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**“ANALYSIS OF DIETARY PATTERNS IN RELATION TO
CARDIOVASCULAR RISK FACTORS”**

Memoria que presenta **Irina Uzhova**, licenciada en Salud Pública, para optar al grado de Doctor Internacional por la Universidad Autónoma de Madrid

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To my husband, Enda Clinton

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“A journey of a thousand miles begins with the first step”
Lao Tzu



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THIS IS TO CERTIFY

That Irina Uzhova, MSc in Public Health awarded by the Karolinska Institutet, has developed the doctoral thesis entitled "**Analysis of dietary patterns in relation to cardiovascular risk factors**" under my supervision and that her work meets the requirements of quality, originality and content necessary to be presented for the defense in order to obtain her Doctoral degree from the Universidad Autónoma de Madrid (UAM).

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Madrid, 27th of May 2018

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I, hereby certify that the submitted work is my own work, was completed while registered as a candidate for the degree stated on the Title Page, and I have not obtained a degree elsewhere on the basis of the research presented in this submitted work.

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Madrid, 27th of May 2018

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ABSTRACT

Background: Compared to the examination of food and nutrient intakes, the identification of dietary patterns provides an overall picture of our diet considering combinations of foods and meals, and contextualizing diet in relation to other lifestyle factors. Due to the inherent heterogeneity in people's eating preferences, accurate characterization of dietary patterns remains a challenge. Efficient strategies to reduce complex and multidimensional nutritional data into meaningful dietary patterns are needed, in particular to study the association between diet and chronic disease.

Aims and objectives: The overall aim of this work is to identify dietary patterns and their association with prevalent cardiometabolic risk factors in different populations. For this, two objectives were defined: 1) To apply "*a-priori*" and "*a-posteriori*" dietary and meal pattern analyses to characterise the nutritional quality of the diet, 2) To quantify the impact of the adherence to distinctive dietary patterns, the type and frequency of meals, eating occasions, and synergistic associations of multiple lifestyles on traditional cardio-metabolic biomarkers, and particularly the presence and extent of subclinical atherosclerosis in asymptomatic adults.

Materials and methods: Analyses were carried out using baseline data from two observational prospective cohorts and a National-representative survey. The Aragon Workers Health Study (AWHS) and the Progression of Early Subclinical Atherosclerosis (PESA) study, both involved populations of middle aged men and women and aimed to assess the determinants of subclinical atherosclerosis progression. The National Adult Nutrition Survey (NANS), measured habitual food and beverage consumption, lifestyle and health status among a National representative sample of adults living in the republic of Ireland.

Results: "*A-posteriori*" (Principal Components Analysis, Cluster Analysis and Latent Class Analysis), "*a-priori*" (adherence to alternate Mediterranean Diet Index) dietary patterns analyses, and quantification of dietary intakes at specific eating occasions (breakfast) were used to describe diet. A Western Dietary Pattern (WDP), characterised by higher intakes of red meat, fast food, dairy and cereals, was associated with lower high-density lipoprotein cholesterol (HDL-c) and apolipoprotein A1 levels. A Mediterranean Dietary Pattern (MDP), characterised by higher intakes of vegetables, fruits, fish, white meat, nuts and olive oil was observed to be related to a more favourable plasma lipid profile and was significantly associated with lower prevalence of plaques in femoral arteries independently of the presence of other conventional risk factors. Higher adherence to MDP combined with non-smoking,

and moderate alcohol consumption resulted in a further reduction in the risk of subclinical atherosclerosis. A Social-Business eating pattern, characterised by high consumption of red meat, pre-made foods, snacks, alcohol, and sugar sweetened beverages, frequent eating out behaviour was associated with a worse CVD risk profile and significantly higher prevalence and extension of subclinical atherosclerosis. Dietary habits significantly differed on weekends. Thus those participants who preferred meat and eggs for breakfast rather than having a cereal, and skipped light meal later during the day, were more likely to follow unhealthy overall dietary pattern, have higher diastolic blood pressure and increased serum ferritin. Moreover, skipping breakfast is not only a marker of overall unhealthy dietary pattern and lifestyle, it is also significantly associated with increased prevalence of non-coronary and generalized atherosclerosis. On the other hand regular breakfast consumption was associated with higher overall dietary quality.

Conclusion: “*A-posteriori*” and “*a-priori*” analyses of dietary patterns are useful techniques to characterise the dietary habits commonly followed within a given population and their relationship with CVD markers. In combination with the investigation of daily meal consumption and at specific eating occasions, this approach could lead to improved public health guidelines and recommendations to improve diet, and overall lifestyle and curb the increasing burden of CVD.

RESUMEN

Introducción: El análisis y la identificación de patrones alimentarios permiten representar una imagen más amplia de nuestra dieta, incluyendo combinaciones de alimentos, comidas y contextualizando la dieta en relación a otros estilos de vida. Debido a la inherente heterogeneidad de nuestras preferencias alimentarias, la caracterización precisa de patrones alimentarios sigue constituyendo un reto y se necesitan estrategias metodológicas efectivas para la reducción de los datos nutricionales, complejos y multidimensionales, en patrones alimentarios que describan las dietas mayoritarias en una población y permitan evaluar la relación de estas con determinantes de salud.

Objetivos: El principal objetivo de este trabajo es identificar los patrones alimentarios principales y determinar su relación con los factores de riesgo cardiometabólico prevalentes en diferentes poblaciones. Para ello se definieron dos objetivos secundarios: 1) aplicar los métodos de identificación de los patrones alimentarios "*a priori*" y "*a posteriori*" para caracterizar la calidad nutricional de la dieta, 2) determinar el impacto de la adherencia a distintos patrones alimentarios, tipos de comidas diarias y su frecuencia y la combinación de múltiples estilos de vida en relación a la presencia de marcadores cardiometabólicos tradicionales, y especialmente a la presencia y extensión de la aterosclerosis subclínica.

Métodos: Los diferentes análisis de identificación de los patrones alimentarios, como el análisis de componentes principales, el análisis de grupos, el análisis de clases latentes, aplicación de aMED score y el análisis de los tipos de comidas se llevaron a cabo utilizando los datos obtenidos de 3 estudios. El Estudio de Salud de los Trabajadores de Aragón (AWHS) y el Estudio de la Progresión y Detección Precoz de Aterosclerosis Subclínica (PESA), dos cohortes prospectivas con el objetivo de investigar los factores determinantes de la presencia y la progresión de la aterosclerosis subclínica en poblaciones de mediana edad en España. La Encuesta de Nutrición en Adultos (NANS) de Irlanda estudia, de forma periódica, la dieta habitual, los estilos de vida y la salud de una muestra representativa de adultos de la República de Irlanda.

Resultados: Un patrón de dieta occidental (WDP), rico en carnes rojas, comida rápida, productos lácteos y cereales, se asocia a niveles más bajos de colesterol unido a lipoproteínas de alta densidad (HDL-c) y apolipoproteína A1. Un patrón de dieta mediterráneo (MDP), rico en verduras, frutas, pescado, carnes blancas, frutos secos y aceite de oliva se asocia con un perfil lipídico más favorable y con una menor prevalencia de placas de aterosclerosis en las arterias femorales de forma independiente de la presencia de otros factores de riesgo

convencionales. La adherencia a MDP combinada con consumo moderado de alcohol y el abandono del hábito de fumar resulta en una reducción adicional en el riesgo de presentar aterosclerosis subclínica. Un tercer patrón de dieta Social-Negocios, caracterizado por un elevado consumo en de carnes rojas, comida rápida, bocadillos, alcohol y bebidas azucaradas, y comidas fuera de casa de forma habitual se relaciona con un riesgo cardiovascular mas elevado, incluida una prevalencia elevada de aterosclerosis subclínica. Los patrones alimentarios difieren significativamente en los fines de semana y los participantes que prefieren carne y huevos para el desayuno en lugar de cereal y omiten la comida ligera más tarde durante el día, presentan un patrón de dieta menos saludable, una presión arterial diastólica más alta y nivel de ferritina elevado. Además, la omisión del desayuno es un marcador de un patrón alimentario y estilo de vida poco saludable y que está asociado con una mayor prevalencia de aterosclerosis no-coronaria y generalizada. Por otro lado, el consumo regular de desayuno se asocia con el patrón dietético más favorable.

Conclusión: Los métodos de identificación de patrones dietéticos establecidos "*a-posteriori*" y "*a priori*" son instrumentos adecuados para caracterizar los hábitos alimentarios mayoritarios dentro de una población determinada. La investigación del tipo de comidas diarias, su frecuencia y la combinación presenta una oportunidad en identificación de los patrones alimentarios y los resultados se podría ser trasladan con mayor facilidad a un público más amplio.

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LIST OF ABBREVIATIONS

AHA: American Heart Association
AHEI: Alternate Healthy Eating Index
aMED: Alternate Mediterranean Diet Index
ApoA1: Apolipoprotein A1
Apo B: Apolipoprotein B
ANNs: Artificial Neural Networks
ANOVA: Analysis of Variance
AWHS: Aragon Workers Health Study
ApoA1: Apolipoprotein A1
BMI: Body Mass Index
CA: Cluster Analysis
CACs: Coronary Artery Calcium Score
CAD: Coronary Artery Disease
CHD: Coronary Heart Disease
cIMT: Carotid Intima Media Thickness
cHDL: High Density Lipoprotein cholesterol
CI: Confidence Interval
cLDL: Low Density Lipoprotein cholesterol
CVD: Cardiovascular Disease
DBP: Diastolic Blood Pressure
DI: Dietary Index
EPIC: European Prospective Investigation into Cancer and Nutrition
EO: Eating Occasion
ESC: European Society of Cardiology
FAO: Food and Agriculture Organization
FBC: Food-based Classification
GGMs: Gaussian graphical models
GBD: Global Burden of Disease
GFPI: German Food Pyramid Index
HEI: Healthy Eating Index
IHD: Ischemic Heart Disease

LCA: Latent Class Analysis
MAI: Mediterranean Adequacy Index
MDS: Mediterranean Diet Score
MDP: Mediterranean Dietary Pattern
MD: Mediterranean Diet
MEDAS: Mediterranean Diet Adherence Screener
MedDiet Score: Mediterranean Diet Score
MEDLIFE: MEDiterranean LIFEstyle Index
MSDPS: Mediterranean Style Dietary Patten Score
MUFA: Monounsaturated Fatty Acids
NANS: National Adults Nutrition Survey
NSIFCS: North-South Ireland Food Consumption Survey
NRF: Nutrient-Rich Food index
OR: Odds Ratio
PCA: Principal Component Analysis
PDP: Prudent Dietary Pattern
PESA: Progression of Early Subclinical Atherosclerosis study
PR: Prevalence Ratio
RRR: Reduced Rank Regression
SBP: Systolic Blood Pressure
SFP: Spanish Food Pyramid
TG: Triglycerides
TT: Treelet Transform
WC: Waist Circumference
WDP: Western dietary pattern
WHO: World Health Organization

INTRODUCTION

1.1. Diet and cardiovascular disease

Cardiovascular disease (CVD) is still one of the leading causes of death worldwide and continues to be a major concern for global health. According to the Global Burden of Disease (GBD) 2016 study 17.3% and 10.1% of total deaths were attributable to ischemic heart disease (IHD) and stroke, respectively (Naghavi *et al.*, 2017). In Europe, an average 3.9 million deaths occur each year due to CVD accounting for 45% of all deaths in the region (Wilkins *et al.*, 2017). CVD including coronary heart disease (CHD), stroke, and other vascular related conditions such as peripheral arterial disease are also a major economic burden in Europe having caused an estimated €210 billions of cumulative economic losses annually from healthcare expenditures, and reduced productivity (Wilkins *et al.*, 2017).

Diet is the major contributing factor to the burden of mortality by CVD in Europe (Naghavi *et al.*, 2017). Approximately 17% and 4% of total deaths from IHD and stroke, respectively, can be attributed to suboptimal diet; compared to smoking which contributed to 4 % and 1.5% of total deaths from IHD and stroke in 2016, respectively (Naghavi *et al.*, 2017). During the last 20 years, Europe, and especially Central Europe, has experienced a significant shift towards a greater consumption of unhealthy foods (Imamura *et al.*, 2015). There is a slight increase by 1.7% in consumption of healthy food and nutrients including fruits, vegetables, beans and legumes, nuts and seeds, whole grains, milk, polyunsaturated fatty acids, fish, plant omega-3 PUFA, and fibre. However, the intakes of red and processed meats, sugar-sweetened beverages, saturated fat, trans fat, dietary cholesterol, and sodium increased by 6.5% (Imamura *et al.*, 2015). Unhealthy diet has been interlinked with smoking and lack physical activity (Fransen *et al.*, 2017), which according to the GBD report from 2016 are the second and the third major contributing factors to the risk of CVD mortality in Europe. This issue is now widely recognized as a public health priority and the importance of introducing healthy eating patterns along with healthy lifestyle, as a fundamental measure to decrease the overall burden of CVD, has never been higher on the agenda of health organizations (WHO, 2015).

The first nutritional guidelines for CVD prevention were issued by American Heart Association (AHA) and focused mainly on nutrients, highlighting the importance of limiting the intake of total fat, saturated fat and cholesterol, as well as limiting salt and increasing total dietary fiber (Mosca *et al.*, 1999). Recent research has moved away from the traditional

epidemiological approach of studying single nutrient – disease relationships and has gathered significant amount of scientific evidence showing the association between different dietary factors and cardiovascular and metabolic risks (Figure 1). Later versions of the AHA guidelines in 2013 (Eckel *et al.*, 2013), and by European Society of Cardiology (ESC) in 2016 (Piepoli *et al.*, 2016) have included food based recommendations for CVD prevention highlighting the importance of consuming a variety of nutrient – rich foods, including fruits and vegetables, whole grains, low fat dairy, lean meat, fish, nuts and legumes, and limiting red meat, dietary sodium, sweets and sugar-sweetened beverages, and alcohol.

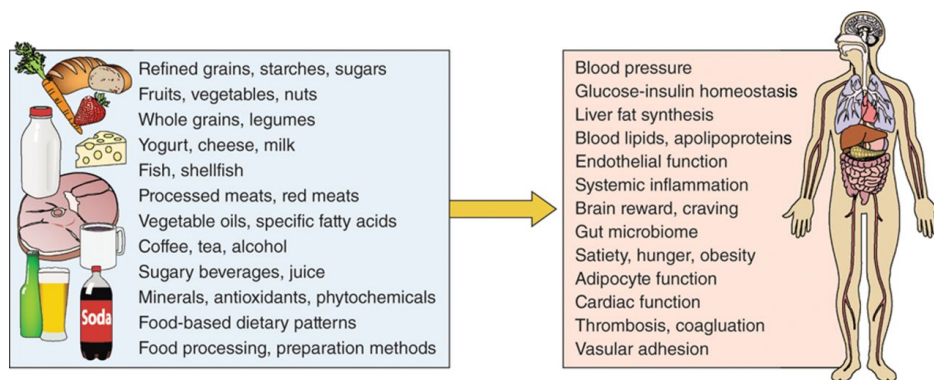


Figure 1: Dietary determinants of cardiovascular and metabolic risks. Illustration retrieved from: Mozaffarian, D. (2016) ‘Dietary and policy priorities for cardiovascular disease, diabetes, and obesity: a comprehensive review’, *Circulation*, 133(2), pp. 187-225.

1.2. Dietary pattern analysis

As previously mentioned, nutritional epidemiology has traditionally focused on the association of single nutrients or foods and disease, not taking into full consideration the complexity of dietary habits (Willet, 2012). Major limitations regarding the focus on single nutrients–disease association have been identified (Hu, 2002; Jacques *et al.*, 2001). For example, due to the high correlation between nutrient intakes (Watts *et al.*, 1990), it is challenging to single out the effect of individual nutrients when using multivariate models (Lee *et al.*, 1988). Also, associations observed in epidemiological studies could be biased due to the presence of unidentified confounding associations between the nutrients and an overall dietary pattern, which might introduce errors in the analysis based on the single nutrients- or food-approach (Hu, 2002). In order to address these limitations, the study the overall dietary patterns

was proposed (Hu, 2002; Jacques *et al.*, 2001). This approach would not only overcome some of the limitations of the single nutrient approach, but it would also reflect more precisely the overall patterns of food consumption, where dietary habits do not consist of isolated nutrients and foods, but rather a combination of the latter consumed as a part of different meals throughout the day. The results of the dietary pattern-based research studies could be used to better formulate relevant messages for informing consumers about the components of a healthy diet, and generating “easy to follow” health and diet-related messages that effectively could promote changes in eating patterns to tackle the burden of CVD among the general population.

1.2.1. “A-priori” defined dietary pattern analysis: Indices of dietary quality, and dietary guidelines

In order to analyse the association between the diet, as a whole, and the risk of CVD, dietary patterns have to be characterised. There are currently several methods for characterizing diets. One of them is the hypothesis-driven, “*a-priori*” defined dietary pattern analysis which assess the degree of adherence of a population diet to a predefined dietary index (DI). The scoring system of DI is usually based on dietary guidelines or developed as a form of dietary assessment tool.

Over the past 20 years a number of DIs have been proposed, and their application has been extensively investigated in relation to numerous CVD outcomes (Kourlaba and Panagiotakos, 2009; Kant, 1996; Wirt and Collins, 2009). In Europe, DIs are used to evaluate compliance with existing dietary guidelines and to assess its association with different disease outcomes. One of them is the Spanish Food Pyramid (SFP) score, which is based on the existing food based dietary guidelines for Spanish population (Aranceta and Serra-Majem, 2001) and considers recommendations for 12 food groups, physical activity and alcohol consumption (Molina-Montes *et al.*, 2014). Higher adherence to SFP was reported to be associated with increased obesity risk in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Granada study (Molina-Montes *et al.*, 2014). Another DI, the so called German Food Pyramid Index (GFPI), was designed to assess the adherence to the German Food Pyramid and reported to be inversely associated with CVD incidence (myocardial infarction or stroke) (von Ruesten *et al.*, 2010).

In light of the emerging scientific evidence on the diet-disease relationship and specifically chronic disease prevention, a number of studies have proved the benefits of adhering to a Mediterranean diet (MD), characterised by a higher consumption of vegetables and fruits, legumes, nuts, fish, and olive oil (Rosato et al., 2017; Grosso et al., 2017). Thus the MD has been internationally proclaimed as a healthy diet, and much effort has been put into measuring individual and population adherence to the MD and the assessment of whether a closer agreement with the Mediterranean Dietary Pattern (MDP) leads to better health. A number of DIs assessing the adherence to MDP have been proposed: Mediterranean Diet Score (MDS) (Trichopoulou *et al.*, 2003), Alternative Mediterranean Diet Index (aMED) (Fung *et al.*, 2005), Mediterranean Adequacy Index (MAI) (Alberti-Fidanza and Fidanza, 2004), Mediterranean Diet Score (MedDiet Score) (Panagiotakos, Pitsavos and Stefanadis, 2006), Mediterranean Style Dietary Pattern Score (MSDPS) (Rumawas *et al.*, 2009), and Mediterranean Diet Adherence Screener (MEDAS) (Schroder *et al.*, 2011). Most of them have a similar scoring system and, depending on the region where the score is developed, would assess the compliance with some of the following dietary characteristics: ratio of mono-unsaturated fatty acids : saturated fat, moderate intake of alcohol in the form of wine, moderate intakes of milk and dairy products, increased consumption of fish, legumes, seeds and nuts, grains (including bread and potatoes), fruits and vegetables, and olive oil, and decreased intakes of meat and meat products. A greater adherence to MDS, aMED, MAI, MedDiet Score, MSDPS and MEDAS DIs led to a significant reduction in the risk of CVD and CHD (Stefler *et al.*, 2017; Reedy *et al.*, 2014; Fidanza *et al.*, 2004; Martínez-González and Bes-Rastrollo, 2014; Lassale *et al.*, 2016; Domínguez *et al.*, 2013)

Although “*a-priori*” defined dietary patterns have proven to successfully establish the association between diet and disease, their applications have some limitations (Hodge and Bassett, 2016). DIs are usually study-specific and often subsequently administered to the participants as part of the dietary assessment. However these DIs are sometimes used in other studies by deriving the score using the population dietary data. For example, the use of a DI measuring the adherence to a MD in a US population where the consumption of typical Mediterranean region food items, such as olive oil and fish, would be quite limited, may fail to capture other sources of healthy fats traditionally consumed in the US. aMED index for instance does not force such data into the score, by classifying individuals into below and above median consumption of MDP components, still presents some limitation (Schwingshackl *et al.*,

2013). Even individuals scoring the highest on adherence to aMED may not reach the level observed in a traditional Mediterranean Diet (MD) and thus may not show the expected associations. Moreover, the use of DIs relies on subjective choice of defining cut off points for the food items intakes which might significantly affect the association with disease outcome (Hu, 2002). Lastly, its use is still limited to the available scientific evidence regarding the diet – disease relationship and therefore they cannot be applied to study the pathogenesis of the disease, and understand the underlying mechanisms and determinants involved in that association (Hu, 2002).

1.2.2. “A-posteriori” defined dietary pattern analysis: Principal Component and Cluster analysis

Dietary patterns defined “*a posteriori*” follow a data-driven approach which was introduced several decades ago (Hu, 2002) and since then has been widely used as a complex nutritional data reduction method to derive dietary patterns (Nettleton *et al.*, 2008; Panagiotakos *et al.*, 2007; Hearty and Gibney, 2008; Osler *et al.*, 2001; van Damm *et al.*, 2002; Stricker *et al.*, 2013). These reduction methods commonly include Principal Component Analysis (PCA) and Cluster Analysis (CA). The principle behind PCA is to derive the common factors expressed by the combinations of the given food groups frequently consumed together (Schwerin *et al.*, 1981; Slattery *et al.*, 1998; Hu *et al.*, 1999; Schulze *et al.*, 2001). The dietary patterns derived by this method reflect the true eating habits of a specific population, regardless of any pre-existing knowledge about diet-disease relationship. The method aggregates predefined food groups on the basis of the degree these food groups are correlated with one another and produce a common underlying dimension (factor) of food consumption (Figure 2). The decision on the number of factors is based on 1) the Eigenvalue, which represent the amount of variance that is accounted for by a given factor; 2) graphic representation of Eigenvalues (Scree plot), and 3) the interpretability of the dietary patterns derived. A factor score is computed for each individual participating in the study by combining standardised food variables with weights.

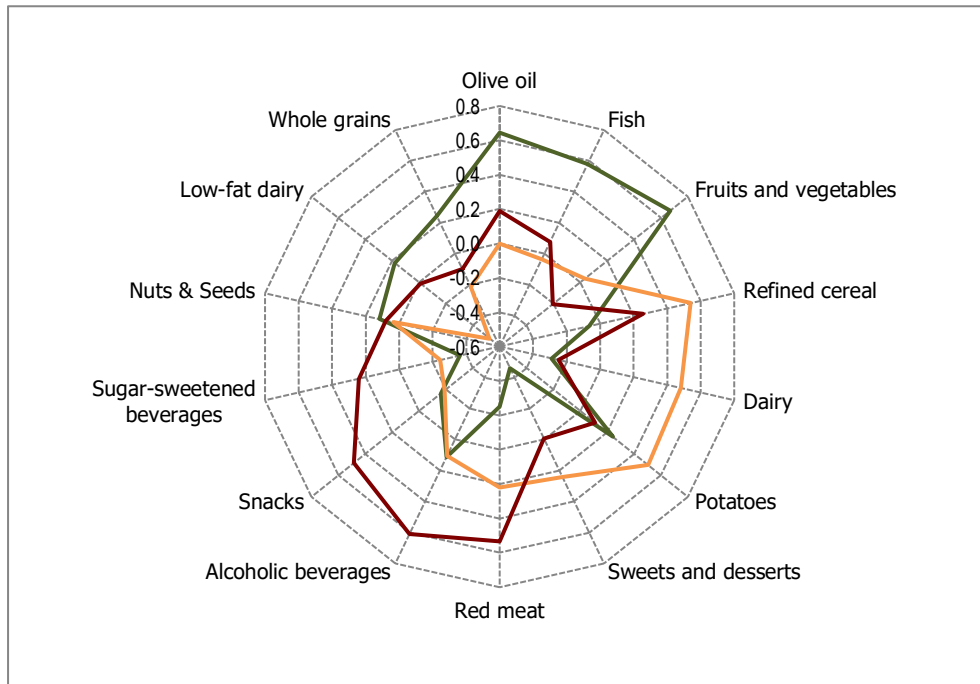


Figure 2: A representation of the main food items characterizing each dietary pattern. Values correspond to factor loadings (the higher the value, the more a particular food item contributes to the dietary pattern). Illustration is retrieved from: Peñalvo, J.L. *et al.* (2016) ‘Association Between a Social-Business Eating Pattern and Early Asymptomatic Atherosclerosis’, *Journal of the American College of Cardiology*, 68 (8), pp. 805-814.

