5): p. 429-37.

112. England, C.Y., et al., A systematic review of brief dietary questionnaires suitable for clinical use in the prevention and management of obesity, cardiovascular disease and type 2 diabetes. Eur J Clin Nutr, 2015.

113. O'Reilly, S.L. and L.R. McCann, Development and validation of the Diet Quality Tool for use in cardiovascular disease prevention settings. Aust J Prim Health, 2012. 18(2): p. 138-47.

114. Bountziouka, V., et al., Validity of dietary patterns derived in nutrition surveys using *a priori* and *a posteriori* multivariate statistical methods. Int J Food Sci Nutr, 2011. 62(6): p. 617-27.

115. Millen, B.E., et al., Validation of a dietary pattern approach for evaluating nutritional risk: the Framingham Nutrition Studies. J Am Diet Assoc, 2001. 101(2): p. 187-94.

116. Lo Siou, G., et al., Exploring statistical approaches to diminish subjectivity of cluster analysis to derive dietary patterns: The Tomorrow Project. Am J Epidemiol, 2011. 173(8): p. 956-67.

117. Castro-Quezada, I., et al., Misreporting in nutritional surveys: methodological implications. Nutr Hosp, 2015. 31 Suppl 3: p. 119-27.

118. Poslusna, K., et al., Misreporting of energy and micronutrient intake estimated by food records and 24 hour recalls, control and adjustment methods in practice. Br J Nutr, 2009. 101 Suppl 2: p. S73-85.

119. Livingstone, M.B. and A.E. Black, Markers of the validity of reported energy intake. J Nutr, 2003. 133 Suppl 3: p. 895S-920S.

120. Bornhorst, C., et al., Diet-obesity associations in children: approaches to counteract attenuation caused by misreporting. Public Health Nutr, 2013. 16(2): p. 256-66.

121. Mendez, M.A., et al., Alternative methods of accounting for underreporting and overreporting when measuring dietary intake-obesity relations. Am J Epidemiol, 2011. 173(4): p. 448-58.

122. Wang, D.D., et al., Trends in dietary quality among adults in the United States, 1999 through 2010. JAMA Intern Med, 2014. 174(10): p. 1587-95.

123. Bigornia, S.J., et al., Sugar-sweetened beverage consumption and central and total adiposity in older children: a prospective study accounting for dietary reporting errors. Public Health Nutr, 2015. 18(7): p. 1155-63.

124. Zheng, M., et al., Substituting sugar-sweetened beverages with water or milk is inversely associated with body fatness development from childhood to adolescence. Nutrition, 2015. 31(1): p. 38-44.

125. Zheng, M., et al., Substitution of sugar-sweetened beverages with other beverage alternatives: a review of long-term health outcomes. J Acad Nutr Diet, 2015. 115(5): p. 767-79.

126. Brown, C.M., A.G. Dulloo, and J.P. Montani, Sugary drinks in the pathogenesis of obesity and cardiovascular diseases. Int J Obes (Lond), 2008. 32 Suppl 6: p. S28-34.

127. Balcells, E., et al., Soft drinks consumption, diet quality and BMI in a Mediterranean population. Public Health Nutr, 2011. 14(5): p. 778-84.

128. Hu, E.A., et al., Lifestyles and risk factors associated with adherence to the Mediterranean diet: a baseline assessment of the PREDIMED trial. PLoS One, 2013. 8(4): p. e60166.

129. Stradling, C., et al., A review of dietary influences on cardiovascular health: part 2: dietary patterns. Cardiovasc Hematol Disord Drug Targets, 2014. 14(1): p. 50-63.

130. Martinez-Gonzalez, M.A. and M. Bes-Rastrollo, Dietary patterns, Mediterranean diet, and cardiovascular disease. Curr Opin Lipidol, 2014. 25(1): p. 20-6.

131. Urpi-Sarda, M., et al., Virgin olive oil and nuts as key foods of the Mediterranean diet effects on inflammatory biomakers related to atherosclerosis. Pharmacol Res, 2012. 65(6): p. 577-83.

132. Abedini, M., E. Falahi, and S. Roosta, Dairy product consumption and the metabolic syndrome. Diabetes Metab Syndr, 2015. 9(1): p. 34-7.

133. Qin, L.Q., et al., Dairy consumption and risk of cardiovascular disease: an updated meta-analysis of prospective cohort studies. Asia Pac J Clin Nutr, 2015. 24(1): p. 90-100.

134. Hu, F.B., Dietary pattern analysis: a new direction in nutritional epidemiology. Curr Opin Lipidol, 2002. 13(1): p. 3-9.

135. Pollak, K.I., et al., Can physicians accurately predict which patients will lose weight, improve nutrition and increase physical activity? Fam Pract, 2012. 29(5): p. 553-60.

136. Kolasa, K.M. and K. Rickett, Barriers to providing nutrition counseling cited by physicians: a survey of primary care practitioners. Nutr Clin Pract, 2010. 25(5): p. 502-9.

Abbreviations

AES – Australian Eating Survey
AHEI – Alternate Healthy Eating Index
ARFS – Australian Recommended Food Score
BMI – Body Mass Index
CHD – Coronary Heart Disease
CVD – Cardiovascular Disease
DASH – Dietary Approach to Stop Hypertension
DQI – Diet Quality Index
HDL-cholesterol – High-Density Lipoprotein Cholesterol
HEI – Healthy Eating Index
HR – Hazard Ratio
FFQ – Food Frequency Questionnaire
IHD – Ischemic Heart Disease
LTPA – Leisure-Time Physical Activity
MDS – Mediterranean Diet Score
MUFA – Monounsaturated Fatty Acids
OR – Odds Ratio
PCA – Principal Component Analysis
PUFA – Polyunsaturated Fatty Acids
REGICOR – Registro Gironí del Cor
RCT – Randomized Clinical Trial
RR – Relative Risk
sDQS – short Diet Quality Screener
SFA – Saturated Fatty Acids
SRRE – Summary Relative Risk Estimates