Cluster analysis (CA) is another commonly used method for “*a-posteriori*” data analysis to aggregate complex dietary data and derive meaningful and interpretable dietary patterns (Hu, 2002). It groups individuals into relatively homogeneous groups with similar dietary characteristics within the group that are different from the ones in the other groups. Most common algorithms used in CA are the K-means algorithm and hierarchical clustering. To group dietary data points of a dataset into clusters, K-means algorithm seeks to subdivide the points based on the nearest mean values, creating the minimal distance between the points, so called high-density cluster (Jain *et al.*, 2010). The process is repeated multiple times assigning the new cluster centers and removing the data points from the low density clusters to the high density regions until no data points change the assignments to the cluster it belong to (Figure 3). Hierarchical clustering, also known as agglomerative, links each data point to its nearest neighbour, creating a cluster (Janssen *et al.*, 2012). At each iteration, two nearest clusters are repeatedly combined into a single larger cluster until the model produce the cluster with low cohesion (Figure 4).

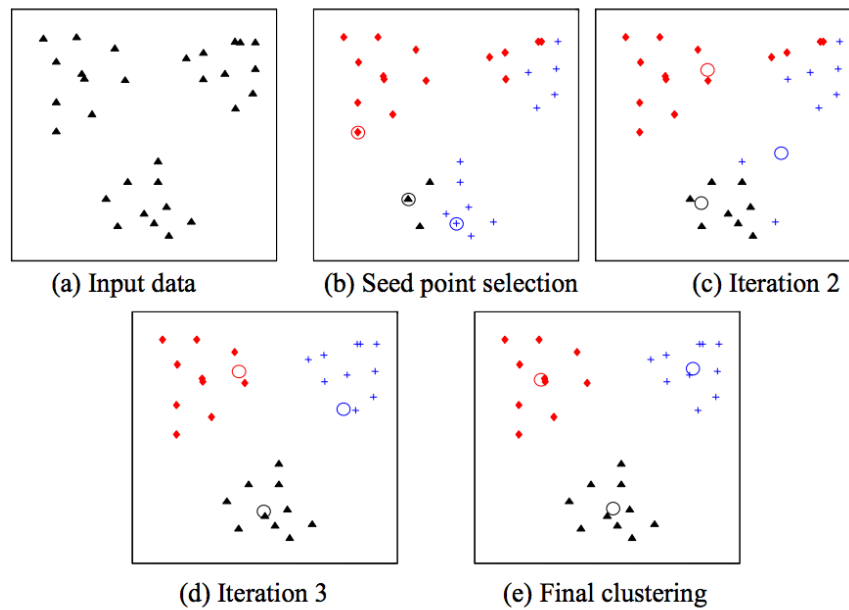


Figure 3: Illustration of K-means algorithm. (a) Data input with three clusters; (b) three seed points selected as cluster centres and initial assignment of the cluster centres; (c) & (d) intermediate iterations updating cluster labels and their centres; (e) final clustering obtained by K-means algorithm. Illustration adapted from: Jain, A. K. *et al.* (2010). “Data clustering: 50 years beyond K-means”. *Pattern recognition letters*, 31(8), pp. 651-666.

The most commonly identified dietary patterns using these “*a-posteriori*” approaches are: ‘Healthy’ or ‘Prudent Dietary Pattern’ (PDP), commonly characterised by higher consumption of vegetables, fruit, legumes, whole grains, fish, and poultry and “Unhealthy”, so-called ‘Western Dietary Pattern’ (WDP), characterised by greater intakes of processed foods, red meat, high-fat dairy products, refined grains, potatoes, and sugar sweetened beverages (Hu *et al.*, 2000; Fung *et al.*, 2001). According to a meta-analysis of observational studies involving 610 691 participants and a recent review of “*a-posteriori*” defined dietary patterns and incident CHD, adherence to a PDP shows a significant association with lower risk for all cardiovascular end points including acute myocardial infarction, incident CHD, total CVD risk and total CVD mortality (Rodríguez-Monforte *et al.*, 2015; Steffen and Hootman, 2016). On the other hand, results for WDP are not as conclusive and the evidence to date does not present a consistent association between an adherence to WDP and incidence of CVD, which highlight the necessity for further investigation (Rodríguez-Monforte *et al.*, 2015).

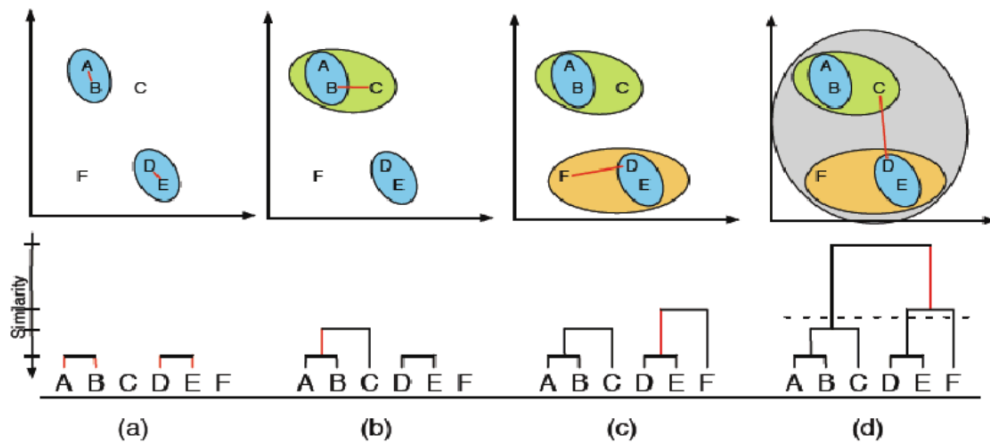


Figure 4: Illustration of hierarchical clustering algorithm. clusters are consecutively merged with the most nearby clusters. The length of the vertical dendogram-lines reflect the nearness. Illustration retrieved from: Janssen, P., Walther, C., and Lüdeke, M. (2012). Cluster analysis to understand socioecological systems: A guideline. In PIK report (Vol. 126, p. 96). Potsdam: Potsdam Institute for Climate Impact Research.

“*A-posteriori*” dietary pattern analysis also has limitations. The major limitation is the labelling of the identified patterns, as they may not be easily classified into healthy or unhealthy. As an example, a PCA-identified dietary pattern, which consisted the mix of healthy and unhealthy food items in particular, beans, tomatoes and refined grains, was still significantly associated with the higher risk of type 2 diabetes (Nettleton *et al.*, 2008). The conclusion derived from the study could have a harmful effect among those who would decide to eliminate tomatoes, which are rich source of antioxidants, and beans, which are rich in folate, fiber and copper, from their daily diet. Moreover, the dietary pattern derived from PCA might limit the generalizability of the findings applications. Thus the “Traditional” dietary pattern derived in Ireland (Hearty *et al.*, 2008) would significantly differ from the “Traditional” dietary pattern in China (Mu *et al.*, 2014). Lastly, if the so-called “unhealthy dietary pattern” would also include high loading of plant food sources, rich in phytochemicals known for its cardio-protective properties, the association with CVD outcomes might not be as evident (Maghsoudi, Ghiasvand and Salehi-Abargouei, 2016).

1.2.3. The combination of “*a-priori*” and “*a-posteriori*” analysis of dietary patterns

Both the application of either DI or PCA/CA for the identification of dietary patterns have limitations, and sometimes their individual use is not completely appropriate to derive meaningful dietary patterns in relation to disease (Osler *et al.*, 2001; Fung *et al.*, 2003; Masaki *et al.*, 2003). A combination data-driven approach with the “*a-priori*” knowledge of a disease has been suggested as having the potential to overcome the limitations that the single methods might pose and provide a better picture of a diet – disease relationship (Hoffmann *et al.*, 2004). In line with this, a method known as reduced rank regression (RRR), is frequently used in nutritional epidemiology. This approach focuses on the identification of the variations in food consumption that are associated with disease outcomes. Specifically, it identifies a disease-specific response variable and identifies the combinations of food intake that explain as much response variation in the disease outcome as possible (Weikert and Schulze, 2016). A number of studies have used RRR to derive dietary patterns, and generated specific hypotheses about foods that might have contributed to the disease risk (Frank *et al.*, 2015; Johns *et al.*, 2015; Livingstone and McNaughton, 2017). A dietary pattern, characterised by higher intakes of meat, margarine, poultry, and low intakes of vegetarian dishes, wine, vegetables, and whole-grain cereals was shown to explain the variation in several coronary artery disease (CAD) associated biomarkers, such as C reactive protein, C peptide and high density lipoprotein cholesterol (cHDL) concentration (Hoffmann *et al.*, 2004). Another study compared the application of RRR with PCA in terms of its efficiency to derive the dietary patterns and how well they could explain the variation in markers of subclinical atherosclerosis (Nettleton *et al.*, 2007). Both techniques derived similar dietary patterns, characterised by high total and saturated fat, low in fiber and micronutrients, however only RRR-derived dietary pattern was significantly associated with increased coronary artery calcium score (CACs) and carotid intima media thickness (cIMT). Despite the possibility of RRR application to shed the light on the potential mechanism in diet – disease relationship, it still holds a major limitation: it might identify a pattern which would be associated with the relevant CVD biomarkers, however may not necessarily exist in reality.

1.2.4. Novel techniques to derive dietary patterns

The challenges in the analysis of population diet resulted in the development and application of novel methods of dietary pattern analysis. Their main aims are to fully elucidate true dietary behaviour, and thus improve our understanding of how the foods are consumed, and what features of those patterns are associated with disease risk. One of these methods is the application of artificial neural networks (ANNs) modelling, which has been commonly used in the field of medicine over the past several decades (Lisboa and Taktak, 2002) to forecast the future event based on the inputs variables, however it is a relatively new to the field of dietary assessment. It is a prediction model which aims to uncover the hidden causal relationships between single or multiple responses and a large set of properties (Zou, Han and So, 2008). ANNs are based on the concept that a highly inter-connected system of simple processing elements can learn (or train) complex inter relationships between independent and dependent variables (Zou, Han and So, 2008). It has been applied in dietary pattern analysis to predict the dietary behaviour, in particular salt consumption, based on participants' clinical variables and knowledge about salt intake (Isma'eel *et al.*, 2015). The same methods were used before to analyse meal patterns and link them to overall dietary quality in the North-South Ireland Food Consumption Survey (NSIFCS) (Hearty and Gibney, 2008). The results demonstrated that ANNs successfully predicted an aspect of dietary quality, using foods consumed together at meals, and therefore shows the promise that it could be further applied into the prediction of health outcomes such as the presence of chronic disease by using dietary variables such as single foods or its combination as inputs.

Introduced nearly a decade ago (Lee, Nadler, and Wasserman, 2007), and also still relatively unused in nutritional epidemiology is the tool for dimensionality reduction, known as treelet transform (TT). It is an exploratory, data driven tool, which combines two analytical methods of data reduction: PCA and hierarchical CA, and clusters all dietary variables regarding their correlations in a hierarchical manner. Currently there is only one study which investigated the association between TT derived dietary pattern and risk of CVD events (Gorst-Rasmussen *et al.*, 2011). The authors derived 7 dietary patterns that were comparable and similar to the ones derived by PCA. Though, TT explained slightly lower factor variance compared to PCA, it was concluded that this technique may be useful in future studies of dietary patterns. Identified by TT dietary pattern characterised by high intakes of processed

meat, mayonnaises, margarines, sugar, butter, and refined cereals, similar to a WDP, was significantly associated with 53% risk of MI.

Probabilistic graphical models, such as Gaussian graphical models (GGMs) (Lauritzen *et al.*, 1996), are widely used to simplify and compress multi-dimensional genetic (Talluri, Baladandayuthapani, and Mallick, 2014) and metabolomics data (Floegel *et al.*, 2014) with the aim to explore distinct underlying pathways. Though it has a great potential in dietary pattern analysis its application in nutritional epidemiology is still relatively unexplored. It is a data-driven method that uses the high degree of correlation between dietary variables to construct networks. Each node in the network represents one dietary variable and each edge between two nodes represents the dependency of two dietary variables reflected by their partial correlation (Figure 5). Recently GGMs was applied to construct the dietary intakes networks using the data from 10,780 men and 16,340 women of the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam cohort (Iqbal *et al.*, 2016). The results provided insights into the dietary pattern intake, showing that red meat and cooked vegetables play a central role in the diet of this population (Iqbal *et al.*, 2016).

Another relatively unexplored tool to derive dietary patterns is latent class analysis (LCA), also known as a particular subtype of finite mixture model, which has been previously used in social sciences to determine different patterns of behaviour (Collins and Lanza, 2013). This technique uses categorical or ordinal type of data to identify number of unobserved, or latent subgroups (classes) within a population based on the observed variables (Formann, 1984). A number of recent studies have applied LCA on qualitative dietary data with the aim to categorize individual's diet into categories of similar dietary behaviour. In a recent study the application of LCA allowed the examination of temporal eating patterns among Australian men and women and evaluation of these patterns according to their sociodemographic profile (Leech *et al.*, 2017). Another study applied LCA to investigate the association between LCA derived patterns of lifestyle behaviour which included physical activity and adherence to a healthy diet with type 2 diabetes related outcomes (Wang *et al.*, 2011). The results showed that the class categorised by higher adherence to unhealthy diet and lack of physical activity was significantly related to longer duration of diabetes, poor glycaemic control and more diabetes-related diseases (Wang *et al.*, 2011).

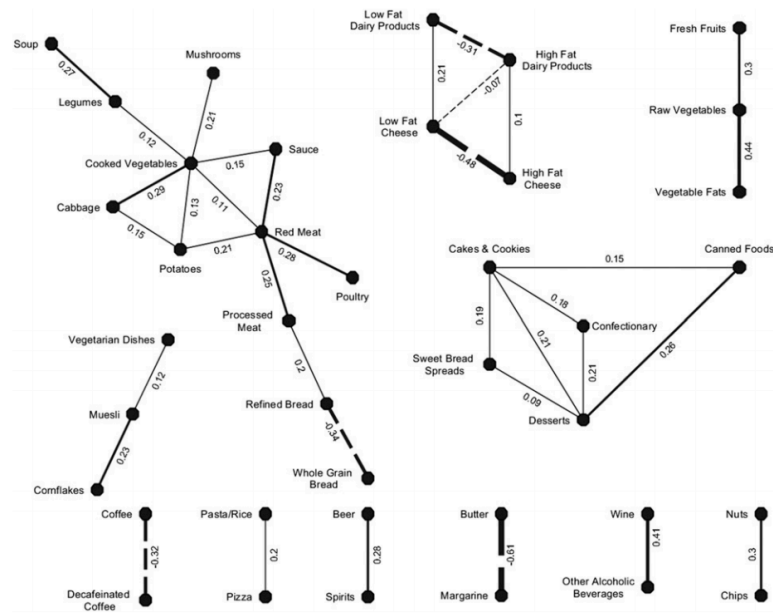


Figure 5: Illustration of Gaussian graphical models (GGMs). Each node in the network represents one dietary variable and each edge between two nodes represents the dependency of two dietary variables reflected by their partial correlation. Illustration retrieved from: Iqbal, K. et al. (2016). Gaussian Graphical Models Identify Networks of Dietary Intake in a German Adult Population–3. *The Journal of nutrition*, 146(3), 646-652.

1.3. The concept of meals and eating occasions

The concept of studying meals, rather than a combination of food groups identified by “*a-priori*” or “*a-posteriori*” defined dietary pattern analysis techniques, has received a significant attention during past years, due to increased scientific evidence showing the effect of timing and frequency of food intake on disease biomarkers (Mattson *et al.*, 2014; St-Onge *et al.*, 2017). Meals consumed at the main eating occasions (breakfast, lunch and dinner) and smaller sized meals such as snacks have been extensively reviewed in terms of patterning (frequency, regularity, timing and skipping), format (types of food combinations, nutrient profile), and context (at home, take-away) (Leech *et al.*, 2015). Due to the lack of a standardized definition, there are different approaches to defining meals: 1) time-of-day, which is defined according to the time-of-day in which food was consumed; 2) participant-identified, which relies on the respondent to identify an eating occasion (EO) as a meal or snack, often from a list of pre-specified meal labels (for example, breakfast, lunch, dinner or snack); 3) food-based classification (FBC) intends to capture both the quantitative and qualitative aspects

of meals. It includes pre-defined food groups with specific nutritional profile, which are combined to form a meal and further classified into the different eating occasions; and 4) neutral, which uses the term “eating event” and describes it in terms of various standardized criteria such as time of the day the meal is consumed, or number of eating events per day (Leech *et al.*, 2015).

Recently, a novel approach of meal identification was introduced by the application of meal coding system which generated generic meals (Woolhead *et al.*, 2015). The authors used the dietary data from 4-days record contained nutritional information on aggregated food groups. The combination of these food groups consumed by one person at a single eating occasion was identified as an individual meal. The combinations of individual meals were matched with a total of 5 meal types such as breakfast, light meals (including lunch light meal and dinner light meal), main meals (lunch main meal and dinner main meal), snack (morning, afternoon, evening, and night snacks), and beverages (alcoholic and non-alcoholic beverages) and the most commonly consumed combinations were then coded into generic meals (Woolhead *et al.*, 2015).

The most extensively investigated meal is breakfast, which is identified as (1) “the first meal of the day that breaks the fast after the longest period of sleep, eaten before or at the start of daily activities (e.g., errands, travel, work), within 2 h of waking, typically no later than 10:00 in the morning, and of an energy level between 20 and 35% of total daily energy need” (Timlin and Pereira, 2007) and (2) “the consumption of food or beverage (excluding water) between 5 and 9 AM” (Haines, Guilkey and Popkin, 1996). A number of studies have shown that breakfast skippers or occasional breakfast consumers have an overall poor nutritional profile, such as low intakes of dietary fiber (Fayet-Moore *et al.*, 2017), increased total energy and sugar intake (Pereira *et al.*, 2017) and not meeting the recommendations for vitamins A and D, calcium, iron and magnesium (Barr *et al.*, 2013; Barr *et al.*, 2014). Several large epidemiological studies support the association between irregular breakfast consumption and increased CVD risk factors (Iqbal *et al.*, 2017), including obesity and weight gain (Horikawa *et al.*, 2011), markers of impaired glucose metabolism, including elevated hemoglobin A1c (Reutrakul *et al.*, 2014), higher fasting plasma glucose (Kollannoor-Samuel *et al.*, 2011), increase in incidence of CHD (Cahill *et al.*, 2015).

1.4. Challenges and perspectives

As previously discussed, some countries of the European Region have experienced a significant shift towards an unhealthier dietary pattern characterised by greater intakes of red and processed meat, sugar-sweetened beverages, saturated fat, trans fat, dietary cholesterol, and sodium (Imamura *et al.*, 2015). In line with this observation, the Food and Agriculture Organization (FAO) reported a decline in the adherence to the traditional dietary pattern of the Mediterranean region (FAO, 2015). It has been noted that the region is undergoing a diet transition towards a Westernized type of diet, resulting in the coexistence of micronutrients deficiencies including vitamin A, zinc, and iron and overnutrition, such as overweight, and obesity (Shrimpton and Rokx, 2012). Health authorities at all levels promote healthy eating, smoking abstinence and increased physical activity level as a number one priority (WHO, 2013), and there is scientific evidence regarding the strategies of how to change health-related behaviour (NICE, 2014). Yet over the years most efforts with respect to changing the lifestyle factors have had only limited success (Marteau, Hollands and Kelly, 2015). Due to inherent heterogeneity of people's eating preferences, the accurate determination of dietary patterns remains a challenge. Moreover, dietary patterns are technically consisted of meals and therefore the investigation of daily meals and specific eating occasions should be taking into consideration when the eating patterns of a population and their relationship with CVD outcomes. There is a clear need for novel and efficient strategies to reduce the complex and multidimensional nutritional data into meaningful patterns/meals describing the diets accurately. This will allow to more precise estimates of the relationship between diet and disease outcomes that will in turn increase the confidence in the messages and public health guidelines and recommendations translated to the consumer and society.

AIM AND OBJECTIVES

Using observational data from two prospective cohorts in Spain and a representative survey from Ireland, the overarching objectives of this doctoral thesis were to 1) apply “*a-priori*” and “*a-posteriori*” dietary and meal pattern analyses to characterise the nutritional composition of habitual diet and its healthfulness, 2) measure the association of the degree of adherence to distinctive dietary patterns, the type and frequency of meals, eating occasions, and the synergistic effect of multiple lifestyles on traditional cardio-metabolic biomarkers, presence and extent of subclinical atherosclerosis.

Specific objectives:

1. Using “*a-posteriori*” methods based on data reduction and data clustering, specifically Principal Component Analysis (PCA) and Cluster Analysis (CA): To identify most common dietary patterns prevalent in two populations, and investigate their association with markers of CVD risk, and the presence and extent of subclinical atherosclerosis in a population of asymptomatic middle-aged adults in Spain (**Publication 1 and Publication 2**).

2. Using “*a-priori*” methods based on a predefined DI measuring adherence to a MD (Alternate Mediterranean Dietary Index (aMED)) characterise the association between different degrees of adherence to a MD and the presence and extent of subclinical atherosclerosis measured by the presence of plaque in multiple vascular territories or the presence of coronary calcium, as well as the impact of other risk factors and lifestyle habits in these associations (**Publication 3 and Publication 4**).

3. To derive meal consumption patterns using the data from the Irish population using generic meal coding and “*a-posteriori*” method of Latent Class Analysis (LCA). To study weekends / weekdays variation in the patterns, and to evaluate its association with CVD biomarkers (**Publication 5**).

4. Using data on eating occasions, to characterise the association between different breakfast patterns and CVD risk factors, and in particular, whether regularly skipping breakfast is associated with subclinical atherosclerosis (**Publication 6**).

5. Using data on eating occasions, to characterise the regularity of different breakfast consumption patterns, and to identify the overall daily dietary patterns associated with them to evaluate its nutritional quality (**Submitted manuscript**).

1st PUBLICATION:

Greater adherence to a Mediterranean dietary pattern is associated with improved plasma lipid profile: the Aragon Health Workers Study cohort.

Rev Esp Cardiol. 2015;68(4):290-7.

GENERAL INTRODUCTION / INDIVIDUAL'S CONTRIBUTION

As it was previously discussed, adherence to a traditional diet of the Mediterranean region is associated with a CVD-related benefits. Moreover, it was reported that there is the recent shift in the dietary patterns within the Mediterranean region (FAO, 2015). Therefore, I aimed to better understand the current dietary patterns within a Spanish population, identify the components of these patterns and their relative proportions, and to study the association between these patterns with CVD disease related outcomes, such as lipids.

In order to determine dietary patterns the “*a-posteriori*” method of analysis was chosen. I used the baseline data from 1290 participants of the Aragon Health Workers Study (AWHS), applied PCA and identified two main dietary patterns prevalent among this population. Those were: a MDP, characterised by greater intakes of vegetables and fruits, fish, white meat, nuts and olive oil, and a WDP, characterised by greater intakes red meat, fast food, dairy and cereals. The analysis provided the correlation matrix of food loadings, which allowed the identification of the main foods consumed and their relative proportions. In order to validate derived dietary patterns I studied their correlation with diet quality indices, such as AHEI (Alternate Healthy Eating Index) (McCullough *et al.*, 2002), aMED (alternate MD Index) (Fung *et al.*, 2009), MEDAS (MD Adherence Screener) (Schroder *et al.*, 2011), and MEDLIFE (MEDiterranean LIFEstyle Index) (Sotos-Prieto *et al.*, 2014). MDP was in agreement with the above indices showing that the greater adherence to the pattern was associated with higher dietary quality score. Further, I applied linear and logistic regressions controlling for confounding factors to determine the relationship between the highest adherence to MDP and WDP with intermediate markers of CVD, including total cholesterol, high density lipoproteins (cHDL), low density lipoproteins (cLDL), triglycerides (TG), TG/cHDL ratio, ApoA1, Apo B, and Lipoprotein (a).

Original article

Greater Adherence to a Mediterranean Dietary Pattern Is Associated With Improved Plasma Lipid Profile: the Aragon Health Workers Study Cohort



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ABSTRACT

Introduction and objectives: There is wide recognition of the importance of healthy eating in cardiovascular health promotion. The purpose of this study was to identify the main dietary patterns among a Spanish population, and to determine their relationship with plasma lipid profiles.

Methods: A cross-sectional analysis was conducted of data from 1290 participants of the Aragon Workers Health Study cohort. Standardized protocols were used to collect clinical and biochemistry data. Diet was assessed through a food frequency questionnaire, quantifying habitual intake over the past 12 months. The main dietary patterns were identified by factor analysis. The association between adherence to dietary patterns and plasma lipid levels was assessed by linear and logistic regression.

Results: Two dietary patterns were identified: a Mediterranean dietary pattern, high in vegetables, fruits, fish, white meat, nuts, and olive oil, and a Western dietary pattern, high in red meat, fast food, dairy, and cereals. Compared with the participants in the lowest quintile of adherence to the Western dietary pattern, those in the highest quintile had 4.6 mg/dL lower high-density lipoprotein cholesterol levels ($P < .001$), 8 mg/dL lower apolipoprotein A1 levels ($P = .005$) and a greater risk of having decreased high-density lipoprotein cholesterol (odds ratio = 3.19; 95% confidence interval, 1.36–7.5; P -trend = .03). Participants adhering to the Mediterranean dietary pattern had 3.3 mg/dL higher high-density lipoprotein cholesterol levels ($P < .001$), and a ratio of triglycerides to high-density lipoprotein cholesterol that was 0.43 times lower ($P = .043$).

Conclusions: Adherence to the Mediterranean dietary pattern is associated with improved lipid profile compared with a Western dietary pattern, which was associated with a lower odds of optimal high-density lipoprotein cholesterol levels in this population.

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La mayor adherencia a un patrón de dieta mediterránea se asocia a una mejora del perfil lipídico plasmático: la cohorte del Aragon Health Workers Study

RESUMEN

Introducción y objetivos: Hay un amplio reconocimiento de la importancia de una dieta saludable para la promoción de la salud cardiovascular. El objetivo de este estudio es identificar los principales patrones alimentarios en la población española y determinar su relación con los perfiles lipídicos plasmáticos.

Métodos: Se llevó a cabo un análisis transversal de los datos obtenidos en 1.290 participantes de la cohorte del Aragon Workers Health Study. Se utilizaron protocolos estandarizados para la obtención de datos clínicos y bioquímicos. Se evaluó la dieta a través de un cuestionario de frecuencia de alimentación, cuantificando el consumo habitual durante los 12 meses previos. Se identificaron los principales patrones de dieta mediante un análisis factorial. Se evaluó la asociación entre la adherencia a los patrones de dieta y las concentraciones plasmáticas de lípidos mediante regresión lineal y logística.

Resultados: Se identificaron dos patrones de dieta: un patrón de dieta mediterránea, rica en verduras, frutas, pescado, carnes blancas, frutos secos y aceite de oliva, y un patrón de dieta occidental, rico en carnes rojas, comida rápida, productos lácteos y cereales. En comparación con los participantes que se

Palabras clave:

Dieta mediterránea

Patrón de dieta

Análisis factorial

Puntuación de dieta

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encontraban en el quintil más bajo de adherencia al patrón de dieta occidental, los que se encontraban en el quintil más alto presentaron cifras de colesterol unido a lipoproteínas de alta densidad 4,6 mg/dl menores ($p < 0,001$) y de apolipoproteína A1, 8 mg/dl menores ($p = 0,005$) y mayor riesgo de mostrar una disminución del colesterol unido a lipoproteínas de alta densidad (*odds ratio* = 3,19; intervalo de confianza del 95%, 1,36–7,5; p de tendencia = 0,03). Los participantes con adherencia al patrón de dieta mediterránea presentaron cifras de colesterol unido a lipoproteínas de alta densidad 3,3 mg/dl mayores ($p < 0,001$) y un cociente de triglicéridos/colesterol unido a lipoproteínas de alta densidad 0,43 veces inferior ($p = 0,043$).

Conclusiones: La adherencia al patrón de dieta mediterránea se asocia a una mejora del perfil lipídico en comparación con lo que se observa con un patrón de dieta occidental, que se asoció a menor probabilidad de que los valores de colesterol unido a lipoproteínas de alta densidad fueran óptimos en esta población.
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Abbreviations

ApoA1: apolipoprotein A1
HDL-C: high density lipoproteins cholesterol
MDP: Mediterranean dietary pattern
TG: triglycerides
WDP: Western dietary pattern

INTRODUCTION

Cardiovascular disease is well recognized as a major public health problem.¹ Given the direct influence of unhealthy dietary habits on its development and progression,² prevention through promoting a healthy way of eating at all population levels is a public health priority.³

The diet-disease relationship can be addressed from different perspectives, from the single nutrient approach to assessment of overall diet quality.⁴ This latter approach accounts for the likely interactions between dietary components and other lifestyle-related habits and may be better suited to identify behavioral determinants of cardiovascular disease rather than explore nutrient-induced etiological mechanisms. Evidence on how the overall diet quality impacts health is also more easily translated to broader audiences and policymakers, helping to underpin effective public health strategies. In this regard, the traditional Mediterranean dietary pattern (MDP), high in plant-based dietary sources, white meat, fish, and olive oil, and low in red meat and processed food, is well known for its cardioprotective effect^{5,6} and is recommended worldwide. Moreover, the traditional MDP has also been proposed as a plausible explanation of the Mediterranean paradox, ie, a high prevalence of cardiovascular disease risk factors along with a low incidence of cardiac events,⁷ and as a priority for primary and secondary cardiovascular disease prevention.⁸

Although the Mediterranean region has recently experienced a transition toward a more westernized dietary pattern and diet varies significantly between the countries of this area, depending on the agricultural and cultural settings, evidence shows that the MDP is associated with improved plasma lipid profile, including increased high-density lipoprotein cholesterol (HDL-C) concentration and decreased levels of low-density lipoproteins, triglycerides (TG), and total cholesterol.^{9–11} Furthermore, the effect of the MDP on apolipoprotein A1 (ApoA1) concentration has also been studied.^{12,13} Some studies have reported an increase of ApoA1 concentrations with Mediterranean diet¹⁴ and reductions in ApoA1 catabolic rate.¹⁵

In view of these findings, our aim was to identify the current major dietary patterns prevalent in a population of Spanish workers, the Aragon Workers Health Study cohort, and to

investigate their association with plasma lipid profile as an intermediate indicator of future cardiovascular outcomes.

METHODS

Study Population

Details of the study design and methodology used have previously been published.¹⁶ In brief, the Aragon Workers Health Study is a prospective cohort aimed at investigating the determinants of the development and progression of subclinical atherosclerosis in a middle-aged population. The study population consisted of a random sample of 5690 employees of the General Motors Spain automobile-assembly plant located in Zaragoza (Spain) who were free of cardiovascular disease at baseline.¹⁶ Each year, a random one-third of the study participants aged 40 to 55 years are selected for subclinical atherosclerosis imaging, clinical and physical examination, and diet, behavior, and lifestyle assessment. The present cross-sectional study was carried out in a subsample of 1593 participants with complete dietary data at baseline. Of these, 104 participants with extreme values for total energy intake (< 800 or > 4200 Kcal, and < 500 or > 3500 for men and women, respectively),¹⁷ and 199 participants with missing data were excluded. The final sample available for analysis consisted of 1290 participants. The study was approved by the central Institutional Review Board of Aragón CEICA (*Comité Ético de Investigación de Aragón*), and all study participants provided written informed consent.¹⁶

Dietary Assessment

Habitual food intakes over the past 12 months were collected through a validated 136-item food-frequency questionnaire, administered by trained dietician.^{18,19} The frequency of consumption varied from “never or almost never” to “more than 6 times per day”. Individuals' total energy and nutrient intakes were derived through a standardized nutrient database (ENDB).²⁰ Using this data, factor analysis was used to determine the main dietary patterns prevalent in our population. Furthermore, to validate the results of factor analysis, previously reported diet quality indices (AHEI [Alternate Healthy Eating Index],²¹ aMED [alternate MD Index],²² MEDAS [MD Adherence Screener],²³ and the recently developed MEDLIFE [MEDiterranean LIFEstyle Index]²⁴) were computed. The details of the indices' development and scoring systems are described elsewhere.^{21–24}

Blood and Urine Collection

At baseline, participants provided a clinical history, including the occurrence of any clinical events and hospitalizations over the

past year, indicating the presence of a personal or family history of early cardiovascular disease, current medication use, and, if diagnosed, hypertension, diabetes, or dyslipidemia. Seated resting blood pressure was measured by using an OMRON M10-IT (OMRON Healthcare Co Ltd; Japan) automatic oscillometric sphygmomanometer. Three measurements were taken and the average of the measurements was used for the analysis. Blood and urine samples were collected at baseline and were processed and stored for further analysis and biobanking. Fasting serum glucose, TG, total cholesterol, and HDL-C concentrations were measured by spectrophotometry (Chemical Analyzer ILAB 650, Instrumentation Laboratory). Low-density lipoprotein cholesterol was calculated using the Friedewald formula. Levels of HDL-C of ≥ 40 mg/dL and ≥ 50 mg/dL for men and women, respectively, were considered optimal.²⁵ Serum ApoA1, B100, and lipoprotein (a) were measured by kinetic nephelometry (Immunochemistry Analyzer IMMAGE 800, Beckman Coulter), and fasting serum insulin by immunoenzymatic chemiluminescence (Access Immunoassay System, Beckman Coulter). Whole blood glycated hemoglobin was measured by reverse-phase cationic exchange chromatography and quantification by double wave-length colorimetry quantification (Analyzer ADAMS A1c HA-810, Arkray Factory). The HOMA (Homeostatic Model Assessment) index was used to assess insulin resistance using glucose and insulin data.²⁶

Physical Activity Assessment

Leisure time physical activity was assessed using the Spanish-validated version²⁷ of the Nurses' Health Study and Health Professionals' Follow-up study physical activity questionnaires.^{28,29} Participants were asked about the average weekly time spent on 17 different types of physical activity, which was multiplied by its typical energy expenditure, expressed in metabolic equivalent transfer units,³⁰ and summed over all activities, to estimate the total level of physical activity spent per week.

Assessment of Other Variables

Anthropometric measurements of body weight, height, and waist circumference were performed at baseline following standardized procedures.³¹ Data were also collected on baseline sociodemographics, education, smoking history, and employment.

Statistical Analysis

The main dietary patterns were determined by factor analysis by deriving factor loading for predefined food groups using Varimax rotation option. Combinations of eigen values, the scree plot, and interpretability were used to determine the number of factors retained. Each factor had an eigen value > 0.3 . Factor scores were computed for each participant for each dietary pattern by summing intakes of food groups weighted by their factor loadings. Based on the score, participants were then divided into quintiles of adherence into the specific dietary pattern. To describe baseline characteristics, categorical variables are presented as count and percentage, and continuous variables as mean (standard deviation). The *P*-trend was tested using the factor adherence as a continuous term in the regression model. The consistency of the factor analysis-derived patterns was tested by comparing factor loadings with *a priori*-defined scores, namely the AHEI, the aMED, the MEDAS, and the recently developed MEDLIFE by studying the strength of the association across quintiles of factor score. Linear regression analyses were conducted between plasma lipid

concentrations and dietary pattern scores after controlling for the following possible confounders: age, sex, education level, dietary energy, physical activity level, plasma lipid-lowering medication, and body mass index. The odds ratio for decreased HDL-C concentration was assessed through logistic regression (adjusted for the same possible confounders as in linear regression) analysis across quintiles of dietary patterns (the first quintile was set as a reference). STATA 12.0 (StataCorp LP; College Station, Texas, United States) was used for all statistical analyses.

RESULTS

Food Consumption Pattern

Food items from the food frequency questionnaire were classified into 17 main food groups (Table 1 supplementary material). Based on the factor loading of the food groups, 2 main dietary patterns were identified (Table 1). The first dietary pattern was characterized by higher intakes of vegetables, fresh fruits, nuts, fish, olive oil and, to a lesser extent, regular consumption of nonfat dairy products and white meat and was named the "Mediterranean dietary pattern" (MDP). The second dietary pattern was characterized by higher intakes of cereals, red meat, full-fat dairy products, fast food, desserts and sweets, and, to a lesser extent, by regular consumption of vegetable oils, soda, coffee, tea and wine/beer and was named the "Western dietary pattern" (WDP). The food group of legume pulses contributed to both patterns and was not considered determinant. These 2 patterns accounted for 22% of the variance of total food intake.

Dietary Patterns and Their Agreement With Diet Quality Indices

The analysis of the association between the 2 dietary patterns identified in our population and previously reported dietary indices capturing a healthy diet/lifestyle and the Mediterranean diet is shown in Table 2. All indices were positively associated with the MDP, indicating that those participants with higher adherence to the MDP also scored highly in distinct *a priori* indices. In contrast, all indices were inversely associated with the WDP,

Table 1
Factor Loading Matrix for Dietary Patterns

Food groups	MDP	WDP
Vegetables	0.6671	0.0350
Fresh fruit	0.4862	-0.1168
Cereals	-0.0164	0.4117
Pulses	0.2180	0.3945
Nuts	0.4339	0.0228
Fish	0.5816	0.0845
White meat	0.3527	-0.0454
Red meat	0.1107	0.6221
Dairy	-0.0613	0.4469
Nonfat dairy	0.2292	-0.4307
Fast food	-0.0110	0.5378
Sweets	-0.0327	0.5482
Olive oil	0.4568	0.0078
Vegetable oils	-0.3630	0.2840
Coffee and tea	0.0567	0.2742
Soda	-0.0517	0.2927
Wine	0.0210	0.1812

MDP, Mediterranean dietary pattern; WDP, Western dietary pattern.

suggesting that this pattern is indeed associated with lower quality diets. This association between *a priori* and *a posteriori*-derived dietary patterns provides additional reliability to the results.

Sociodemographic Characteristics and Cardiovascular Risk Factors

Baseline characteristics according to quintiles of the 2 major dietary patterns (MDP and WDP) among the 1290 participants are shown in Table 3. As energy intake was not considered during factor analysis, adherence to both factors increased with energy intake due to the wider variety of foods consumed by those with higher caloric intake. On average, those participants in the highest quintile of adherence to the MDP were slightly older ($P = .01$), were either less likely to currently smoke ($P < .001$) or were more likely to be former smokers ($P < .001$), were more physically active ($P < .001$), and had higher energy intake ($P < .001$) compared with those in the lowest quintile. Adherence to none of the patterns was associated with cardiovascular risk factors or biochemistry indicators, either across the quintiles of distribution (P -trend $> .05$) or when we compared the samples in extreme quintiles ($P > .05$). In contrast, participants with the highest adherence to the WDP were slightly younger men, belonged to families of 3 or more members, and had a lower education level ($P < .05$). No differences were found for cardiovascular risk factors except for a lower percentage of medication use ($P < .05$ for all medication) and a higher prevalence of smoking ($P < .001$) among those adhering more closely to the WDP.

Dietary Pattern Adherence and Plasma Lipids

Plasma lipid concentrations across quintiles of adherence to the MDP and WDP are shown in Table 4. Fully adjusted linear regression models were used for comparison. On average, participants adhering more closely to the MDP had higher HDL-C (quintile 1 = 51.5 mg/dL; quintile 5 = 54.8 mg/dL; $P < .001$) and a lower TG/HDL-C ratio (quintile 1 = 3.38; quintile 5 = 2.95; $P = .043$). In contrast, participants who scored high on the WDP had significantly ($P < .001$) lower HDL-C (quintile 1 = 54.5 mg/dL; quintile 5 = 49.9 mg/dL), and serum ApoA1 (quintile 1 = 147 mg/dL; quintile 5 = 139 mg/dL; $P = .005$). Figure shows the risk of having HDL-C lower than 40 mg/dL for men and lower than 50 mg/dL for women across quintiles of adherence to the WDP and MDP. The risk of having lower HDL-C increased with greater adherence to the WDP (quintile 5 vs quintile 1, odds ratio = 3.19; 95%

confidence interval, 1.36–7.50; P -trend = .03). Regarding the MDP, the trend across quintiles was not significant (quintile 5 vs quintile 1, odds ratio = 0.603; 95% confidence interval, 0.329–1.100; P -trend = .203).

DISCUSSION

In this study, we investigated the relationship between overall diet quality expressed by adherence to *a posteriori*-derived dietary patterns and plasma lipid profile as an intermediate indicator of cardiovascular risk. Previous studies have suggested that adherence to a healthy diet is linked to improved health-related behaviors.³² Analysis of the dietary patterns of a Dutch population revealed that individuals who followed a pattern high in dairy products, meat, and processed food were more likely to be less educated, less physically active, and heavy smokers.³³ In contrast, those with a higher intake of vegetables and vegetable oils, pasta, rice, fish, white meat, and wine were likely to have a higher education level and to be more physically active.³³

There is scientific evidence that choosing a healthier dietary pattern depends on socioeconomic status.³⁴ This is true in Spain, where a recent study among university graduates found that the Mediterranean diet, to some extent, was expensive.³⁵ In our study, individuals with a lower education and income might face economic barriers to the MDP, and are thus prone to the WDP or a similar high-fat pattern, giving preference to a less expensive and less healthy diet, whereas a more highly educated population with a higher income might be more able to afford certain healthier food items, which on average are considered expensive. Additionally, more highly educated participants might have better nutrition knowledge and greater awareness of the effects of exposure to a low-quality diet on risk of disease progression; therefore they would develop the healthier dietary pattern. As for lifestyle habits, in our study only physical activity was positively associated with increased adherence to the MDP and smoking with a lower or higher adherence to MDP or WDP, respectively. Current smokers tended to be less health-conscious and to have a lower degree of self-awareness of their health-related behaviors, including diet, than those who either never smoked or gave up smoking.³⁶ Smoking causes an increased turnover of micronutrients,³⁷ thus putting smokers who follow the WDP at greater risk of chronic disease compared with nonsmokers, not only due to the unhealthy dietary intake characterized by the WDP, but also due to malnutrition.³⁸

Table 2
Association Between *a Posteriori*-defined Dietary Patterns and *a Priori*-defined Dietary Indices

	Quintiles of adherence to the pattern					<i>P</i> -trend
	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	
<i>MDP</i>						
MEDLIFE	9.31 (2.32)	10.40 (2.00)	11.10 (2.32)	12.10 (2.17)	13.30 (2.28)	<.001
MEDAS	4.95 (1.44)	6.01 (1.30)	6.57 (1.36)	7.10 (1.35)	7.94 (1.56)	<.001
AHEI	42.4 (7.25)	46.1 (6.67)	49.6 (6.61)	51.8 (7.10)	58.2 (7.51)	<.001
aMED	2.61 (1.37)	3.34 (1.47)	3.82 (1.50)	4.45 (1.44)	5.45 (1.48)	<.001
<i>WDP</i>						
MEDLIFE	11.6 (2.69)	11.3 (2.69)	11.3 (2.75)	11.1 (2.60)	10.9 (2.29)	.001
MEDAS	6.94 (1.78)	6.64 (1.71)	6.54 (1.69)	6.35 (1.72)	6.09 (1.63)	<.001
AHEI	50.2 (9.40)	50.1 (8.93)	50.1 (8.89)	49.4 (8.70)	48.3 (8.05)	.002
aMED	4.56 (1.56)	4.18 (1.81)	4.07 (1.61)	3.59 (1.80)	3.29 (1.65)	<.001

AHEI, Alternate Healthy Eating Index; aMED, alternate MD Index; MEDAS, MD Adherence Screener; MEDLIFE, MEDiterranean LIFEstyle Index; MDP, Mediterranean dietary pattern; WDP, Western dietary pattern.
The results are expressed as mean (standard deviation).

Table 3
Baseline Characteristics by Quintiles of Adherence

	Quintiles* of adherence to MDP					P-trend	P-value (quintile 1 vs quintile 5)
	Quintile 1 [†] 258 (-3.08 to 0.84)	Quintile 2 258 (-0.84 to -0.28)	Quintile 3 258 (-0.28 to 0.23)	Quintile 4 258 (0.23-0.79)	Quintile 5 258 (0.80-3.84)		
Demographics							
Age, mean (SD), y	50.8 (3.80)	51.1 (3.66)	51.7 (3.6)	51.4 (3.61)	51.5 (3.45)	.010	.021
Number of family members, mean (SD)	3.20 (1.06)	3.23 (1.03)	3.21 (0.89)	3.16 (0.93)	3.19 (0.96)	.669	.862
Number of children, mean (SD)	1.50 (0.82)	1.49 (0.734)	1.52 (0.755)	1.51 (0.74)	1.53 (0.72)	.392	.648
Gender (female)	7 (2.71)	16 (6.20)	12 (4.65)	18 (6.98)	11 (4.3)	.392	.337
CVD history	2 (0.78)	2 (0.79)	0 (0)	1 (0.41)	3 (1.2)	.427	.637
Education level							
≤ high school	240 (93.0)	246 (95.3)	244 (94.5)	238 (92.6)	245 (95)	.952	.354
> high school	18 (6.98)	12 (4.65)	14 (5.43)	19 (7.39)	13 (5.04)		
CVD risk factors							
Medication: Dyslipemia	36 (14.2)	38 (15.1)	31 (12.4)	35 (14.5)	35 (14.1)	.979	.970
Medication: Diabetes	11 (4.40)	10 (3.98)	8 (3.19)	7 (2.89)	11 (4.5)	.327	.977
Medication: Hypertension	60 (23.6)	54 (21.4)	47 (18.8)	51 (21)	56 (22.5)	.556	.763
Body mass index, mean (SD), kg/m ²	27.8 (3.56)	27.7 (3.62)	27.7 (3.74)	28.1 (3.67)	28.0 (3.33)	.370	.575
Waist circumference, mean (SD), cm	98.0 (9.70)	97.9 (9.47)	96.9 (10.5)	97.8 (10)	97.7 (8.9)	.628	.687
Systolic blood pressure, mean (SD), mmHg	125.0 (14.0)	126.0 (14.7)	125.1 (13.5)	125.9 (14.9)	126 (14.6)	.864	.379
Diastolic blood pressure, mean (SD), mmHg	83.5 (9.2)	83.7 (9.5)	82.8 (9.2)	84.3 (10.1)	83.4 (9.80)	.790	.664
Smoking status							
Smoker	103 (40.1)	74 (29.0)	85 (33.2)	90 (35.9)	53 (20.9)	<.001	<.001
Nonsmoker	82 (31.9)	90 (35.3)	92 (35.9)	81 (32.3)	86 (33.9)	.913	.639
Former smoker	72 (28.0)	91 (35.7)	79 (30.9)	80 (31.9)	115 (45.3)	<.001	<.001
Lifestyle							
Energy intake, mean (SD), kcal	2540 (673)	2697 (655)	2797 (573)	2875 (622)	3069 (578)	.000	<.001
Physical activity, mean (SD), METs-h/week	30.5 (18.3)	33.3 (19.6)	31.7 (18.2)	34.6 (20.3)	38.2 (22.4)	<.001	<.001
Sleep, mean (SD), h, business d	6.36 (0.92)	6.25 (0.92)	6.17 (0.89)	6.25 (1.07)	6.27 (1.03)	.619	.302
Biochemistry							
Glucose, mean (SD), mg/dL	98.7 (15.8)	101.0 (21.7)	98.6 (18.3)	98.7 (17.3)	99.9 (18.2)	.904	.458
Insulin, mean (SD), uU/mL	8.71 (7.01)	7.68 (5.80)	7.28 (4.9)	8.14 (5.6)	7.75 (5.02)	.230	.087
Glycated hemoglobin, mean (SD), %	5.56 (0.47)	5.56 (0.60)	5.54 (0.5)	5.55 (0.5)	5.57 (0.56)	.836	.890
Insuline resistance, mean (SD), HOMA	2.18 (2.00)	1.95 (1.70)	1.82 (1.4)	2.07 (1.7)	1.96 (1.49)	.342	.178
C-reactive protein, mean (SD), mg/dL	0.320 (0.73)	0.262 (0.20)	0.322 (0.4)	0.273 (0.3)	0.24 (0.29)	.081	.105
	Quintiles* of adherence to WDP					P-trend	P-value (quintile 1 vs quintile 5)
	Quintile 1 258 (-3.09 to -0.85)	Quintile 2 258 (-0.84 to -0.25)	Quintile 3 258 (-0.25 to 0.25)	Quintile 4 258 (0.25-0.83)	Quintile 5 258 (0.84-4.41)		
Demographics							
Age, mean (SD), y	51.6 (3.60)	51.3 (3.85)	51.7 (3.44)	51.0 (3.57)	50.8 (3.69)	.003	.012
Number of family members, mean (SD)	3.00 (0.98)	3.20 (0.97)	3.17 (0.99)	3.29 (0.97)	3.33 (0.92)	<.001	<.001
Number of children, mean (SD)	1.38 (0.76)	1.53 (0.74)	1.51 (0.79)	1.55 (0.76)	1.59 (0.69)	<.001	.001
Sex (female)	30 (11.60)	17 (6.59)	14 (5.43)	2 (0.77)	1 (0.39)	<.001	<.001
CVD history	4 (1.61)	0 (0.00)	1 (0.40)	2 (0.81)	1 (0.39)	.310	.168
Education level							
≤ high school	236 (91.5)	237 (92.2)	245 (94.9)	244 (94.5)	251 (97.3)	.002	.004
> high school	22 (8.50)	20 (7.78)	13 (5.04)	14 (5.43)	7 (2.70)		
CVD risk factors							
Medication: dyslipemia	50 (20.40)	31 (12.50)	42 (16.90)	31 (12.60)	21 (8.24)	<.001	<.001
Medication: diabetes	17 (7.00)	8 (3.24)	8 (3.21)	8 (3.25)	6 (2.36)	.026	.014
Medication: hypertension	71 (28.9)	49 (19.7)	67 (26.7)	47 (19.0)	34 (13.3)	<.001	<.001
Body mass index, mean (SD), kg/m ²	28.1 (3.71)	27.8 (3.69)	27.8 (3.60)	27.9 (3.64)	27.5 (3.27)	.123	.054
Waist circumference, mean (SD), cm	97.6 (10.80)	97.3 (10.40)	97.9 (9.36)	97.8 (9.52)	97.7 (8.50)	.637	.983
Systolic blood pressure, mean (SD), mmHg	126 (14.9)	125 (14.2)	125 (14.6)	126.8 (14.1)	125 (14.0)	.626	.742
Diastolic blood pressure, mean (SD), mmHg	83.4 (9.80)	82.8 (9.30)	83.8 (9.90)	84.3 (9.80)	83.5 (8.96)	.281	.874
Smoking status							
Smoker	54 (20.9)	77 (30.2)	73 (29.1)	83 (32.9)	118 (45.9)	<.001	<.001

Table 3 (Continued)
Baseline Characteristics by Quintiles of Adherence

	Quintiles* of adherence to WDP					P-trend	P-value (quintile 1 vs quintile 5)
	Quintile 1 258 (-3.09 to -0.85)	Quintile 2 258 (-0.84 to -0.25)	Quintile 3 258 (-0.25 to 0.25)	Quintile 4 258 (0.25-0.83)	Quintile 5 258 (0.84-4.41)		
Nonsmoker	107 (41.5)	86 (33.7)	82 (32.7)	93 (36.9)	63 (24.5)	<.001	<.001
Former smoker	97 (37.60)	92 (36.19)	96 (38.20)	76 (30.20)	76 (29.60)	.014	.054
Lifestyle							
Energy intake, mean (SD), kcal	2093 (455)	2518 (413)	2769 (448)	3085 (410)	3514 (408)	<.001	<.001
Physical activity, mean (SD), METs-h/week	31.4 (19.3)	35.2 (20.9)	33.6 (20.7)	34.7 (20.5)	33.3 (18.2)	.194	.266
Sleep, mean (SD), h, business d	6.25 (0.91)	6.21 (1.01)	6.31 (0.92)	6.26 (1.10)	6.27 (0.91)	.808	.847
Biochemistry							
Glucose, mean (SD), mg/dL	99.9 (18.0)	98.5 (14.5)	99.8 (17.1)	99.2 (19.9)	99.1 (21.3)	.915	.657
Insulin, mean (SD), uU/mL	7.49 (6.03)	7.6 (4.48)	7.88 (5.90)	8.32 (6.28)	7.87 (5.90)	.919	.902
Glycated hemoglobin, mean (SD), %	5.56 (0.57)	5.53 (0.46)	5.56 (0.52)	5.56 (0.59)	5.55 (0.55)	.721	.828
Insuline resistance, mean (SD), HOMA	2.00 (1.75)	1.89 (1.26)	2.00 (1.70)	2.13 (1.89)	1.98 (1.77)	.863	.909
C-reactive protein, mean (SD), mg/dL	0.290 (0.39)	0.337 (0.35)	0.272 (0.35)	0.276 (0.31)	0.250 (0.21)	.131	.235

CVD, cardiovascular disease; HOMA, Homeostatic Model Assessment; MDP, Mediterranean dietary pattern; WDP, Western dietary pattern. Data are expressed as No. (%) or median (standard deviation).

* Quintiles are described by No. (minimum-maximum).

The results of our study on HDL-C are in agreement with both observational studies and clinical trials.^{9,10,39} Overall, a higher intake of refined and processed food, which characterizes the WDP, is associated with a lower HDL-C level, while closer adherence to the MDP will more likely result in increased HDL-C. One plausible underlying mechanism explaining the association between the WDP and lower HDL-C could be greater consumption of refined carbohydrates among those who follow the WDP. Refined carbohydrates tend to increase visceral adiposity, decrease insulin sensitivity, and stimulate hepatic *de novo* lipogenesis, which result in reduced HDL-C levels.⁴⁰ In contrast, the favorable effect of the MDP on HDL-C could be due to higher consumption of olive oil among the MDP population. Olive oil has been related to higher levels of trienoic prostaglandins, resulting in amelioration of plasma lipid profile, such as an increase in serum HDL-C.⁴¹

Some observational studies have shown an independent beneficial effect of physical activity on lipid profile.⁴² The role played by physical activity in altering the association between diet and lipid profile was to some extent observed in our study. Controlling for physical activity in the model slightly weakened the association between the MDP and TG/HDL-C ratio, and ApoA1 among those who followed the MDP, indicating that physical activity could indeed be partly associated with improvement of some components of the lipid profile.

Some authors have claimed that the effect of adherence to the healthy MDP on lipid profile is due to its favorable effect on obesity.⁴³ Although greater compliance with the MDP was seen to be associated with a lower obesity risk, adjustment for body mass index as a proxy of obesity, according to our results, only slightly altered the association between diet and lipid profile, indicating

Table 4
Plasma Lipids by Quintiles of Adherence to Main Dietary Patterns

	TG, mg/dL	Cholesterol, mg/dL	HDL-C, mg/dL	LDL-C, mg	TG/HDL-C ratio	ApoA1, mg/dL	ApoB, mg/dL	Lipoprotein (a), mg/dL
MDP								
Quintile 1	156 (145-168)	222 (218-227)	51.5 (50.1-52.9)	141 (137-144)	3.38 (3.07-3.69)	143 (141-146)	105 (102-108)	30.8 (26-35.5)
Quintile 2	157 (146-168)	221 (217-225)	52.5 (51.1-53.8)	137 (134-141)	3.33 (3.03-3.63)	146 (143-148)	104 (101-107)	34.6 (29.8-39.4)
Quintile 3	146 (135-157)	221 (217-225)	52.2 (50.9-53.6)	141 (137-144)	3.13 (2.82-3.43)	143 (140-145)	104 (101-107)	35.4 (29.8-39.1)
Quintile 4	152 (141-163)	228 (223-232)	54.5 (53.1-55.9)	144 (140-148)	3.09 (2.78-3.40)	147 (145-150)	108 (105-111)	35.8 (31-40.7)
Quintile 5	145 (134-157)	226 (222-231)	54.8 (53.4-56.2)	143 (139-147)	2.95 (2.64-3.27)	147 (144-149)	107 (104-110)	33.3 (28.4-38.2)
P-trend	.139	.066	<.001	.081	.043	.069	.276	.384
WDP								
Quintile 1	152 (138-166)	224 (218-229)	54.5 (52.7-56.2)	141 (136-146)	3.09 (2.71-3.47)	147 (144-150)	104 (101-108)	32.6 (26.6-38.7)
Quintile 2	146 (134-158)	226 (221-230)	55.0 (53.6-56.4)	142 (138-146)	2.96 (2.64-3.27)	149 (146-151)	106 (103-109)	33.3 (28.4-38.2)
Quintile 3	151 (140-162)	226 (222-230)	53.4 (52.0-54.8)	143 (139-147)	3.15 (2.85-3.46)	146 (144-148)	107 (104-110)	36.6 (31.8-41.5)
Quintile 4	153 (141-164)	224 (219-228)	52.7 (51.3-54.2)	141 (137-145)	3.21 (2.89-3.53)	144 (141-146)	106 (103-109)	31.1 (26.2-36)
Quintile 5	155 (142-169)	219 (214-225)	49.9 (48.2-51.6)	139 (134-144)	3.47 (3.09-3.85)	139 (136-142)	105 (102-109)	35.1 (29-41.2)
P-trend	.419	.189	<.001	.414	.101	.005	.963	.992

Apo, apolipoprotein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MDP, Mediterranean dietary pattern; TG, triglycerides; WDP, Western dietary pattern.

Adjusted means (95% confidence interval) for age, sex, education level, energy intake, physical activity level, plasma lipid-lowering medication, and body mass index.

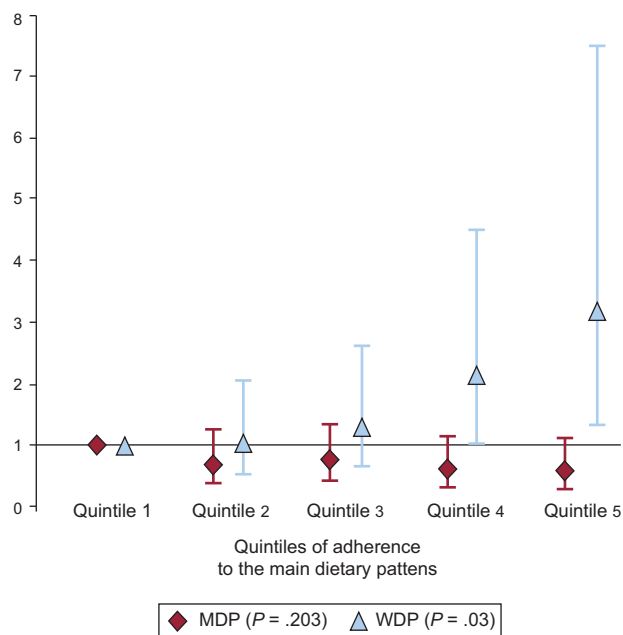


Figure. Odds ratio and 95% confidence interval for low high-density lipoprotein cholesterol levels across quintiles of adherence to the main dietary patterns. MDP, Mediterranean dietary pattern; WDP, Western dietary pattern.

that the improvements in plasma lipids observed were more likely to be due to adherence to the dietary pattern rather than to a change in body mass index.

Although not directly observed in our study, diet influences serum TG level, which has been reported in several studies.⁴⁴ In our study, we observed an inverse association between the MDP and the TG/HDL-C ratio, known as a surrogate index directly related to coronary heart risk, especially myocardial infarction.⁴⁵ Omega-3 polyunsaturated fatty acids, which are abundant in the MDP, are known to reduce TG synthesis, which will result in a lower TG/HDL-C ratio,⁴⁶ therefore adherence to the MDP, even with nonincreasing HDL-C concentration, will likely result in a lower risk for the development of coronary heart disease.

Even though a direct association between adherence to the MDP and ApoA1 was observed in our study, as well as in several other studies,^{11,14,47} the evidence regarding this relationship is still inconsistent. Taking into account the importance of apolipoproteins in cardiovascular disease prediction,⁴⁸ future studies are required to confirm our results.

Over the years, epidemiological studies have demonstrated an inverse association between HDL-C levels and cardiovascular risk,⁴⁹ such as in the Framingham Heart Study, where an increase of 5 mg/dL in HDL-C concentrations was associated with a 21% lower cardiovascular risk.⁵⁰ However, some recent studies have challenged this evidence, showing that increases in HDL-C are not necessary related to a lower risk of myocardial infarction and that its protective effect still needs to be further investigated.⁵¹

A *posteriori*-derived dietary patterns could only partially explain the variance in total food intake in this population, and therefore the influence of other minor dietary patterns attenuating the observed associations cannot be ruled out. In addition, the results of studies based on factor analyses largely depend on subjective decisions taken by the researchers regarding the grouping of food items into food categories. In our study, consumption of food items such as legume pulses, cereals, and wine was associated with a high-fat, high-carbohydrate diet (WDP). These items were originally considered as essential parts of

the traditional MDP, and could indicate an ongoing dietary transition from the traditional MDP to the current Mediterranean-based pattern followed in Spain. It is known that the same dietary pattern significantly differs between distinct populations, suggesting, for instance, that the MDP studied in 2 different countries might also differ.⁵²

Strengths and Limitations

An advantage of our study is the validation of an *a posteriori*-defined dietary pattern through comparison with *a priori* defined indices of diet quality. Based on our results, it can be concluded that factor analysis-defined dietary patterns are valid tools to assess the relationship between overall diet quality and biomarkers of diseases. In relation to the associations found, and due to the cross-sectional design of our study, a causal relationship cannot be established. In addition, this sample may not be representative of the general population, because these participants were mostly men and active workers and therefore healthy or at least without disabling diseases. As in most diet-related studies, reporting biases due to social desirability of overreporting healthier food items and underreporting less favorable foods also cannot be ruled out.

CONCLUSIONS

Higher adherence to the MDP was associated with improved plasma lipid profile while adherence to a WDP decreased the odds of optimal HDL-C levels in this cohort of Spanish workers.

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CONFLICTS OF INTEREST

None declared.

SUPPLEMENTARY MATERIAL



Supplementary material associated with this article can be found in the online version available at [doi:10.1016/j.rec.2014.09.019](https://doi.org/10.1016/j.rec.2014.09.019).

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Greater Adherence to a Mediterranean Dietary Pattern Is Associated With Improved Plasma Lipid profile: the Aragon Health Workers Study Cohort

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Supplementary Material Table 1: Food items classification into main food groups

Food groups	Food items
Vegetables	Green beans, lettuce, tomato, carrots, eggplant, peppers, asparagus, gazpacho-soup, salad, onions, garlic, parsil, mushrooms, fresh peas, cabbage, chard
Fruit	Orange, apple, strawberry, peach, cherry, watermelon, melon, kiwi, grapes, banana, fresh juice, dried fruits, other juices
Cereal-derived (including whole grain)	Bread, breakfast cereal, pasta, rice, whole grain cookies
Pulses	Lentils, chickpeas, beans
Nuts	Walnuts, hazelnuts, almonds
Fish	White- and blue-fish, seafood
White meat	Chicken, rabbit, low-fat ham
Red meat and eggs	Beef, pork, lamb, liver, paté, cured ham, minced meat
Dairy	Milk, yogurt, cream, milkshakes, condensed milk, butter, cheese
Low-fat dairy	Skimmed milk, skimmed yogurt, low fat cheese
Pre-made, frozen foods, fast food and dressings	French fries, chips and snacks, mayonnaise, mustard, ketchup
Sweets	Cookies, chocolate, cakes, donought, fried dough (churro) muffins, almond paste (turrón)
Olive oil	Extra-virgin, virgin, refined, olives
Vegetable oils	Sunflower, corn, margarine
Coffee and tea	Coffee, tea, decaf coffee
Soda	Any flavor soda including diet soda and sweetened juices
Beer and wine	Red wine, rosé wine, white wine, beer

2nd PUBLICATION:

**Association Between a Social-Business Eating Pattern and Early
Asymptomatic Atherosclerosis.
*J Am Coll Cardiol. 2016;68(8):805-14.***

GENERAL INTRODUCTION / INDIVIDUAL'S CONTRIBUTION

Taking into account the specificity of dietary patterns within a given population, the findings obtained from the AWHs study were considered for replication in a different population. Thus, I used the baseline data from 4082 middle age asymptomatic participants of the Progression of Early Subclinical Atherosclerosis (PESA) study to derive “*a-posteriori*” dietary patterns. Compared to AWHs participants, PESA participants presented slightly different socio-economic and traditional CVD risk factors profile: they were younger, with higher education level, more likely to be engaged in moderate or vigorous physical activity, and have a unique lifestyle which included frequent eating out and business trips. Therefore, it was hypothesized that the application of PCA would allow to derive different patterns from the ones derived among AWHs participants, and on the other hand, which might be shared by other populations in the same socio-economic stratum.

I used the dietary data collected from the computerized dietary history, classified foods into predefined food groups based on their nutritional content and submitted them to PCA in order to derive the most common underlying dietary patterns. Further based on the analysis of Eigen values and scree plot, 3 factor solution was determined which reflected the existence of 3 major dietary pattern specific for PESA population. In order to classify the individuals into non-overlapping and mutually exclusive groups of population with distinctive dietary patterns, clustering Euclidean distances between observations by using the K-mean method was performed. The combination of PCA and CA identified 3 mutually exclusive dietary patterns. Among those, there were 2 dietary patterns with similar characteristics to the ones observed among AWHs population: MDP and WDP. It was observed that participants with the highest adherence to MDP had the greatest intakes of fruits and vegetables, as well as whole grains and olive oil, while WDP was characterised by higher intakes of refined cereal products, legumes, and dairy products as well as sweets and desserts. Reflective to the distinctive lifestyle, socio-economic status and risk factors profile among PESA participants, a unique dietary pattern was identified, which was named “Social Business” eating pattern. It grouped the participants with the highest habitual intakes red meat and shellfish, pre-made foods, appetizers, and snacks, alcoholic and sugar-sweetened beverages. In order to assess the lifestyle and demographic determinants of those dietary patterns I performed a stepwise logistic regression.

Further, I studied association between the derived dietary patterns and the markers of subclinical atherosclerosis, including a presence of atherosclerotic plaques in carotid, ilio-femoral arteries and increased coronary artery calcium score (CACs), as well as the atherosclerosis disease extension by performing the logistic regression controlled for demographic, lifestyle and traditional CVD risk factors.



Association Between a Social-Business Eating Pattern and Early Asymptomatic Atherosclerosis

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ABSTRACT

BACKGROUND The importance of a healthy diet in relation to cardiovascular health promotion is widely recognized. Identifying specific dietary patterns related to early atherosclerosis would contribute greatly to inform effective primary prevention strategies.

OBJECTIVES This study sought to quantify the association between specific dietary patterns and presence and extent of subclinical atherosclerosis in a population of asymptomatic middle-aged adults.

METHODS The PESA (Progression of Early Subclinical Atherosclerosis) study enrolled 4,082 asymptomatic participants 40 to 54 years of age (mean age 45.8 years; 63% male) to evaluate the presence of subclinical atherosclerosis in multiple vascular territories. A fundamental objective of this cohort study was to evaluate the life-style-related determinants, including diet, on atherosclerosis onset and development. We conducted a cross-sectional analysis of baseline data, including detailed information on dietary habits obtained as part of the overall life-style and risk factor assessment, as well as a complete vascular imaging study that was performed blinded to the clinical information.

RESULTS Most PESA participants follow a Mediterranean (40% of participants) or a Western (41%) dietary pattern. A new pattern, identified among 19% of participants, was labeled as a social-business eating pattern, characterized by a high consumption of red meat, pre-made foods, snacks, alcohol, and sugar-sweetened beverages and frequent eating-out behavior. Participants following this pattern presented a significantly worse cardiovascular risk profile and, after adjustment for risk factors, increased odds of presenting subclinical atherosclerosis (odds ratio: 1.31; 95% confidence interval: 1.06 to 1.63) compared with participants following a Mediterranean diet.

CONCLUSIONS A new social-business eating pattern, characterized by high consumption of red and processed meat, alcohol, and sugar-sweetened beverages, and by frequent snacking and eating out as part of an overall unhealthy life-style, is associated with an increased prevalence, burden, and multisite presence of subclinical atherosclerosis. (Progression of Early Subclinical Atherosclerosis [PESA]; [NCT01410318](https://doi.org/10.1016/j.jacc.2016.05.080)) (J Am Coll Cardiol 2016;68:805-14)
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ABBREVIATIONS AND ACRONYMS

BMI = body mass index

CAC = coronary artery calcification

cIMT = carotid intima-media thickness

CT = computed tomography

CVD = cardiovascular disease

DH-E = Dietary History-Enrica

Cardiovascular disease (CVD) is responsible for about 32% and 47% of all deaths in the United States (1) and Europe (2), respectively. Smoking, physical inactivity, and unhealthy dietary habits (3) are the main targets for effective population-based primary prevention strategies to decrease CVD's high prevalence (1). Evidence suggests that studying the overall quality of diet, rather than focusing on single nutrients and foods, enables identification of dietary patterns associated with CVD risk (2-5).

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Several studies have investigated the association between dietary patterns and presence of atherosclerosis. However, most studies have evaluated the presence of atherosclerosis by measuring carotid intima-media thickness (cIMT) and/or coronary artery calcification (CAC) (4-6), with no study directly evaluating the presence of plaques, a hallmark of atherosclerosis. There is accumulating evidence that cIMT is a vascular process different from atherosclerosis, which calls into question its validity for individual cardiovascular risk stratification (7-9). Conversely, although CAC is clearly linked to atherosclerosis progression, it appears at later stages of the disease. Moreover, given atherosclerosis' systemic nature, rather than focusing on a single vascular bed, a more extensive analysis including multiple sites and a direct assessment of plaques may potentially provide a more accurate estimation of the presence, extent, and factors related to subclinical atherosclerosis.

The PESA (Progression of Early Subclinical Atherosclerosis) study, which aims to understand the determinants of the onset and progression of subclinical atherosclerosis diagnosed by noninvasive vascular imaging in multiple vascular sites (10), offers a unique opportunity to further define the association between dietary patterns and early atherosclerosis in a sample of middle-aged adults who may be optimal targets for primary prevention.

METHODS

STUDY POPULATION. PESA is an ongoing observational prospective cohort study. Details of the study

design and methodology have been previously described (11). The PESA cohort consists of 4,082 employees of the Santander Bank in Madrid. Male and female subjects 40 to 54 years of age, free of known CVD, were examined at baseline by vascular ultrasound and noncontrast computed tomography (CT) and will be followed at 3 and 6 years. Additionally, each visit includes clinical interviews, physical examination, a fasting blood draw, urine sample, and a 12-lead electrocardiogram. Of the 4,082 participants recruited at baseline, 26 subjects with missing data on diet, psychosocial factors, physical activity, ultrasound, or CT, and 4 participants reporting extreme values of total energy intake (<800 or >4,200 kcal and <500 or >3,500 kcal for men and women, respectively) (12) were excluded. Therefore, the final sample available for cross-sectional analysis was 4,052 participants. The study was approved by the ethics committee of the Instituto de Salud Carlos III, and all study participants provided written informed consent (11).

ASSESSMENT OF DEMOGRAPHIC, LIFE-STYLE, AND CVD RISK FACTORS. During the baseline visit, per PESA protocol (11), traditional risk factors such as family history of CVD, smoking habits, hypertension, dyslipidemia, and diabetes, as well as the use of any medication, were documented as part of the participant's medical history. At physical examination, blood pressure at rest was measured using OMRON HEM-907 automatic oscillometric sphygmomanometer (OMRON Healthcare, Kyoto, Japan). The anthropometric measurements were performed by standardized procedures, and obesity was defined as body mass index (BMI) ≥ 30 kg/m². Physical activity was assessed through triaxial accelerometers (Acti Trainer, Actigraph, Pensacola, Florida) that were placed on each participant's waist for 1 week. Venous blood was collected after 8 h of fasting, and samples were tested for high-density lipoprotein cholesterol, total cholesterol, low-density lipoprotein cholesterol, triglycerides, glucose, whole blood hemoglobin A_{1c}, and markers of inflammation. Additionally, blood aliquots were processed and stored at -80°C for further analysis. Participants also completed questionnaires to assess dietary habits and sleep patterns. Depression and perceived stress at home and at work were

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evaluated using CES-D (Center for Epidemiological Studies Depression) and PSS (Perceived Stress Scale) questionnaires. All interviews were conducted blinded to clinical, laboratory, and imaging results by trained technicians.

DIETARY ASSESSMENT. Habitual food intake was assessed by trained dietitians using the computerized Dietary History-Enrica (DH-E) questionnaire developed and validated for the Spanish population within the ENRICA (Estudio de Nutrición y Riesgo Cardiovascular) study (13). Briefly, during an approximately 1-h interview, participants were asked to report all of the foods usually consumed in the previous year, taking into consideration 6 eating occasions (on waking up, breakfast, mid-morning, lunch, mid-afternoon, and dinner). A food was considered to be “usually consumed” when it was eaten at least once every 15 days, and participants were guided to report foods that represented a typical week. Conversion factors were then used to transform the weekly frequency to year frequency depending on the number of months in which the food was consumed (13). The DH-E questionnaire can collect standardized information on 861 foods (including 127 sets of digitized photographs to help participants estimate portion size). Additionally, the DH-E includes 184 recipes for traditional or commonly consumed dishes in Spain. Finally, for quality control during the interview, the DH-E generates warnings when registering unrealistic energy intake or when foods that are usually part of the main eating occasions are not reported (13).

DEFINITION OF DIET CLUSTERS. Foods reported during the dietary assessment were classified into 21 food groups on the basis of nutrient content and submitted to factor analysis to identify common underlying dimensions (factors or patterns) of food consumption by deriving factor loadings for each pre-defined food group. Factors were subsequently rotated using a Varimax procedure to maintain uncorrelated factors. Analysis of eigenvalues, scree plot, and the interpretability of the factor solution were used to support a final decision on retaining a 3-factor solution, where each factor had an eigenvalue >0.3. A factor score for each participant was calculated by summing the daily intake of each food group weighted by their factor loadings. Derived factor scores were then analyzed for clustering on the basis of Euclidean distances between observations by using the K-mean method, allowing study participants to be grouped into nonoverlapping, mutually exclusive clusters reflecting their dietary patterns. We explored solutions with 2 to 4 clusters to evaluate which set

TABLE 1 Energy-Adjusted* Daily Intake of Selected Foods

	Total (N = 4,052)	Cluster 1 (Mediterranean) (n = 1,615)	Cluster 2 (Western) (n = 1,668)	Cluster 3 (Social-Business) (n = 769)
Energy, kcal	2,314 ± 468	2,087 ± 399†‡	2,421 ± 421§¶	2,562 ± 492†§
Vegetable products, g				
Vegetables	204 ± 102	253 ± 110†‡	168.0 ± 75.8§¶	179 ± 91.1†§
Refined grains	194.0 ± 66.8	179.0 ± 67.1†	214.0 ± 63.0§¶	184.0 ± 63.7†
Fruits	177 ± 138	217 ± 157†‡	162 ± 120§¶	128 ± 107†§
Legumes	37.7 ± 46.9	43.2 ± 60.8†‡	36.1 ± 34.8§¶	29.7 ± 32.2†§
Potatoes	32.4 ± 19.3	27.8 ± 18.4†‡	36.1 ± 18.6§¶	34.3 ± 20.6§
Whole grains	9.40 ± 22.10	16.9 ± 28.9†‡	4.10 ± 13.70§	5.50 ± 14.50§
Nuts	6.60 ± 9.50	6.80 ± 9.70†	5.80 ± 8.20§¶	7.80 ± 11.20†
Pickled olives	3.90 ± 5.90	3.69 ± 5.12†‡	2.78 ± 3.72§¶	7.00 ± 9.44†§
Animal products, g				
Whole-fat dairy	171 ± 120	134 ± 105†	227 ± 125§¶	126.0 ± 84.2†
Low-fat dairy	83.3 ± 119.0	137 ± 144†‡	36.2 ± 68.4§¶	72.3 ± 102.0†§
Lean meat	62.1 ± 30.9	74.4 ± 37.5†‡	55.0 ± 23.0§	53.6 ± 23.6§
Fish	61.1 ± 34.0	72.3 ± 36.3†‡	52.0 ± 28.9§¶	57.2 ± 32.6†§
Red meat	52.1 ± 27.6	46.8 ± 26.5†‡	53.7 ± 26.5§¶	59.7 ± 29.8†§
Processed meat	35.9 ± 23.0	33.5 ± 22.5†	35.4 ± 22.1†	42.2 ± 24.5†§
Eggs	19.2 ± 12.3	21.5 ± 14.4†‡	17.5 ± 10.0§	18.3 ± 11.3§
Shellfish	8.70 ± 8.40	7.90 ± 7.60†	7.30 ± 7.00†	13.3 ± 10.6†§
Oils and fats, g				
Olive oil	26.9 ± 11.0	31.7 ± 11.5†‡	23.40 ± 9.40§	24.20 ± 9.60§
Other vegetables oils	4.60 ± 4.90	4.52 ± 5.03	4.65 ± 4.89	4.69 ± 4.46
Animal fat	2.20 ± 3.80	2.20 ± 4.10	2.30 ± 3.80†	1.90 ± 3.40†
Processed foods, g				
Commercial bakery	59.1 ± 37.4	49.5 ± 33.8†‡	71.0 ± 38.7§¶	53.3 ± 34.4†§
Pre-cooked meals	40.3 ± 29.6	34.1 ± 27.3†‡	44.1 ± 29.0§	45.1 ± 32.8§
Fast food	7.00 ± 10.40	6.01 ± 9.31†‡	7.79 ± 11.20§	7.24 ± 10.70§
Chips	5.30 ± 7.10	4.64 ± 5.95†‡	3.68 ± 4.74§¶	10.2 ± 10.6†§
Beverages, servings				
Beer	0.27 ± 0.42	0.19 ± 0.30†	0.20 ± 0.31†	0.61 ± 0.63†§
Wine	0.05 ± 0.19	0.05 ± 0.16†‡	0.03 ± 0.12§¶	0.10 ± 0.31†§
Distilled spirits	0.08 ± 0.17	0.05 ± 0.13†	0.06 ± 0.14†	0.17 ± 0.26†§
SSB	0.41 ± 0.57	0.39 ± 0.58†	0.39 ± 0.52†	0.49 ± 0.66†§

Values are mean ± SD. *Energy adjusted = g/2,000 kcal. †p < 0.5 versus cluster 2. ‡p < 0.5 versus cluster 3. §p < 0.5 versus cluster 1. ||Servings/2,000 kcal: beer = 12 oz, ~350 ml; wine = 5 oz, ~150 ml; distilled spirits = 1.5 oz, ~45 ml; sugar-sweetened beverages (SSB) = 12 oz, ~330 ml.

of clusters was more meaningful to define dietary patterns. The 3-cluster solution was determined most appropriate on the basis of power requirements and sufficient representation of interpretable dietary patterns. As per protocol, diet and life-style evaluation was performed independently and blinded to risk factor assessment and subclinical atherosclerosis findings.

ASSESSMENT OF SUBCLINICAL ATHEROSCLEROSIS. Presence and significance of atherosclerotic plaques in carotids, infrarenal aorta, and both iliofemoral territories were assessed using vascular ultrasound (Philips iU22 ultrasound, Philips Healthcare, Bothell, Washington) as previously described (11). Plaque was

TABLE 2 Demographic and Life-Style-Related Characteristics

	Total (N = 4,052)	Cluster 1 (Mediterranean) (n = 1,615)	Cluster 2 (Western) (n = 1,668)	Cluster 3 (Social-Business) (n = 769)
Demographics				
Male	2,564 (63.3)	713 (44.1)*†	1,224 (73.4)‡	627 (81.5)*‡
Age, yrs	45.80 ± 4.27	45.60 ± 4.19†	45.70 ± 4.28†	46.50 ± 4.36*‡
Marital status				
Married	3,081 (76.0)	1,204 (74.6)	1,293 (77.5)	584 (75.9)
Divorced	207 (5.1)	89 (5.5)	70 (4.2)	48 (6.2)
Separated	74 (1.8)	27 (1.7)	31 (1.9)	16 (2.1)
Single	306 (7.6)	138 (8.5)	113 (6.8)	55 (7.2)
Education level				
High school degree	1,013 (25.2)	326 (20.4)*†	441 (26.6)‡	246 (32.2)*‡
Graduate degree	604 (15.0)	242 (15.1)	250 (15.1)	112 (14.7)
Post-graduate degree	2,405 (59.8)	1,033 (64.5)*†	966 (58.3)‡	406 (53.1)‡
Income level				
Low	1,430 (35.5)	624 (38.6)†	596 (35.7)†	210 (27.3)*‡
Medium	1,191 (29.4)	437 (27.1)†	500 (30.0)	254 (33.0)‡
High	1,431 (35.3)	554 (34.3)†	572 (34.3)†	305 (39.7)*‡
Job category				
Technicians/ operators/security	1,362 (34.2)	579 (36.5)	543 (33.0)	240 (31.7)
Administrative staff	154 (3.9)	69 (4.3)	63 (3.8)	22 (2.9)
Managers	1,113 (27.9)	431 (27.1)	487 (29.6)	195 (25.8)
Executives	1,359 (34.1)	509 (32.1)†	550 (33.5)†	300 (39.6)*‡
Years working for the company	18.50 ± 9.08	17.90 ± 8.60†	18.60 ± 9.30	19.30 ± 9.60‡
Physical activity, min/day				
Moderate	43.5 ± 16.6	42.5 ± 16.7†	43.6 ± 16.3†	45.3 ± 17.1*‡
Vigorous	3.80 ± 7.10	3.80 ± 7.00	3.70 ± 7.00	3.90 ± 7.20
MVPA	47.3 ± 19.6	46.3 ± 19.8†	47.3 ± 19.1	49.3 ± 20.1‡
Resting (sleeping) patterns, h				
<7	2,121 (52.3)	809 (50.1)†	885 (53.1)	427 (55.5)‡
7-8	1,630 (40.2)	678 (42.0)	662 (39.7)	290 (37.7)
>8	301 (7.40)	128 (7.90)	121 (7.30)	52 (6.80)
Insomnia	40 (1.00)	17 (1.10)	13 (0.80)	10 (1.30)
Obstructive apnea	78 (1.90)	21 (1.30)†	32 (1.90)	25 (3.30)‡
Smoking status				
Nonsmokers	1,574 (38.8)	660 (40.9)†	707 (42.4)†	207 (26.9)*‡
Active smokers	833 (20.6)	284 (17.6)*†	356 (21.3)‡	193 (25.1)‡
Social smokers	340 (8.39)	138 (8.54)	124 (7.43)	78 (10.1)
Former smokers	1,305 (32.2)	533 (33.0)*	481 (28.8)‡	291 (37.8)*
Cigarettes/day§	8.23 ± 11.40	7.27 ± 10.30†	7.67 ± 11.10†	11.5 ± 13.3*‡
Frequency of eating out, lunch 				
Rare	581 (14.3)	232 (14.4)	264 (15.8)†	85 (11.1)*
Sometimes	332 (8.2)	130 (8.0)	142 (8.5)	60 (7.8)
Often	3139 (77.5)	1253 (77.6)	1262 (75.7)†	624 (81.1)*

Continued on the next page

defined as any focal protrusion into the arterial lumen >0.5 mm of thickness or >50% of the surrounding intima-media thickness, or a diffuse thickness >1.5 mm measured between the media-adventitia and intima-lumen interfaces (14). CAC score was obtained by noncontrast electrocardiogram-gated prospective acquisition with a 16-slice CT scanner and quantified using Agatston scoring (15). Subclinical

atherosclerosis was defined as any plaque in at least 1 explored territory (right/left carotid, aorta, right/left iliofemoral) and/or a CAC score ≥1. Extension of atherosclerosis was classified as focal (1 site affected), intermediate (2 to 3 sites), or generalized (4 to 6 sites) disease (10). All imaging acquisitions were performed at the PESA examination center (11), and imaging analysis was centralized in the Central Imaging Core laboratory at the Centro Nacional de Investigaciones Cardiovasculares (CNIC) Carlos III.

STATISTICAL ANALYSIS. All statistical analyses were performed with STATA version 12 (StataCorp LLP, College Station, Texas). Baseline characteristics of the study population are presented as counts and percentages for categorical variables and as mean ± SD for continuous variables. Analysis of variance with multiple-testing correction (Bonferroni) for continuous variables or chi-square test (for categorical variables) was used to compare participants' characteristics across dietary clusters. A forward-stepwise regression was used to identify life-style-related determinants of adherence to a dietary pattern among the factors identified by the American Heart Association as main targets for CVD prevention. The strength of these relationships was assessed using categorical covariates and odds ratios (ORs). Adjusted logistic regression models were used to assess the relationship among dietary patterns, the presence of plaque in the carotid or iliofemoral territories, and CAC score. Age (years), sex (male or female), energy intake (kcal), education (high school, college, or university degree), income, physical activity (compliance with World Health Organization recommendations), CVD risk factors (dyslipidemia, current smoking, hypertension, and diabetes), other life-style factors (sleep duration, use of discretionary salt, and frequency of eating out [lunch or dinner]), and frequency of traveling for business were considered as potential confounding factors and, therefore, were used to adjust models. Additionally, to assess the potential differential effect by sex, a stratified analysis was performed using Poisson regression (using the same progressive adjustment of the models), reporting the results as prevalence ratios to provide a more conservative estimate of the association.

RESULTS

DIETARY PATTERNS, FOODS, AND NUTRIENTS. Factor and cluster analyses identified 3 mutually exclusive dietary patterns among the PESA participants. The main food items characterizing each of these clusters are shown in Table 1 and Online Table 1.

Participants in cluster 1 (n = 1,615; 40%) had the highest consumption of fruits and vegetables, as well as whole grains and olive oil. They also showed the highest intake of low-fat dairy products, lean meat, and fish, and the lowest consumption of processed food (Table 1). Coffee and tea consumption was also highest in this group. On a nutrient basis, this group was strongly correlated with dietary fiber, potassium, magnesium, carotenoids, and vitamins (except for retinoids), and was negatively correlated with saturated fat (Online Table 2).

Participants within cluster 2 (n = 1,668; 41%) were characterized by the highest consumption of refined cereal products, legumes, and dairy products as well as sweets and desserts (Table 1). Participants in this cluster also showed high intakes of red meat and pre-made foods. This dietary pattern was strongly correlated with total and saturated fat, carbohydrates (including sugars and polysaccharides), dietary fiber, iron, and sodium (Online Table 2).

Finally, cluster 3 (n = 769; 19%) grouped participants with the highest consumption of red meat and shellfish, pre-made foods, appetizers, and snacks (including pickled olives and salted nuts), and alcoholic and sugar-sweetened beverages (Table 1). This diet correlated positively with total and animal protein, fat (including saturated, monounsaturated, and polyunsaturated fatty acids), cholesterol, alcohol, iron, sodium, phosphorus, and vitamins B3 and B12 (Online Table 2).

CHARACTERISTICS ACCORDING TO DIETARY PATTERNS.

Per the demographic characteristics and life-style-related variables for each dietary pattern (Table 2), sex was almost equally distributed within cluster 1, whereas participants in clusters 2 and 3 were predominantly men. A small but significant difference in age was observed for participants in cluster 3 (approximately 1 year older than clusters 1 or 2). No difference in relation to marital status was observed between clusters. Regarding education, mostly post-graduate participants formed all 3 clusters, reflecting the overall sample, but cluster 3 included the highest percentage of individuals with a high school degree. Participants in cluster 3 also had the highest level of income, on average worked 1.5 years longer in the company, and were more likely to hold director and executive positions within the company.

Cluster 3 showed marked differences in life-style factors compared with the other clusters. For example, 76% of cluster 3 participants were current, social, or former smokers, and they dedicated more time to moderate or moderate-to-vigorous physical activity. This group (cluster 3) also had the highest proportion of participants reporting short sleep (<7 h)

TABLE 2 Continued

	Total (N = 4,052)	Cluster 1 (Mediterranean) (n = 1,615)	Cluster 2 (Western) (n = 1,668)	Cluster 3 (Social-Business) (n = 769)
Frequency of eating out, dinner				
Rare	2,731 (67.4)	1,071 (66.3)*	1,186 (71.1)†‡	474 (61.6)*
Sometimes	1,221 (30.1)	508 (31.5)*	457 (27.4)†‡	256 (33.3)*
Often	100 (2.5)	36 (2.2)†	25 (1.5)†	39 (5.1)*‡
Work travel¶				
Never	3,106 (76.7)	1,261 (78.1)†	1,316 (78.9)†	529 (68.8)*‡
Sometimes	501 (12.4)	194 (12.0)	194 (11.6)	113 (14.7)
Often	445 (11.0)	160 (9.9)†	158 (9.5)†	127 (16.5)*‡
Frequency use of discretionary salt				
Always on salads	722 (17.8)	264 (16.3)†	277 (16.6)†	181 (23.5)*‡
Always on vegetables	493 (12.2)	185 (11.5)†	182 (10.9)†	126 (16.4)*‡
Always on other dishes	495 (12.2)	168 (10.4)†	199 (11.9)†	128 (16.6)*‡

Values are n (%) or mean ± SD (Bonferroni correction was applied for categorical variables [p < 0.017]). *p < 0.5 versus cluster 2. †p < 0.5 versus cluster 3. ‡p < 0.5 versus cluster 1. §For both active and former smokers. ||Rare: <3 times/month; sometimes: 1 to 2 times/week; often: >3 times/week. ¶Sometimes: 1 time/year to 2 times/trimester; often: >1 time/month.
MVPA = moderate-to-vigorous physical activity.

and obstructive sleep apnea, more frequent business trips, and the highest frequency of eating out. Cluster 3 also included the highest percentage of individuals using discretionary salt (Table 2).

The relationships between the identified clusters and the American Heart Association targets for CVD prevention are summarized in Table 3. Although adherence to cluster 1 was associated with female sex, moderate consumption of alcohol, and nonsmoker status, adherence to cluster 2 was associated with male sex, normal weight, and moderate consumption of alcohol. Finally, adherence to cluster 3 was associated with alcohol consumption above the recommended intake as well as male sex, obesity, and smoker status.

DIETARY PATTERNS AND CARDIOVASCULAR RISK FACTORS.

The relationship between adherence to the identified diet clusters and markers of CVD risk is presented in Table 4. Hypertension, dyslipidemia, and diabetes were most prevalent among participants in cluster 3. Accordingly, the values of systolic and diastolic blood pressure, as well as the plasma lipid profile and indicators of glycemia homeostasis, were worse among participants adhering to the cluster 3 pattern, and a trend in the same direction was observed for those in cluster 2. Also, obesity was more prevalent among cluster 3 participants. Furthermore, inflammation markers, such as C-reactive protein and P-selectin, were higher in clusters 2 and 3. These differences in individual markers of CVD risk were also reflected in a lower score on the classical algorithms for CVD risk classifications of cluster 1 participants (Framingham 10-year risk score: 4.71 ± 3.77%)

compared with those in cluster 3 ($7.75 \pm 5.13\%$). No significant differences in psychosocial factors were observed between clusters (Table 4).

DIETARY PATTERNS AND SUBCLINICAL ATHEROSCLEROSIS. Participants belonging to cluster 1 showed a lower prevalence and extent of subclinical atherosclerosis compared with the overall sample mean values (Table 5). Stratifying by sex, these differences persisted and were more pronounced among men (Online Table 3). In contrast, participants in cluster 3 presented a higher prevalence of plaques in any of the explored vascular territories using vascular ultrasound and higher values of CAC score. Almost one-half (48.7%) of participants in cluster 3 presented plaques in the femoral territory versus 35.5% in cluster 2 and 29.3% in cluster 1. Using logistic regression and after adjustment for potential confounders including CVD risk factors (dyslipidemia, current smoking, hypertension, diabetes, BMI, and life-style and dietary habits), participants adhering to a cluster 3 pattern had a significantly higher prevalence (OR: 1.31; 95% confidence interval [CI]: 1.06 to 1.63) of subclinical atherosclerosis and a borderline significant higher prevalence of plaques in the iliofemoral territory (OR: 1.22; 95% CI: 0.99 to 1.50) (Table 6). Nonsignificant associations were observed for the presence of carotid plaques (OR: 1.18; 95% CI: 0.96 to 1.45) and abdominal aorta (OR: 1.06; 95% CI: 0.85 to 1.33). These results also held true when using Poisson regression to estimate prevalence ratios using the same covariates for adjustment (Online Table 4). Finally, the extent of subclinical atherosclerosis was also associated with adherence to a particular diet (Central Illustration). Participants who were free of disease (43%) concentrated in

cluster 1, whereas participants with generalized atherosclerosis (more than 3 territories affected) were more frequently included in cluster 3 (Central Illustration). A similar association was observed after stratification by sex (Online Figures 1 and 2).

DISCUSSION

We report a new dietary pattern that we have labeled the “social-business eating pattern,” characterized by eating away from home more frequently, either as a job requirement or due to busier schedules. This eating pattern is followed by 19% of the PESA cohort participants, among whom a higher prevalence and extent of subclinical atherosclerosis was observed. This social-business eating pattern was characterized by a low consumption of vegetables and fruits and high increased consumption of red and processed meat, pre-made meals, snacks, and alcoholic and sugar-sweetened beverages. Although there have been reports identifying a dietary pattern with high intake of alcoholic beverages (16) labeled as a drinker pattern in other studies (17), to the best of our knowledge, this social-business eating pattern has not been singled out before. The sociability aspect of the newly described social-business dietary pattern, as well as the high degree of correlation with other life-style factors, made it different from other unhealthy patterns reported previously. This dietary pattern was characterized by more frequent eating outside of the home (restaurants, cafeterias, convenience food, and so on). Participants following this pattern were less likely to eat homemade meals and more likely to engage in unhealthy habits, such as smoking, excess salt consumption, and alcohol intake beyond moderation. Taking into consideration the

TABLE 3 Determinants of Dietary Patterns

Categories	Cluster 1 (Mediterranean)	p Value	Cluster 2 (Western)	p Value	Cluster 3 (Social-Business)	p Value	
	OR (95% CI)		OR (95% CI)		OR (95% CI)		
Sex	Women (ref)	—	—	—	—	—	
	Men	0.25 (0.21-0.28)	<0.001	2.53 (2.18-2.95)	<0.001	2.57 (2.06-3.21)	<0.001
Smoking status	Nonsmoker (ref)	—	—	—	—	—	
	Current smoker	0.76 (0.66-0.89)	<0.001	—	—	1.35 (1.12-1.62)	0.001
BMI	Normal-weight (ref)	—	—	—	—	—	
	Overweight	—	—	0.79 (0.68-0.92)	0.002	1.69 (1.36-2.10)	<0.001
	Obese	—	—	0.61 (0.49-0.75)	<0.001	2.77 (2.14-3.59)	<0.001
Alcohol intake	≤1 and ≤2 servings/day for women and men (ref)	—	—	—	—	—	
	>1 and >2 servings/day for women and men	0.47 (0.37-0.61)	<0.001	0.27 (0.20-0.35)	<0.001	7.95 (6.28-10.06)	<0.001

Determinants made on the basis of a forward stepwise regression model. All variables are categorical.
BMI = body mass index; CI = confidence interval; OR = odds ratio; ref = reference.

sociocultural characteristics of eating in Spain, this pattern might reflect the consumption of small amounts of food accompanied by an alcoholic beverage, frequently wine or beer, and mainly consumed in a social-related environment, hence the name social-business eating pattern.

A closer look at the energy intake of participants adhering to this group revealed that this group consumed approximately 475 more kcal/day compared with cluster 1 and 140 more kcal/day compared with cluster 2. Although this caloric excess might partially explain the higher prevalence of obesity and overweight in this group, which may lead to worse levels of CVD risk markers (18), the data reported here pointed to an overall unhealthy life-style and, in particular, a low-quality diet as potentially the main reason responsible for the observed association. Indeed, participants in this social-business eating group presented increased values of CVD risk markers and increased presence and extent of subclinical atherosclerosis, including plaques in the carotid and the iliofemoral territories and calcification in the coronaries, independently of other risk factors. The association was significantly modified after adjusting for CVD risk factors, such as hypertension, dyslipidemia, diabetes, and smoking, indicating that these were the main potential mediators of the dietary effect. The fact that this association held after adjusting for the previously mentioned CVD risk factors, as well as BMI and several life-style determinants, points to this eating pattern—which was a combination of dietary intake and eating habits—as an important life-style to be avoided and as an appealing target for primary prevention strategies.

The social-business dietary pattern characterized in this sample serves as a proxy for an overall life-style, with the “business” component as an essential part of this group’s behavior. To understand the possible underlying cause of this behavior, we analyzed other life-style characteristics, such as marital status, job situation, stress, and depression. This cluster of participants included the highest proportion of directors and higher-income staff. The prevalence of stress in this cluster was not different from that of the other clusters, nor was the prevalence of depression, which was overall low. Cluster 3 participants reported shorter sleeping times, which has been associated with increased BMI in some studies (19). However, these observations did not fully support the idea of this eating behavior being a reaction to psychosocial conditions; therefore, we can only hypothesize that the higher status within the company entailed obligations (e.g., lunch meetings, frequent travel) that affected and limited dietary

TABLE 4 CVD Risk Factors

	Total (N = 4,052)	Cluster 1 (Mediterranean) (n = 1,615)	Cluster 2 (Western) (n = 1,668)	Cluster 3 (Social-Business) (n = 768)
Family history of CVD	641 (15.8)	263 (16.3)	241 (14.4)	137 (17.8)
Obesity	594 (14.7)	181 (11.2)†	218 (13.1)†	195 (25.4)*‡
BMI, kg/m ²	26.20 ± 3.83	25.40 ± 3.63*‡	26.10 ± 3.69†‡	27.8 ± 4.0*‡
Hypertension	480 (11.8)	139 (8.61)*‡	200 (12.0)†‡	141 (18.3)*‡
Systolic BP, mm Hg	116.0 ± 12.5	114.0 ± 12.5*‡	117.0 ± 12.1†‡	121.0 ± 12.3*‡
Diastolic BP, mm Hg	72.50 ± 9.44	70.9 ± 9.1*‡	72.60 ± 9.35†‡	75.6 ± 9.53*‡
Dyslipidemia	1,681 (41.5)	561 (34.7)*‡	741 (44.4)‡	379 (49.3)‡
Total cholesterol, mg/dl	201.0 ± 33.3	199.0 ± 32.0†	199.0 ± 33.0†	209.0 ± 35.2*‡
LDL-C mg/dl	132.0 ± 29.7	130.0 ± 29.0†	132.0 ± 29.3†	139.0 ± 31.1*‡
HDL-C mg/dl	49.0 ± 12.2	51.9 ± 12.4*‡	47.0 ± 11.5‡	47.5 ± 11.9‡
TG, mg/dl	95.0 ± 57.2	83.7 ± 48.7*‡	98.8 ± 58.4†‡	110.0 ± 65.9*‡
Inflammation				
C-reactive protein, mg/dl	0.18 ± 0.37	0.16 ± 0.30†	0.19 ± 0.50	0.20 ± 0.30‡
P-selectin, ng/ml	131.0 ± 40.1	126.0 ± 39.1*‡	133.0 ± 40.3‡	137.0 ± 40.3‡
Urinary isoprostanes-to-creatinine ratio	1.17 ± 6.30	1.07 ± 0.70	1.27 ± 9.80	1.16 ± 0.70
Diabetes	79 (1.95)	24 (1.49)†	30 (1.80)	25 (3.25)‡
Fasting glucose, mg/dl	90.6 ± 13.7	89.0 ± 13.9*‡	90.4 ± 12.0†‡	94.2 ± 16.0*‡
HbA _{1c} , %	5.44 ± 0.48	5.40 ± 0.44†	5.44 ± 0.47†	5.52 ± 0.51*‡
CVD risk scales				
FRS-10	5.94 ± 4.45	4.71 ± 3.77*‡	6.30 ± 4.36†‡	7.75 ± 5.13*‡
FRS-30	18.0 ± 11.9	14.7 ± 10.5*‡	18.8 ± 11.6†‡	23.1 ± 13.1*‡
EU-SCORE	0.57 ± 0.61	0.42 ± 0.51*‡	0.59 ± 0.56†‡	0.82 ± 0.77*‡
REGICOR	2.07 ± 1.52	1.69 ± 1.28*‡	2.17 ± 1.50†‡	2.65 ± 1.79*‡
Psychosocial characteristics				
CES-D score§	6.07 ± 6.23	6.12 ± 6.15	5.99 ± 6.21	6.14 ± 6.45
Being depressed (CES-D score ≥16)	298 (7.4)	124 (7.7)	114 (6.8)	60 (7.8)
PSS	21.90 ± 5.79	22.1 ± 5.8	21.7 ± 5.8	21.7 ± 5.9
Being stressed (PSS ≥25)	1,199 (29.6)	495 (30.7)	474 (28.4)	230 (29.9)

Values are n (%) or mean ± SD (Bonferroni correction was applied for categorical variables [p < 0.017]). *p < 0.5 versus cluster 2. †p < 0.5 versus cluster 3. ‡p < 0.5 versus cluster 1. §Score range: 10 to 60; the higher the score, the greater the depression. ||Score range: 10 to 50; the higher the score, the greater the stress.

BP = blood pressure; CES-D = Center for Epidemiological Studies Depression; EU-SCORE = European Systematic Coronary Risk Evaluation; FRS = Framingham risk score; HbA_{1c} = glycosylated hemoglobin; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; PSS = perceived stress scale; REGICOR = Registre Gironi del Cor; TG = triglycerides; other abbreviations as in Table 3.

TABLE 5 Cardiovascular Imaging

	Total (N = 4,052)	Cluster 1 (Mediterranean) (n = 1,615)	Cluster 2 (Western) (n = 1,668)	Cluster 3 (Social-Business) (n = 769)
Coronary calcium (Agatston score)	14.8 ± 84.5	12.8 ± 82.8†	11.7 ± 69.9†	25.4 ± 111.0*‡
CAC score ≥1	734 (18.1)	225 (13.9)*‡	294 (17.6)†‡	215 (28)*‡
CAC score >100	142 (3.5)	47 (2.90)†	49 (2.90)†	46 (6.00)*‡
CAC score >400	29 (0.71)	13 (0.80)	7 (0.42)	9 (1.17)
Presence of any plaque	2,407 (60.3)	880 (55.1)*‡	1,011 (61.4)†‡	516 (69.0)*‡
Aorta	992 (24.6)	384 (23.8)†	388 (23.4)†	220 (28.9)*‡
Carotid arteries	1,276 (31.5)	448 (27.7)*‡	538 (32.3)†‡	290 (37.8)*‡
Femoral artery	1,437 (35.5)	471 (29.3)*‡	592 (35.5)†‡	374 (48.7)*‡
Iliac arteries	840 (21.0)	316 (19.7)†	334 (20.2)†	190 (25.3)*‡
Any plaque and CAC score ≥1	2,496 (62.5)	906 (56.7)*‡	1,047 (63.6)†‡	543 (72.6)*‡

Values are mean ± SD or n (%) (Bonferroni correction was applied for categorical variables [p < 0.017]). *p < 0.5 versus cluster 2. †p < 0.5 versus cluster 3. ‡p < 0.5 versus cluster 1.

CAC = coronary artery calcification.

TABLE 6 Prevalent Subclinical Atherosclerosis*

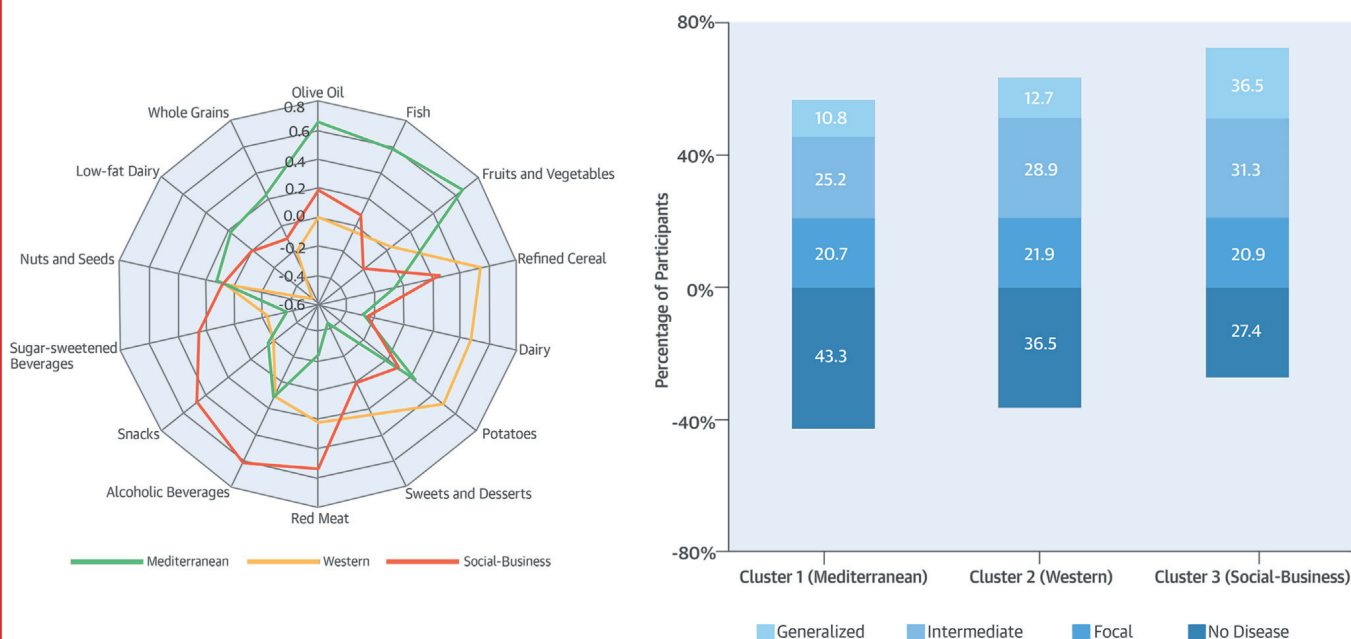
	Cluster 1 (Mediterranean)	Cluster 2 (Western) Adjusted OR (95% CI)	Cluster 3 (Social-Business) Adjusted OR (95% CI)
Plaque in Carotids			
Model 1	Ref	1.10 (0.94-1.30)	1.28 (1.04-1.56)†
Model 2	Ref	1.07 (0.91-1.26)	1.19 (0.97-1.46)
Model 3	Ref	1.06 (0.90-1.26)	1.20 (0.98-1.47)
Model 4	Ref	1.07 (0.90-1.26)	1.18 (0.96-1.45)
Plaque in Iliofemoral Territories			
Model 1	Ref	1.00 (0.96-1.17)	1.36 (1.11-1.65)‡
Model 2	Ref	0.95 (0.81-1.12)	1.22 (1.00-1.50)
Model 3	Ref	0.94 (0.80-1.11)	1.23 (1.00-1.51)†
Model 4	Ref	0.95 (0.80-1.11)	1.22 (0.99-1.50)
Any Plaque or CAC >1			
Model 1	Ref	1.10 (0.94-1.29)	1.46 (1.18-1.80)‡
Model 2	Ref	1.05 (0.89-1.24)	1.33 (1.07-1.65)‡
Model 3	Ref	1.05 (0.89-1.23)	1.33 (1.07-1.65)‡
Model 4	Ref	1.05 (0.89-1.23)	1.31 (1.06-1.63)†

*Models were adjusted as follows: Model 1: sex, age, energy intake, education, income, and physical activity (compliance with recommendations). Model 2: Model 1 plus prevalent dyslipidemia, hypertension, diabetes, and current smoking. Model 3: Model 2 plus BMI. Model 4: Model 3 plus sleep duration, use of discretionary salt, frequency of eating out (lunch and dinner), and frequency of traveling. †p < 0.05 versus cluster 1. ‡p < 0.01 versus cluster 1.
Abbreviations as in Tables 3 and 5.

choices. Restaurants do not always provide healthy meals and, in fact, eating out was reported to be associated with on average excess intake of 200 kcal/day (20). Participants clustering around this eating pattern exercised slightly more but also were more likely to smoke and had a higher consumption of alcoholic beverages than participants in other clusters, possibly as a consequence of networking events.

The majority (81%) of PESA participants were classified into either cluster 1 or 2. These 2 dietary patterns have been observed in previous studies in Spain (16,21-23), including the Spanish AWHs (Aragon's Workers Health Study) cohort (24), and have been respectively labeled as a "Mediterranean diet" (characterized by a higher intake of vegetables, fruits, legumes, whole grains, fish, and olive oil) and a "Western diet" (characterized by a higher intake of processed meat, red meat, butter, high-fat dairy products, eggs, and refined grains). As suggested in previous reports in Spain (16,21,22), the Western pattern (cluster 2), which was present in 41% of the PESA population, might represent a shift from the traditional Mediterranean diet toward a more

CENTRAL ILLUSTRATION Social Eating and Atherosclerosis: Comparison of Mediterranean Pattern, Western Pattern, and Social-Business Pattern



Peñalvo, J.L. et al. J Am Coll Cardiol. 2016;68(8):805-14.

(Left) A representation of the main food items characterizing each dietary cluster. Values correspond to factor loadings (the higher the value, the more a particular food item contributes to the dietary cluster). (Right) The distribution of the extent of atherosclerosis within each identified cluster is represented. The group of participants (19% of the PESA population) following the social-business dietary pattern presents the highest proportion of individuals with generalized (>3 territories affected) or intermediate (2 to 3 territories) atherosclerosis, whereas the majority of individuals (43%) classified into the Mediterranean pattern present no sign of disease.

Westernized diet, characterized by higher consumption of refined cereal products and other processed food, red meat, full-fat dairy, and sweets (e.g., pastries and ice cream). To an extent, it represents a step away from the CVD-protective traditional Mediterranean diet and, in fact, is associated with higher rates of hypertension, dyslipidemia, and diabetes compared with the Mediterranean diet cluster in our population sample. The evidence of a relationship between a Western dietary pattern and an increased risk of CVD has been reported to be inconsistent (23). In our study, participants classified as following this Western pattern (cluster 2) presented a higher cardiovascular risk as per risk-stratification scales, together with higher CAC scores and plaque presence (compared with cluster 1), although no statistical association with subclinical atherosclerosis was found when adjusting for traditional risk factors, suggesting that the effect of this dietary pattern on atherosclerosis is mediated by traditional cardiovascular risk factors. In MESA (Multi-Ethnic Study of Atherosclerosis), a diet characterized by high intakes of processed meat, fat, sweets, fried foods, and sugar-sweetened beverages was positively associated with CAC score and cIMT (4). Similarly, a diet high in sugar-sweetened beverages, red meat, desserts, and saturated fat, but low in vegetables, fruits, fiber, and micronutrient density, was associated with a 2-fold increased prevalence of carotid atherosclerosis in the Framingham cohort (6).

The beneficial association between the Mediterranean diet, evaluated by this approach (factor- and cluster-derived dietary patterns), and lower prevalence of subclinical atherosclerosis has not been previously reported in observational studies. In MESA, a diet high in whole grains, fruits, and nuts was associated with neither cIMT thickness nor CAC score (25). Similarly, no significant association between a healthier (greater intakes of fruits and vegetables, rye, legumes, and nuts) diet and cIMT thickness was observed in an analysis from the Cardiovascular Risk in Young Finns study (26). Considering the depreciated value of cIMT for improving CVD risk prediction (9), the lower prevalence of plaques and coronary calcification found in participants following a Mediterranean-type diet in our study helped to better understand the clinical benefit attributed to this dietary pattern. This is of interest as recent systematic reviews of epidemiological studies assessed using cluster or factor analysis concluded that, although limited, dietary patterns characterized by vegetables, fruits, whole grains, fish, and low-fat dairy products are associated with decreased risk of CVD in

adults (23). Moreover, most randomized controlled trials on diet interventions showed that a Mediterranean/prudent diet may be effective in atherosclerosis regression in subjects with an initially moderate or severe burden of coronary heart disease (27,28).

STUDY LIMITATIONS. Middle- to high-income office workers with somewhat unique dietary habits constituted the PESA cohort; therefore, generalizability of our observations is limited. This can be regarded as a limitation but also as a strength, because this characteristic allowed us to identify a specific dietary pattern that might be shared by other populations in the same socioeconomic stratum and can help target preventive strategies toward this collective. Factor and cluster analyses are data-driven approaches in which the consolidation of food items into food groups, the number of factors or clusters to extract, and the labeling of components are subjective. Patterns derived from either factor or cluster analysis may not be reproducible across studies because elements of dietary patterns and analytic decisions differ. Also, due to the cross-sectional nature of our study, causality cannot be addressed. Furthermore, the use of ORs may overestimate the strength of the association due to the relatively high prevalence of plaques in the PESA population. Finally, because of the multicausal etiology of atherosclerosis, residual confounding cannot be ruled out despite of the use of fully adjusted models.

CONCLUSIONS

Approximately 1 in 5 participants enrolled in the PESA cohort followed a social-business eating pattern that, as a part of an overall unhealthy life-style, was associated with increased CVD risk and a higher prevalence and extension of subclinical atherosclerosis. These results suggested that diet and overall life-style habits were important in early atherosclerosis and could inform strategies to reduce the burden of CVD in similar populations after the results are validated when prospective data become available in the PESA cohort.

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PERSPECTIVES

COMPETENCY IN PATIENT CARE AND

PROCEDURAL SKILLS: Identification of dietary patterns associated with specific socioeconomic strata may help targeting strategies for prevention of CVD.

TRANSLATIONAL OUTLOOK:

Randomized trials of strategies that address dietary and other life-style habits for primordial and primary prevention of CVD should include assessments of the presence and severity of subclinical atherosclerosis.

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KEY WORDS cardiovascular disease, dietary patterns, plaque, subclinical atherosclerosis

APPENDIX For supplemental tables and figures, please see the online version of this article.

SUPPLEMENTAL TABLES AND FIGURES

Association between a social-business eating pattern and early asymptomatic atherosclerosis

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Table 1 (supplemental): Factor loading* matrix for three main dietary clusters

	Cluster 1 (Mediterranean)	Cluster 2 (Western)	Cluster 3 (Social business)
Factor 1**	0.43 ± 1,02 ^a	-0,39 ± 0,81 ^b	-0,05 ± 0,96 ^c
Factor 2**	-0,70 ± 0,65 ^a	0,69 ± 0,83 ^b	-0,04 ± 0,91 ^c
Factor 3**	-0,40 ± 0,60 ^a	-0,31 ± 0,58 ^b	1,52 ± 0,96 ^c
Fruits and vegetables	0.670	0.033	-0.205
Potatoes and tubers	0.243	0.503	0.111
Whole grain	0.243	-0.216	-0.100
Refined grains	-0.067	0.540	0.256
Legumes	0.170	0.590	0.060
Nuts	0.117	0.035	0.079
Olive oil	0.645	-0.001	0.189
Vegetable oils¹	-0.111	0.224	0.074
Lean meat	0.191	-0.244	0.216
Red meat	-0.251	0.220	0.533
Fish	0.581	-0.037	0.075
Shellfish	0.146	0.046	0.508
Low fat dairy	0.179	-0.526	-0.011
Whole fat dairy	-0.290	0.481	-0.249
Pickled olives	0.147	0.009	0.437
Nibbles	-0.161	-0.189	0.485
Fries and chips	-0.242	0.379	0.356
Sweets and desserts	-0.461	0.243	-0.008
Coffee and tea	0.234	-0.159	-0.057
Alcoholic beverages	0.113	0.107	0.610
SSBs	-0.365	-0.246	0.242

* Extreme loadings (lower than -0.3 or higher than 0.3) are highlighted as main contributors to the dietary pattern. ** Mean ± SD for factor scores (p<0.005 by variance analysis), values with different superscripts in the same row were significantly different by Duncan's multiple range test at p<0.05. ¹ vegetable oils other than olive oil.

Table 2 (Supplemental): Correlations between individual nutrient intake and factor loadings by dietary cluster among PESA participants

Nutrients	Cluster 1 (Mediterranean)	Cluster 2 (Western)	Cluster 3 (Social business)
Total protein	0.1554*	0.2862*	0.5050*
Animal protein	0.0915*	0.0757*	0.4709*
Vegetable protein	0.1795*	0.5456*	0.2517*
Total fat	0.0186	0.3440*	0.4859*
SFA ¹	-0.3042*	0.3464*	0.3144*
PUFA ²	0.0179	0.2796*	0.4279*
MUFA ³	0.2617*	0.2542*	0.4872*
Cholesterol	-0.1893*	0.2402*	0.4151*
Carbohydrates	-0.1229*	0.5584*	0.1613*
Total sugar	-0.0939*	0.2925*	-0.0677*
Polysaccharides	-0.1016*	0.5653*	0.2917*
Fiber	0.4222*	0.4091*	0.0428*
Ethanol	0.1437*	0.0958*	0.5794*
Iron	0.2728*	0.4090*	0.4010*
Calcium	-0.0399*	0.1431*	-0.0387*
Sodium	-0.0680*	0.3000*	0.5545*
Potassium	0.4443*	0.2769*	0.2461*
Magnesium	0.3788*	0.2728*	0.2819*
Phosphorus	0.1118*	0.2050*	0.3368*
Zinc	0.0055	0.2256*	0.2640*
Vitamin A	0.1458*	0.1187*	-0.0205
Carotenoids	0.4467*	0.0297	-0.0967*
Retinoids	-0.2385*	0.1316*	0.0752*
Vitamin D	0.2548*	-0.0118	0.0636*
Vitamin E	0.3679*	0.1089*	0.1970*
Vitamin B1	0.1331*	0.1136*	0.2278*
Vitamin B2	0.0580*	0.0664*	0.0649*
Vitamin B3	0.2952*	0.1150*	0.4592*
Vitamin B6	0.4152*	0.1204*	0.2402*
Folic acid	0.5303*	0.2045*	0.0361*
Vitamin B12	0.1420*	0.1187*	0.4159*
Vitamin C	0.4653*	0.0298	-0.1634*

¹Saturated fatty acids; ² Mono-unsaturated fatty acids; ³ Poly-unsaturated fatty acids.

Table 3 (Supplemental): Gender-stratified description of cardiovascular imaging findings of PESA participants by dietary clusters

	Men				Women			
	Total N=2564	Cluster 1 (Mediterranea n) N=713	Cluster 2 (Western) N=1224	Cluster 3 (Social business) N=627	Total N=1488	Cluster 1 (Mediterranean) N=902	Cluster 2 (Western) N=444	Cluster 3 (Social business) N=142
Coronary artery score								
CAC score ≥ 1	22.5 \pm 104.9 666 (26.0)	27.2 \pm 121.9 187 (26.2) ^c	15.4 \pm 81.0 ^c 272 (22.2)	31.0 \pm 122.9 ^b 207 (33.0) ^{a,b}	1.50 \pm 14.4 68 (4.6)	1.53 \pm 16.46 38 (4.2)	1.68 \pm 11.84 22 (5.0)	0.76 \pm 4.44 8 (4.6)
CAC score > 100	139 (5.4)	44 (6.2)	49 (4.0) ^c	46 (7.3) ^b	5 (0.3)	3 (0.3)	2 (0.5)	0 (0.0)
CAC score > 400	28 (1.1)	12 (1.7)	7 (0.6)	9 (1.4)	1 (0.1)	1 (0.1)	0 (0.0)	0 (0.0)
Presence of any plaque, n (%)								
Aorta	667 (26.2)	181 (25.4)	310 (25.5)	176 (28.4)	325 (21.9)	203 (22.5)	78 (17.6) ^c	44 (31.0) ^b
Carotids	929 (36.3)	257 (36.0)	424 (34.7)	248 (39.6)	347 (23.3)	191 (21.2)	114 (25.7)	42 (29.6)
Femoral	1174 (45.8)	312 (43.8) ^c	520 (42.5) ^c	342 (54.6) ^{a,b}	263 (17.7)	159 (17.7)	72 (16.2)	32 (22.5)
Iliacs	576 (22.8)	157 (22.2)	262 (21.6)	157 (25.8)	264 (17.8)	159 (17.7)	72 (16.3)	33 (23.2)
Any plaque & CAC score ≥ 1, n (%)	1784 (70.8)	494 (69.9) ^c	827 (68.6) ^c	463 (76.4) ^{a,b}	712 (48.3)	412 (46.2)	220 (49.9)	80 (56.3)

^a significant difference from the cluster 1 (p-value<0.05); ^b significant difference from the cluster 2 (p-value<0.05); ^c significant difference from the cluster 3 (p-value<0.05). Bonferroni correction was applied (p-value<0.017).

Table 4 (Supplemental). Adjusted prevalence* ratios (PR, 95%CI) for prevalent subclinical atherosclerosis according to dietary cluster.

	Cluster 1 (Mediterranean)	Cluster 2 (Western)	Cluster 3 (Social business)
Plaque in carotids			
Model 1	ref	1.09 (0.97; 1.21)	1.17 (1.02; 1.33) *
Model 2	ref	1.06 (0.95; 1.19)	1.11 (0.98; 1.27)
Model 3	ref	1.06 (0.95; 1.18)	1.11 (0.98; 1.27)
Model 4	ref	1.06 (0.95; 1.19)	1.11 (0.97; 1.26)
Plaque in ilio-femorals			
Model 1	ref	1.01 (0.93; 1.09)	1.15 (1.05; 1.26) *
Model 2	ref	0.98 (0.91; 1.06)	1.09 (1.00; 1.19)
Model 3	ref	0.98 (0.90; 1.06)	1.09 (1.00; 1.20)
Model 4	ref	0.99 (0.91; 1.07)	1.09 (1.00; 1.20)
Any plaque or CAC>1			
Model 1	ref	1.04 (0.98; 1.10)	1.12 (1.05; 1.19) *
Model 2	ref	1.02 (0.97; 1.08)	1.09 (1.02; 1.16) *
Model 3	ref	1.02 (0.97; 1.08)	1.09 (1.02; 1.16) *
Model 4	ref	1.03 (0.97; 1.09)	1.08 (1.02; 1.15) *

Model 1: adjusted for gender, age, EI, education, income, physical activity (compliance with recommendations); Model 2: additionally adjusted for prevalent dyslipidemia, hypertension, diabetes, and current smoking; Model 3: additionally adjusted for BMI; Model 4: additionally adjusted for sleep duration, use of discretionary salt, frequency of eating out (lunch and dinner), frequency of travelling. * p < 0.05; † p < 0.01. P value given represents significant difference from cluster 1.

Figure 1 (Supplemental)

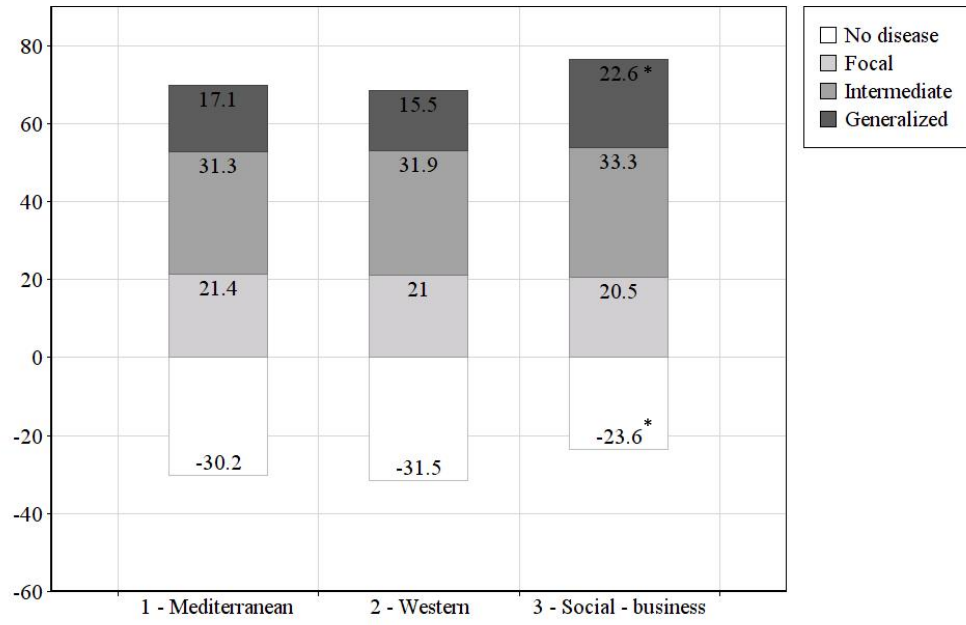
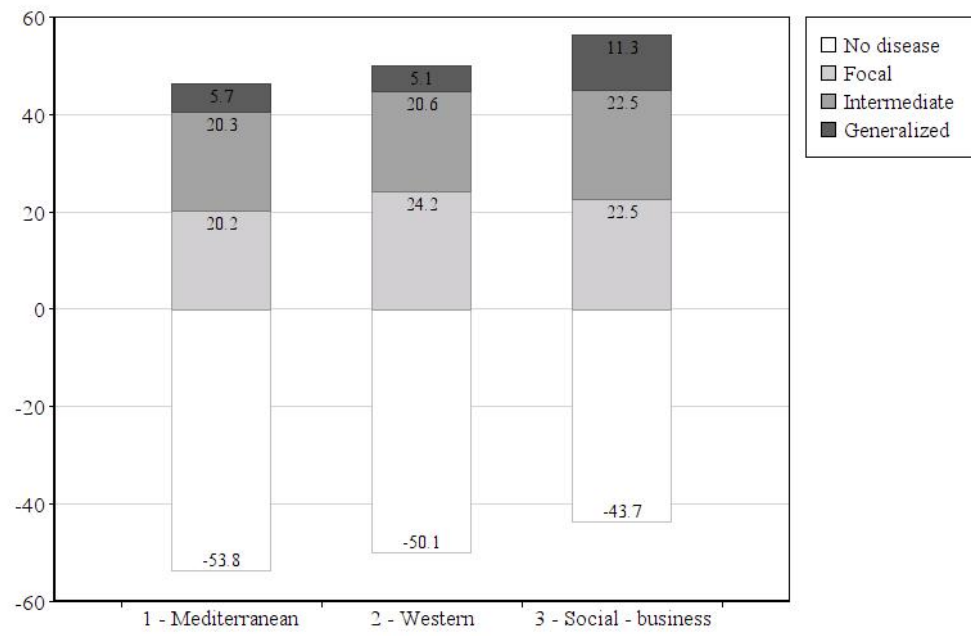


Figure 2 (Supplemental)



3rd PUBLICATION:

Adherence to a Mediterranean diet is associated with the presence and extension of atherosclerotic plaques in middle-aged asymptomatic adults: The AWHHS Study.

J Clin Lipidol. 2017;11(6):1372-1382.

GENERAL INTRODUCTION / INDIVIDUAL'S CONTRIBUTION

It was concluded from the Publication 1 and Publication 2 that neither PESA nor AWHS participants followed a traditional MDP, but rather a Mediterranean-like dietary pattern characterised by greater intakes of olive oil, fruits and vegetables, and fish, and low intakes of components traditional for MDP, such as nuts and seeds, whole grains and legumes. Considering the beneficial properties of compliance with MDP seen from first two publications, it was aimed at identifying the degree of an adherence to a traditional MDP among AWHS participants and whether that level of a compliance is associated with lower prevalence of atherosclerotic plaques, which are considered to be dominant causes of CVD.

In order to determine the compliance with traditional MDP, I used “*a-priori*” method of dietary analysis. Based on the predefined food groups and nutrients a score of adherence to MDP (aMED score) was computed. The food groups and nutrients used in the analysis were fruits, vegetables, nuts, legumes, whole grains, fish, the ratio of monounsaturated to saturated fatty acids, red and processed meat and alcohol. Median intakes were calculated and the participants were assigned one point for each of the mentioned food groups if their intakes for fruits, vegetables, nuts, legumes, whole grains, fish, the ration of monounsaturated versus saturated fatty acids were above the median, intakes of red and processed meat were below the median and the alcohol consumption was within the range of 5 to 25 g/d and 0 to 50 g/d for women and men respectively. The total aMED score ranged from 0 to 9, reflecting the lowest and the highest compliance with MDP. Further, the quartiles of the score were computed and studied in relation to the presence and quantity of atherosclerotic plaques in different vascular territories, including carotid artery, femoral artery, and aorta.

Adherence to a Mediterranean diet is associated with the presence and extension of atherosclerotic plaques in middle-aged asymptomatic adults: The Aragon Workers' Health Study



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KEYWORDS:

Mediterranean diet;
Atherosclerosis;
Plaques;
Femoral;
Carotid;
Aorta

BACKGROUND: The Mediterranean diet (MeDi) is known to prevent cardiovascular events but the mechanisms mediating this association are not fully understood.

OBJECTIVE: The objective of the study was to examine the association between MeDi adherence and the presence and extent of atherosclerotic plaques in carotid, femoral, and aorta territories and its relationship with risk factors in asymptomatic middle-aged adults.

METHODS: Cross-sectional analysis of the Aragon Workers' Health Study, a cohort of 2588 subjects (94.9% men aged 51.3 ± 3.89 years) without previous cardiovascular history. Participants underwent carotid, femoral, and aorta ultrasound for the quantification of number and thickness of plaques and intima-media thickness. To estimate the participant's adherence to MeDi, we computed the Alternative MEDiterranean index (aMED).

RESULTS: The overall aMED score was 4.19 ± 1.70 , representing a moderate adherence to MeDi. aMED score was associated with the presence of plaque in femoral arteries (odds ratio highest vs

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lowest aMED score quartile: 0.63; 95% confidence interval: 0.48–0.83; P trend = .045) independently of risk factors and mediators. The strongest association between aMED quartiles and presence of plaque was found among smokers, both in femoral (0.39 [0.22–0.69]; P trend = .001) and in any territory (0.33 [0.14–0.79], P trend = .008). aMED was inversely associated with the number of plaques in all territories except for carotids.

CONCLUSION: MeDi adherence showed a dose-dependent protective association with the presence, number, and thickness of plaques independent of other risk factors. The association was strongest for femoral arteries and among smokers.

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Introduction

Lifestyle plays an essential role in the prevention of non-communicable diseases and especially cardiovascular diseases (CVD), the main cause of morbidity and mortality in most countries.^{1,2} Among lifestyle behaviors, diet is directly related to major CVD risk factors such as dyslipidemia, hypertension, and diabetes.³ Mediterranean countries share dietary characteristics that are globally known as Mediterranean diet (MeDi), which has been related to health benefits such as CVD prevention.^{4–8} In agreement with this concept, Mediterranean countries show low CVD rates (mainly coronary disease) in spite of high prevalence of cardiovascular risk factors. This suggests a lower impact of these factors on CVD development in these countries.^{9,10} The PREvención con DIeta MEDiterránea (PREDIMED) trial showed that among individuals at high cardiovascular risk, a MeDi supplemented with extra-virgin olive oil or nuts reduced the incidence of major cardiovascular events.⁸ However, the mechanisms responsible for this benefit are not completely known. It is not fully understood if diet is solely responsible or if dietary patterns interrelate with other environmental factors by modulating the impact of cardiovascular risk factors on CVD.

The inverse association of MeDi with CVD could be mediated by the attenuation of atherosclerotic plaque development and progression.⁸ Some studies have investigated the effect of MeDi on carotid intima-media thickness (IMT) and have produced divergent results.^{11–14} The PREDIMED trial showed carotid atherosclerosis regression as IMT thinning in those participants after a MeDi supplemented with olive oil or nuts, and a baseline carotid IMT above 0.9 mm.¹¹ In the DIRECT-Carotid study, those subjects more compliant with the MeDi showed a decrease of 37.7 mm³ in the carotid vessel wall volume. However, it appeared to be mediated mainly by the weight loss–induced decline in blood pressure.¹³

Arterial IMT carotid wall volume is the result of multiple factors including age, blood pressure, and lipid and non-lipid depositions, and its measurement has been questioned as a predictor of CVD.^{15,16} In contrast, arterial plaques in medium size arteries are the hallmark of atherosclerosis

and their presence is predictive of CVD independent of traditional risk factors in several prospective studies.¹⁷ The presence of plaques has been mostly studied in carotid arteries by ultrasonography. Recently, in 2 large different studies, iliofemoral plaques identified by ultrasonography have demonstrated to be more frequent, better correlated with CVD risk factors, and highly associated with coronary calcium, even more than carotid plaques.^{18,19} The association of iliofemoral plaques to MeDi adherence has not been previously studied. So, we sought to examine the impact of MeDi adherence on presence and extent of atherosclerosis plaques in carotid, aorta, and iliofemoral territories and the impact of risk factors in these associations in the Aragon Workers' Health Study (AWHS) participants.

Material and methods

Study design

This study is a cross-sectional analysis of baseline imaging data from AWHS. The protocol for AWHS has been previously explained in depth.^{19,20} Briefly, the AWHS is a Spanish cohort, which aims to characterize the factors associated with metabolic abnormalities and subclinical atherosclerosis in a middle-aged population free of clinical CVD, who may be optimal target for primary prevention. The study involved 5400 employees of a car assembly plant in Figueruelas (Zaragoza, Spain) that were recruited during annual checkup in 2009 to 2012 and have undergone clinical examinations, laboratory assays, collection of biological materials for biobanking, and cardiovascular imaging screening (carotid, femoral, and abdominal ultrasonography and ankle–arm blood pressure score) every 3 years. Among 5400 employees who consented for participation into the study, subclinical atherosclerosis imaging was carried out to study participants who were aged 40 to 60 years at baseline.²⁰ As shown in Figure 1, 2571 subjects (2440 men and 131 women aged 40–60 years) were finally recruited into the AWHS imaging study from January 2011 to December 2014. Forty-seven subjects were excluded because they had previous history of CVD. The Aragon regional government's Ethics Committee for Clinical

Research approved the study and all participants signed a written informed consent. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

Subclinical atherosclerosis imaging

Carotid and femoral imaging acquisition has been previously described.¹⁹ Briefly, Philips IU22 ultrasound system (Philips Healthcare, Bothell, WA) was used to assess the presence of plaques in 3 vascular territories (carotid, femoral, and aorta). We acquired ultrasound images by linear high-frequency 2-dimensional probes (Philips Transducer L9-3; Philips Healthcare), using the BioImage Study protocol for the carotid arteries and a specifically designed protocol for the femoral arteries.¹⁵ Inspection sweeps and 3 measurements of IMT were obtained in right and left sides for 3 sites of carotid (common, internal, and bulb) and femoral territories, and aorta. We calculated the mean and maximum values of IMT for each territory and we recorded plaque number and plaque thickness if applicable in all territories. All measurements were analyzed using electrocardiogram-gated frames corresponding to end-diastole (R-wave).¹⁷

Our main outcome was presence of subclinical atherosclerosis, which was defined as the detection of at least 1 plaque in any of the segments studied (both sides of carotid and femoral arteries and aorta) with vascular ultrasound (Fig. 1). Plaque was defined as a focal structure protruding ≥ 0.5 mm into the lumen or reaching a thickness $\geq 50\%$ of the surrounding intima. If subjects had no plaque in 2 territories (carotids, femorals, and/or aorta) and no information in the third one, we considered as absence of atherosclerosis.

If the data were available only for 1 territory, subject was excluded from the final analysis. Among these subjects ($N = 2523$), plaque in “any territory” was defined when presence of plaque was noted in any of the 5 territories (both sides of carotid and femoral arteries and aorta). The extent of subclinical atherosclerosis was defined by 4 categories: disease-free (0 vascular sites affected), having focal (1 site), intermediate (2–3 sites), or generalized atherosclerosis (≥ 4 sites).

Clinical, sociodemographic, and biochemical characteristics

Clinical and laboratory data were obtained during the annual medical examination. We included in the analysis the one corresponding to the year that subclinical atherosclerosis and diet were assessed. The data collection procedures have been previously described in detail.^{19,20} In brief, clinical data included medical history, current use of medication, anthropometry, and blood pressure. Blood samples were collected in fasting (>8 h) conditions. Fasting serum glucose, triglycerides, total cholesterol, and high-density lipoprotein (HDL) cholesterol were measured by spectrophotometry (Chemical Analyzer ILAB 650; Instrumentation Laboratory SpA, Bedford, MA). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula. Whole blood glycosylated hemoglobin was measured by reverse-phase cation exchange chromatography and quantified by double wavelength colorimetry (ADAMS A1c HA-810 Analyzer; Arkray Factory, Minneapolis, MN.). C-reactive protein

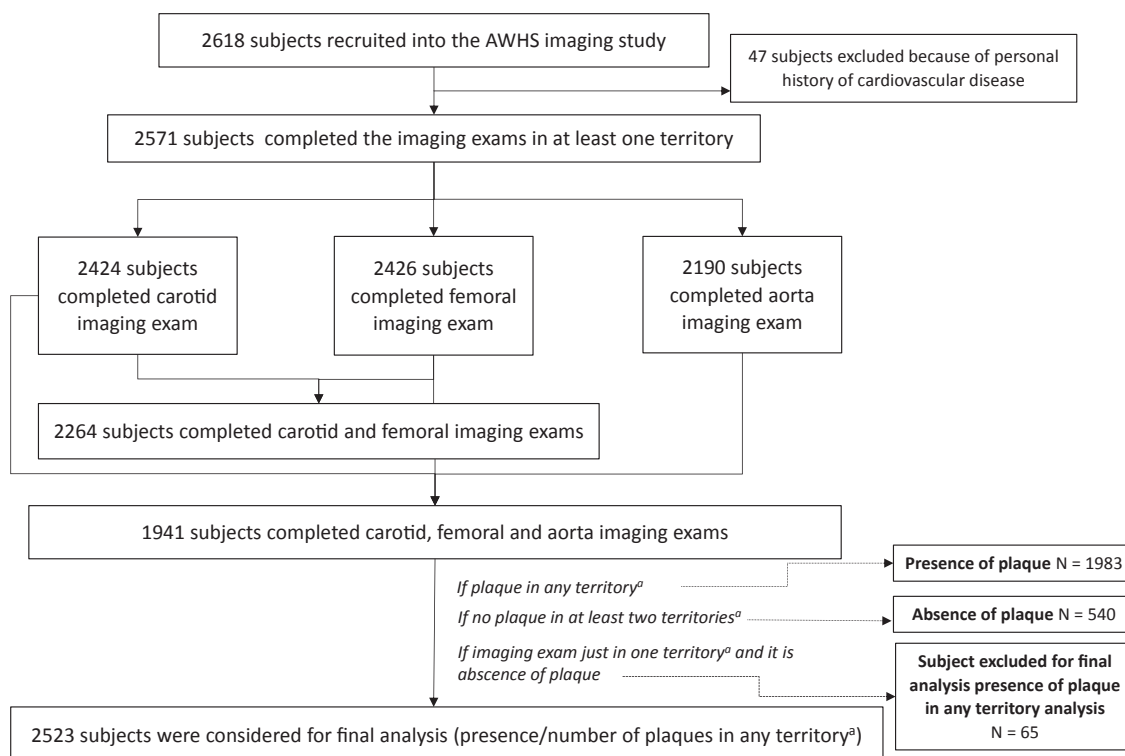


Figure 1 Study flowchart of subclinical atherosclerosis imaging. ^aFive territories have been considered (both sides of carotid and femoral arteries and aorta) when assessing the presence of plaque.

(CRP) and lipoprotein(a) were determined by nephelometry using the IMMAGE 800 system (Beckman Coulter). We defined dyslipidemia when total cholesterol ≥ 240 mg/dL, LDL cholesterol ≥ 160 mg/dL, HDL cholesterol < 40 mg/dL in men or HDL cholesterol < 50 mg/dL in women, or self-reported use of lipid-lowering drugs.²¹ Diabetes was defined as fasting plasma glucose ≥ 126 mg/dL or self-reported treatment with hypoglycemic medication.²² Hypertension was defined as systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg or self-reported treatment with

antihypertensive medication.²² Current smoking was defined as smoking in the present or having smoked in the last year. Being former smoker was defined as a subject having smoked at least 50 cigarettes in his lifetime, but not having smoked in the last year. The variable "Number of CV risk factors" was created counting how many of the following 4 risk factors were present: hypertension, dyslipidemia, diabetes, and current smoking. Sociodemographic data included education level that was classified as less than high school, high school or some college, college degree, or other higher education degree.

Table 1 Clinical and biochemical characteristics of AWHs participants according to quartiles of aMED score*

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	<i>P</i>
	0–2 points	3–4 points	5 points	6–9 points	
	N = 434	N = 1015	N = 535	N = 582	
Age, y	50.9 \pm 4.03	51.3 \pm 3.87	51.3 \pm 3.87	51.7 \pm 3.72	.011
Gender, n (% males)	409 (94.2)	971 (95.7)	507 (94.8)	553 (95.0)	.678
Weight, kg	81.4 \pm 12.9	81.6 \pm 12.1	82.2 \pm 12.3	81.5 \pm 11.8	.792
BMI, kg/m ²	27.7 \pm 3.79	27.7 \pm 3.57	28.0 \pm 3.62	27.6 \pm 3.38	.313
Waist circumference, cm	97.6 \pm 10.5	97.6 \pm 9.75	97.4 \pm 9.40	96.4 \pm 9.82	.115
Smoking habit					
Non-smoker	100 (23.4)	228 (23.1)	114 (22.0)	142 (26.4)	
Current smoker	188 (44.0)	366 (37.1)	185 (35.6)	136 (25.3)	<.001
Former smoker	139 (32.6)	393 (39.8)	220 (42.4)	260 (48.3)	
Education level					
Less than high school	218 (50.2)	524 (51.6)	248 (46.4)	274 (47.2)	
High school or some college	191 (44.0)	430 (42.4)	242 (45.2)	263 (45.3)	
College degree	25 (5.80)	56 (5.50)	41 (7.70)	43 (7.40)	.222
Other	0	5 (0.50)	4 (0.70)	1 (0.20)	
Hypertension, n (%)	99 (22.8)	199 (19.6)	128 (23.9)	120 (20.7)	.197
Systolic blood pressure, mm Hg	125 \pm 14.5	125 \pm 14.3	124 \pm 14.1	125 \pm 14.2	.559
Diastolic blood pressure, mm Hg	82.5 \pm 9.52	82.5 \pm 9.49	82.3 \pm 9.69	82.6 \pm 9.42	.924
Ankle–brachial score	1.09 \pm 0.09	1.10 \pm 0.09	1.11 \pm 0.10	1.11 \pm 0.09	.063
Diabetes, n (%)	18 (4.20)	38 (3.70)	19 (3.60)	26 (4.50)	.849
Glucose, mg/dL	97.7 \pm 14.9	97.1 \pm 17.2	98.6 \pm 19.5	98.9 \pm 16.4	.166
HbA1c, %	5.57 \pm 0.50	5.54 \pm 0.50	5.57 \pm 0.65	5.55 \pm 0.58	.902
Dyslipidemia, n (%)	71 (16.4)	182 (17.9)	109 (20.4)	108 (18.6)	.424
Total cholesterol, mg/dL	219 \pm 37.2	219 \pm 36.7	221 \pm 35.8	224 \pm 35.6	.096
HDL cholesterol, mg/dL	51.9 \pm 11.7	52.7 \pm 11.3	53.3 \pm 11.8	55.4 \pm 11.9	<.001
Triglycerides, mg/dL	154 \pm 92.3	149 \pm 96.7	153 \pm 91.5	146 \pm 104	.494
LDL cholesterol, mg/dL	138 \pm 31.7	138 \pm 31.2	138 \pm 30.4	140 \pm 30.2	.495
Non-HDL cholesterol, mg/dL	167 \pm 35.5	166 \pm 35.3	168 \pm 34.8	168 \pm 34.8	.803
Lipoprotein(a), mg/dL [†]	36.8 \pm 28.8	39.6 \pm 30.3	39.2 \pm 32.2	41.2 \pm 33.1	.549
C-reactive protein, mg/dL [†]	0.44 \pm 0.61	0.38 \pm 0.33	0.36 \pm 0.37	0.33 \pm 0.35	<.001
Number of CV risk factors [‡]					
0	70 (16.4)	167 (16.9)	78 (15.1)	109 (20.3)	
1	238 (55.9)	552 (56.0)	275 (53.2)	272 (50.6)	
2	185 (20.0)	198 (20.1)	123 (23.8)	121 (22.5)	.322
>2	33 (7.70)	69 (7.00)	41 (7.90)	36 (6.70)	

BMI, body mass index; CV, cardiovascular; HDL, high-density lipoprotein; LDL, low-density lipoprotein cholesterol.

*Data expressed as mean \pm standard deviation or N (%) as appropriate. *P* refers to differences calculated by analysis of variance, Kruskal–Wallis, or chi-squared tests as appropriate. AWHs denotes Aragon Workers' Health Study.

[†]Lipoprotein(a) and C-reactive protein were imputed by multiple imputation method because of 53.6% and 45.6% of missing values, respectively.

[‡]Risk factors include smoking habit, hypertension, diabetes, and dyslipidemia.

Dietary and physical activity assessment

Diet was assessed by a face-to-face interview through a 137 items semi-quantitative food frequency questionnaire (FFQ) that was previously validated in Spanish population.²³ Participants were asked about the average frequency of consumption and the serving size over the previous year. Food and nutrient intakes were calculated as frequency \times nutrient composition of specified portion sizes, where frequencies were measured in nine categories (never, 1–3 times a month, 1 time a week, 2–4 times a week, 5–6 times a week, 1 time a day, 2–3 times a day, 4–6 times a day, and >6 times a day) for each food item. The total energy and nutrients intakes were derived from Spanish food composition tables.²⁴

We computed the Alternative MEDiterranean score (aMED) based on the FFQ as previously described.⁶ The aMED score involved the following food groups and nutrients: (1) fruit; (2) vegetables; (3) nuts; (4) legumes; (5) whole grains; (6) fish; (7) ratio of monounsaturated to saturated fat; (8) red and processed meats; and (9) alcohol. Participants whose intakes were above the sex-specific AWHs sample medians for fruit, vegetables, nuts, legumes, whole grains, fish, or the ratio of monounsaturated fat to saturated fat received 1 point for each dietary component. The consumption of red and processed meats below the median was given 1 point. Those women consuming 5 to 25 g/d of alcohol received 1 point and those men with an alcohol intake of 10 to 50 g/d were given 1 point. The total score could range from 0 to 9 with higher scores reflecting higher MeDi adherence.

Table 2 Energy-adjusted intake of nutrients and food groups and physical activity of AWHs participants according to quartiles of aMED score*

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	<i>P</i>
	0–2 points	3–4 points	5 points	6–9 points	
	N = 434	N = 1015	N = 535	N = 582	
Nutrients					
Energy, kcal	2648 \pm 695	2830 \pm 732	2924 \pm 705	3109 \pm 781	<.001
Protein, %	14.7 \pm 2.26	15.1 \pm 2.42	15.4 \pm 2.33	15.6 \pm 2.27	<.001
Carbohydrates, %	46.6 \pm 6.85	45.0 \pm 6.68	44.8 \pm 6.48	43.8 \pm 6.00	<.001
Fiber, g/d	24.1 \pm 3.93	25.1 \pm 4.14	25.6 \pm 3.99	26.7 \pm 4.42	<.001
Fat, %	34.3 \pm 5.57	34.8 \pm 5.34	34.7 \pm 5.20	35.7 \pm 5.00	<.001
Saturated fatty acids, %	10.5 \pm 2.28	10.2 \pm 2.12	9.93 \pm 1.98	9.77 \pm 1.91	<.001
MUFA, %	15.8 \pm 3.16	16.1 \pm 3.13	16.0 \pm 3.19	16.4 \pm 3.05	.044
PUFA, %	5.40 \pm 1.65	5.60 \pm 1.63	5.70 \pm 1.48	6.27 \pm 1.68	<.001
Ratio unsaturated/saturated fatty acids	2.08 \pm 0.39	2.18 \pm 0.45	2.24 \pm 0.44	2.37 \pm 0.45	<.001
Ratio MUFA/PUFA	3.15 \pm 0.97	3.06 \pm 0.87	2.96 \pm 0.82	2.77 \pm 0.78	<.001
Trans fatty acids, %	0.30 \pm 0.13	0.26 \pm 0.12	0.25 \pm 0.11	0.24 \pm 0.11	<.001
Cholesterol, mg/d	439 \pm 86.7	462 \pm 91.2	473 \pm 87.9	496 \pm 97.4	<.001
Marine omega-3 fatty acids, g/d	0.68 \pm 0.04	0.69 \pm 0.04	0.70 \pm 0.04	0.71 \pm 0.05	<.001
Non-marine omega-3 fatty acids, g/d	1.51 \pm 0.35	1.60 \pm 0.37	1.65 \pm 0.35	1.74 \pm 0.39	<.001
Omega-6 fatty acids, g/d	13.3 \pm 3.15	14.1 \pm 3.31	14.5 \pm 3.19	15.3 \pm 3.53	<.001
Ratio omega-6/3 fatty acids	7.72 \pm 3.45	6.70 \pm 2.60	5.91 \pm 2.03	5.74 \pm 1.80	<.001
Food groups					
Vegetables, g/d	317 \pm 19.6	322 \pm 20.7	325 \pm 19.9	330 \pm 22.0	<.001
Fruits, g/d	296 \pm 26.7	303 \pm 28.1	307 \pm 27.1	314 \pm 30.0	<.001
Legumes, g/d	15.6 \pm 1.39	15.9 \pm 1.46	16.1 \pm 1.41	16.5 \pm 1.56	<.001
Regular cereals, g/d	171 \pm 82.1	192 \pm 86.4	203 \pm 83.3	225 \pm 92.0	<.001
Whole-grain cereals, g/d	30.4 \pm 3.50	29.4 \pm 3.69	29.0 \pm 3.56	28.0 \pm 3.93	<.001
Whole dairy products, g/d	106 \pm 44.4	117 \pm 46.8	123 \pm 45.1	135 \pm 49.9	<.001
Skimmed dairy products, g/d	193 \pm 2.88	99.0 \pm 34.8	104 \pm 33.6	113 \pm 37.1	<.001
Meat products, g/d	174 \pm 32.2	183 \pm 33.9	187 \pm 32.7	196 \pm 36.2	<.001
Fish, g/d	85.8 \pm 4.92	87.1 \pm 5.18	87.8 \pm 4.99	89.1 \pm 5.53	<.001
Olive oil, g/d	27.2 \pm 1.79	27.6 \pm 1.88	27.9 \pm 1.81	28.4 \pm 2.01	<.001
Alcohol, g/d	18.7 \pm 5.51	20.1 \pm 5.80	20.9 \pm 5.59	22.3 \pm 6.19	<.001
Sweetened beverages, g/d	91.3 \pm 33.0	99.0 \pm 34.8	104 \pm 33.6	113 \pm 37.1	<.001
Physical activity, METs-h/wk	28.3 \pm 20.8	30.4 \pm 22.8	33.2 \pm 22.8	35.8 \pm 23.5	<.001

aMED, alternative MEDiterranean; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

*Data expressed as mean \pm standard deviation or N (%) as appropriate. *P* refers to differences calculated by analysis of variance or Kruskal-Wallis as appropriate. AWHs denotes Aragon Workers' Health Study.

Physical activity was assessed by a Spanish validated version of the Nurses' Health Study and Health Professionals' Follow-up exercise questionnaires.²⁵ Subjects were asked about the time and frequency devoted to the practice of 17 different sports during the previous year. We computed the volume of activity for each patient by assigning a specific metabolic cost based on Ainsworth's criteria, which was multiplied by the time the subject spent in each activity.²⁶ We obtained an overall weekly metabolic equivalent of task per hour (METs-h) value from the sum of all activities.

Statistical analysis

Data are expressed as mean ± standard deviation or percentage for continuous and categorical variables, respectively. Differences between groups of categorical variables were assessed using a Pearson chi-square test. Analysis of variance and Kruskal–Wallis tests were performed to multiple independent variables comparison. We grouped aMED score in quartiles after verifying the linear relationship between aMED score and IMT for each territory by cubic spline regression. Quartiles have unequal numbers of participants because of ties in diet scores. Missing data for CRP (45.6%) and lipoprotein(a) (53.6%) were imputed using the chained-

equations approach²⁷; 5 complete data sets were simulated, and estimates were combined, to get appropriate analysis.

We examined the association between the presence and number of plaques and quartiles of aMED score with the use of multivariable logistic regression model. Potential confounders were included in the model: age, gender, energy intake (kcal), education level (less than high school, high school or some college, college degree, and other), smoking (nonsmoker, current smoker, and former smoker), physical activity (METs-h/wk), body mass index, LDL cholesterol, systolic and diastolic blood pressure, diabetes mellitus, CRP, and lipoprotein(a). We conducted tests of linear trend by entering the median of aMED score for each quartile as a continuous scale. All statistical analyses were performed with SPSS software version 24.0 (Chicago, IL) using a significance level of *P* < .05.

Results

Mean aMED score was 4.24 ± 1.72 in women and 4.19 ± 1.70 in men (*P* = .762). Compared with individuals in the lowest quartile of aMED, AHWS participants with the highest MeDi scores were, on average, older, less likely to

Table 3 Association between aMED score and presence of plaque in different territories in AWHS participants

		OR (95% CI)				<i>P</i> for trend
		Quartile 1	Quartile 2	Quartile 3	Quartile 4	
		0–2 points N = 434	3–4 points N = 1015	5 points N = 535	6–9 points N = 582	
Carotid						
Age, gender-adjusted, N = 1511/864*	Ref.	1.02 (0.79–1.31)	0.99 (0.75–1.32)	1.02 (0.75–1.35)	.922	
Multivariable-adjusted 1†, N = 1463/826*	Ref.	1.06 (0.86–1.37)	1.06 (0.79–1.43)	1.16 (0.87–1.57)	.311	
Multivariable-adjusted 2‡, N = 1354/751*	Ref.	1.19 (0.90–1.56)	1.18 (0.87–1.62)	1.31 (0.95–1.80)	.119	
Femoral						
Age, gender-adjusted, N = 1069/1035*	Ref.	0.86 (0.67–1.10)	0.76 (0.58–1.01)	0.63 (0.48–0.83)§	<.001	
Multivariable-adjusted 1†, N = 1036/1249*	Ref.	0.89 (0.68–1.16)	0.79 (0.59–1.07)	0.69 (0.51–0.93)¶	.008	
Multivariable-adjusted 2‡, N = 1036/1249*	Ref.	0.89 (0.68–1.18)	0.80 (0.59–1.10)	0.74 (0.54–1.02)	.045	
Aorta						
Age, gender-adjusted, N = 1029/1124*	Ref.	0.87 (0.67–1.11)	0.68 (0.51–0.89)§	0.72 (0.55–0.94)¶	.006	
Multivariable-adjusted 1†, N = 988/1096*	Ref.	0.91 (0.70–1.17)	0.71 (0.53–0.94)¶	0.84 (0.63–1.12)	.125	
Multivariable-adjusted 2‡, N = 896/1022*	Ref.	0.97 (0.74–1.27)	0.76 (0.56–1.03)	0.89 (0.66–1.21)	.303	
Any territory						
Age, gender-adjusted, N 3533/1968*	Ref.	0.99 (0.74–1.32)	0.78 (0.57–1.07)	0.75 (0.55–1.03)	.022	
Multivariable-adjusted 1†, N = 514/1898*	Ref.	1.02 (0.75–1.38)	0.82 (0.59–1.15)	0.85 (0.61–1.20)	.183	
Multivariable-adjusted 2‡, N = 465/1741*	Ref.	1.04 (0.76–1.44)	0.87 (0.61–1.25)	0.93 (0.64–1.33)	.471	

aMED, alternative MEDiterranean; AWHS, Aragon Workers' Health Study; CI, confidence interval; OR, odds ratio.

*N, Number of subjects without/with plaque.

†Adjusted by age, gender, energy intake, education level (less than high school, high school or some college, college degree, and other), tobacco consumption (non-smoker, current smoker, and former smoker), and physical activity.

‡Adjusted by age, gender, energy intake, education level (less than high school, high school or some college, college degree, and other), tobacco (non-smoker, current smoker, and former smoker), physical activity, body mass index, low-density lipoprotein cholesterol, systolic and diastolic blood pressure, diabetes mellitus, C-reactive protein (imputed), and lipoprotein(a) (imputed).

§Denotes *P* < .010 with respect to first quartile.

¶Denotes *P* < .050 with respect to first quartile.

be current smokers, have higher levels of HDL cholesterol and lower concentration of CRP, and be more physically active (Table 1). As expected, because of the method to calculate the MeDi score, participants in the highest quartile reported a healthier dietary pattern (Table 2). They had, on average, higher intakes of vegetables, fruits, legumes, cereals, whole-grain cereals, and olive oil. After energy-consumption adjustment, participants from the fourth quartile of aMED still showed higher consumption of dairy, meat, fish, and sugar-sweetened beverages. Those subjects in the highest aMED quartile were more likely to have higher intakes of protein, fiber, fat (mainly from monounsaturated and polyunsaturated fatty acids), cholesterol, omega 3 and omega 6 fatty acids, and alcohol while reporting less consumption of carbohydrates, saturated and *trans* fatty acids. Participants in the top quartile of aMED also reported the highest physical activity level.

Among AWHs participants, the prevalence of atheroma plaque was 36.4% in carotid, 54.8% in femoral, and 52.3% in aorta territories. There was a significant linear association between aMED score and presence of plaque in any territory although the significance disappeared after adjustment for potential confounders including demographics, physical activity, traditional CVD risk factors, and other clinical variables (Table 3). When compared with participants with

low adherence to MeDi, the highest adherence presented a lower probability of prevalent plaque in femoral territories (odds ratio [OR]: 0.63; 95% confidence interval [CI]: 0.48–0.83) with a linear trend across quartiles. This trend remained significant ($P = .045$) in the fully adjusted model although the odds between lowest and highest quartiles were attenuated (OR: 0.74; 95% CI: 0.54–1.02). The same association was observed in aorta territories (OR: 0.72; 95% CI: 0.55–0.94; P for trend = .006). The statistical significance disappeared in the fully adjusted model. The presence of plaque in carotid territories was not associated with adherence to MeDi. This association remained significant when only including men in the regression analysis. In sensitivity analyses stratifying participants by smoking status (Table 4, Supplemental Tables 1–2), the highest independent association between aMED score and presence of plaque was observed in femoral and in any territory for current smokers (OR: 0.39; 95% CI: 0.22–0.69 and OR: 0.33; 95% CI: 0.14–0.79, respectively, highest vs lowest quartile; Table 4). We also found significant relationship between MeDi adherence and presence of plaque in femoral in former smokers although the significance disappeared in the fully adjusted model (Supplemental Table 2).

Assessing the different contribution of the aMED score components toward disease presence, we observed that red

Table 4 Association between aMED score and presence of plaque in different territories in smoker AWHs participants

	OR (95% CI)				<i>P</i> for trend
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
	0–2 points	3–4 points	5 points	6–9 points	
	N = 188	N = 366	N = 185	N = 136	
Carotid					
Age, gender-adjusted, N = 471/347*	Ref.	1.15 (0.79–1.67)	1.02 (0.66–1.57)	1.09 (0.67–1.75)	.855
Multivariable-adjusted 1 [†] , N = 471/347*	Ref.	1.15 (0.79–1.68)	1.01 (0.65–1.57)	1.11 (0.68–1.81)	.819
Multivariable-adjusted 2 [‡] , N = 432/307*	Ref.	1.34 (0.88–2.02)	1.17 (0.73–1.91)	1.26 (0.74–0.15)	.482
Femoral					
Age, gender-adjusted, N = 237/592*	Ref.	0.89 (0.58–1.37)	0.73 (0.45–1.18)	0.45 (0.27–0.75) [§]	.001
Multivariable-adjusted 1 [†] , N = 237/592*	Ref.	0.85 (0.55–1.31)	0.67 (0.41–1.10)	0.42 (0.25–0.70) [§]	<.001
Multivariable-adjusted 2 [‡] , N = 220/529*	Ref.	0.77 (0.48–1.23)	0.65 (0.38–1.10)	0.39 (0.22–0.69) [§]	.001
Aorta					
Age, gender-adjusted, N = 304/422*	Ref.	0.62 (0.41–0.93) [¶]	0.48 (0.30–0.76) [§]	0.65 (0.39–1.07)	.035
Multivariable-adjusted 1 [†] , N = 304/422*	Ref.	0.64 (0.43–0.96) [¶]	0.50 (0.31–0.80) [§]	0.71 (0.3–1.19)	.095
Multivariable-adjusted 2 [‡] , N = 267/384*	Ref.	0.66 (0.42–1.03)	0.51 (0.31–0.84) [§]	0.83 (0.47–1.46)	.258
Any territory					
Age, gender-adjusted, N = 109/753*	Ref.	0.58 (0.30–1.11)	0.39 (0.20–0.78) [§]	0.34 (0.17–0.71) [§]	.001
Multivariable-adjusted 1 [†] , N = 109/753*	Ref.	0.62 (0.32–1.19)	0.41 (0.20–0.83) [¶]	0.38 (0.18–0.79) [§]	.004
Multivariable-adjusted 2 [‡] , N = 95/67*	Ref.	0.46 (0.21–0.98) [¶]	0.33 (0.15–0.74) [§]	0.33 (0.14–0.79) [¶]	.008

*N, Number of subjects without/with plaque.

[†]Adjusted by age, gender, energy intake, education level (less than high school, high school or some college, college degree, and other), tobacco consumption (non-smoker, current smoker, and former smoker), and physical activity.

[‡]Adjusted by age, gender, energy intake, education level (less than high school, high school or some college, college degree, and other), tobacco (non-smoker, current smoker, and former smoker), physical activity, body mass index, low-density lipoprotein cholesterol, systolic and diastolic blood pressure, diabetes mellitus, C-reactive protein (imputed), and lipoprotein(a) (imputed).

[§]Denotes $P < .010$ with respect to first quartile.

[¶]Denotes $P < .050$ with respect to first quartile.

meat consumption above the sample median was significantly associated with a greater probability of prevalent plaque in carotid (OR: 1.31; 95% CI: 1.07–1.61; [Supplemental Table 3](#)). Fruits consumption above the sample median was observed to be associated with lower prevalence of plaque in femoral territories (OR: 0.75; 95% CI: 0.62–0.92), whereas ratio of monounsaturated fatty acids:saturated fatty acids above the median was independently associated with lower risk of having plaque in aorta (OR: 0.80; 95% CI: 0.66–0.98). Moderate alcohol intake, compared with those who did not consume alcohol or consumed it excessively, was independently associated with lower risk of having plaque in aorta and in any territory (OR: 0.80; 95% CI: 0.66–0.96 and OR: 0.78; 95% CI: 0.62–0.96 respectively).

We observed an inverse linear association between maximum plaque thickness and MeDi adherence in carotid territories on the fully adjusted model (P trend = .027)

([Supplemental Table 4](#)). However, there was no significant association between MeDi adherence and mean or maximum IMT. Conversely, in femoral territories, we observed a significant relationship between mean (0.93 ± 0.49 and 0.85 ± 0.44 mm in lowest and highest quartiles; P trend = .035) and maximum IMT (1.01 ± 0.59 and 1.12 ± 0.66 mm in lowest and highest quartiles; P trend = .025) and MeDi adherence, but no linear trend was observed for maximum plaque thickness.

The number of plaques observed was inversely associated with MeDi adherence in all territories except in carotids ([Fig. 2](#)). The strongest association between the number of plaques and aMED quartiles was observed for femoral territories (average aMED scores: 4.33 ± 1.71 , 4.18 ± 1.69 , 4.06 ± 1.67 , and 3.90 ± 1.57 among those with no plaques, 1–2, 3–4, or more, adjusted P linear trend < .001). There was also significant association

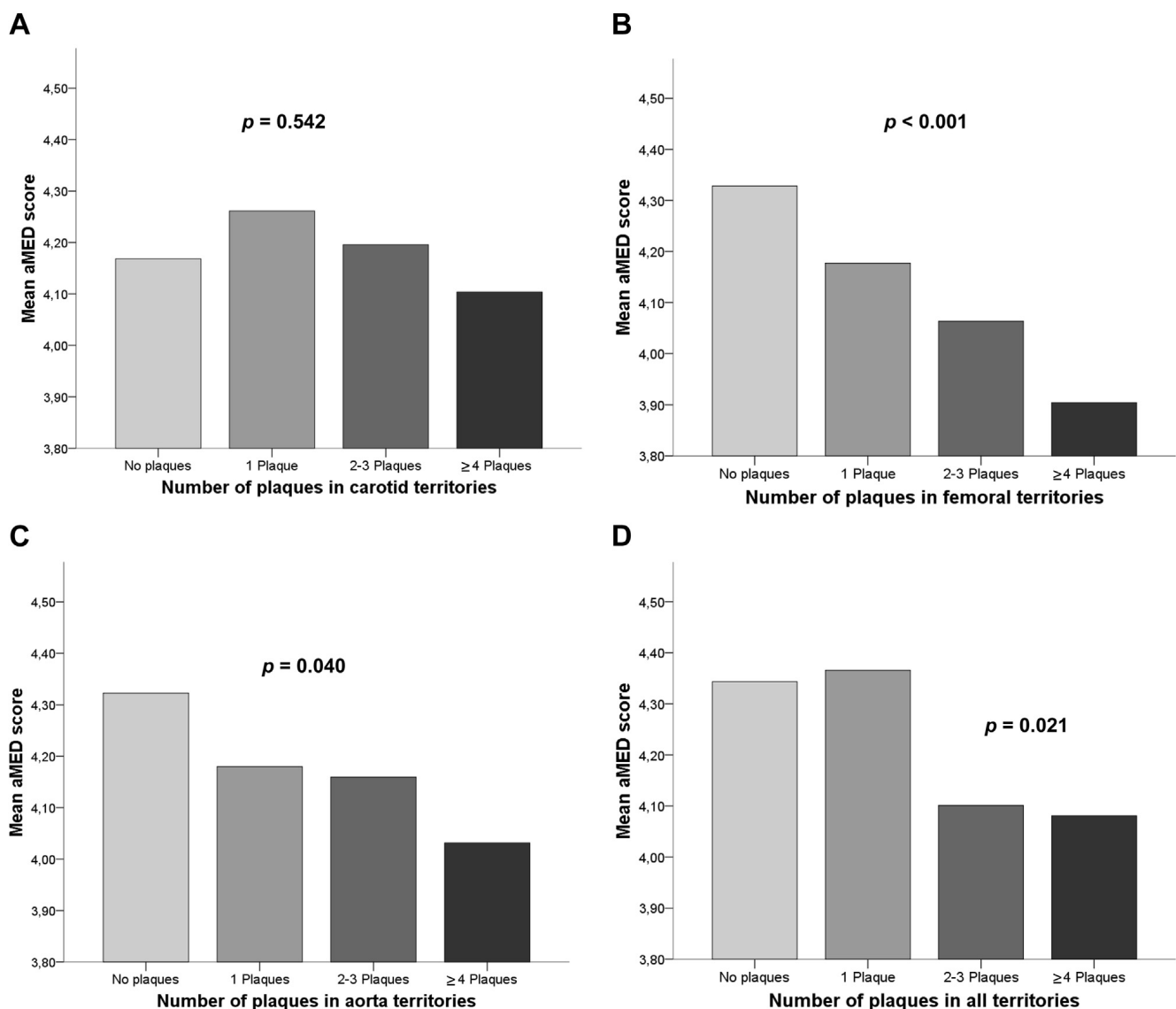


Figure 2 aMED score by number of plaques in carotid (A) femoral (B) aorta (C), and all territories (D).^a P refers to differences calculated by linear regression with adjustment for age, gender, energy intake, education level (less than high school, high school or some college, college degree, and other), tobacco (non-smoker, current smoker, and former smoker), physical activity, body mass index, low-density lipoprotein cholesterol, systolic and diastolic blood pressure, diabetes mellitus, C-reactive protein (imputed), and lipoprotein(a) (imputed).

between the number of plaques and aMED in aorta (average aMED scores: 4.32 ± 1.68 , 4.18 ± 1.74 , 4.16 ± 1.70 , and 4.03 ± 1.76 among those with no plaques, 1–2, 3–4, or more, adjusted *P* linear trend = .040) and any territory (average aMED scores: 4.34 ± 1.70 , 4.37 ± 1.71 , 4.10 ± 1.6 , and 4.08 ± 1.73 among those with no plaques, 1–2, 3–4, or more adjusted *P* linear trend = .021). The number of plaques in carotid territories was not associated to MeDi adherence.

Conclusions

Main finding of this study is MeDi adherence induces a dose-dependent protection with presence, number, and thickness of plaques independent of other risk factors. The association was strongest for femoral arteries and among smokers. MeDi includes nutritional and, probably, other environmental components associated with the way the food is eaten.^{4,5,28}

Beyond food consumption, physical activity, adequate rest, sociability, and conviviality are the other factors comprised in the Mediterranean lifestyle definition.²⁸ Among traditional foods consumed by the Mediterranean population, olive oil, fruits, and vegetables are considered the most characteristic elements of MeDi. The mechanisms involved in CVD protection associated with MeDi have not been fully elucidated.⁵ Proposed mechanisms include the retardation of atherosclerosis development and progression through the improvement of CVD risk factors such as decrease in LDL cholesterol and blood pressure, or increase in HDL cholesterol. Other proposed mechanisms involve the stabilization of established atherosclerotic plaques that increase intravascular fibrosis, decrease in inflammation and cellular necrosis inside plaques, and preventing plaque rupture and thrombosis.^{7,29,30} Our study shows that a higher adherence to a MeDi is directly associated with lower presence and magnitude of atherosclerosis indicating that MeDi reduces CVD, at least in part, by preventing the development of atherosclerotic plaques. Interestingly, this atherosclerosis protection is independent of traditional risk factors like hypertension, dyslipidemia, or diabetes.

Reduction of atherosclerosis burden and progression as a mechanism of CVD prevention was previously described in the Northern Manhattan and the PREDIMED studies, respectively.^{11,12} The Northern Manhattan Study is an observational study, which included 1374 participants who show an inverse association between MeDi adherence and 75th percentile of plaque thickness and median of plaque, which remained significant after adjusting for mediators and risk factors.¹² As we showed in our study, they did not find any association between MeDi adherence and carotid IMT. The PREDIMED study is a randomized clinical trial exploring the effect of MeDi enriched with extra-virgin olive oil or nuts in CVD.^{8,29} They observed a carotid atherosclerosis regression, as IMT thinning, only in those subjects with a baseline IMT over 0.9 mm.¹¹ Together, the results of these different approaches suggest

that MeDi has a substantial influence in plaque development and progression.

An important finding in our study is the different relationships between MeDi and atherosclerosis plaques depending on the arterial location. This issue has not been analyzed previously, and we demonstrate that MeDi associated mainly with femoral and aortic plaques and rarely with carotid atherosclerosis. This is important because the femoral artery is the artery most frequently affected by atherosclerosis among different peripheral vascular sites.^{18,19,31} Previous studies have shown that both arteries differ regarding the type of arterial remodeling process as well as the morphologic and histologic traits of the plaques.^{32,33} It is unknown whether these differences could lead to different clinical implications.³⁴ Different studies have demonstrated that femoral atherosclerosis showed higher association with traditional risk factors and coronary calcium than carotid atherosclerosis. However, the value of femoral atherosclerosis in the prediction of CVD events is not completely elucidated.^{19,35} Mechanisms responsible for the different impact of risk factors depending on the type of artery are poorly understood. High LDL cholesterol is a major risk factor mainly for coronary disease.³⁶ Familial hypercholesterolemia (FH) is a paradigmatic example of this strong association because FH subjects suffer from premature coronary disease early in life but peripheral disease and ischemic stroke do not show any evident increased rate with respect to non-FH subjects.³⁷ In contrast, hypertension predisposes mainly to ischemic stroke and tobacco consumption to peripheral disease.³⁸ Thus, the association of MeDi with a specific territory would suggest that the beneficial effect on atherosclerosis may be related to the modulation of the impact of certain risk factors.

In agreement with the previous concept, we have found a protective role of MeDi in femoral territories only in smoker subjects. The protective effect of MeDi against smoking has been previously hypothesized based on experimental data.³⁹ Cigarette smoking has a high content of free radicals and other oxidants and plasma dietary antioxidants play a substantial role against this oxidative stress.⁴⁰ Among other important oxidative processes, smoking induces oxidation of atherogenic lipoproteins, and circulating products of lipid peroxidation and oxidized LDL particles are significantly increased in smokers.^{41,42} MeDi has demonstrated to inhibit this lipoprotein modification and contribute to atherosclerosis protection.⁴³ MeDi is rich in antioxidants with beneficial effects preventing lipoprotein modification, the formation of nitrolipid compounds, and modulating gene expression of proatherogenic genes.^{29,44,45} MeDi adherence is also characterized by higher consumption of omega-3 fatty acids with beneficial effects on lipoprotein oxidation, systemic inflammation, and membrane composition.⁴ Furthermore, omega-3 polyunsaturated fatty acids supplementation modifies HDL particle proteome in active smokers, increasing the HDL content of clusterin, paraoxonase, and apo AI. These are all well-defined antiatherogenic HDL proteins that are

usually reduced in smokers and suggest that these protein changes improve the HDL functionality.⁴⁶

Our study has some limitations including (1) the observational and cross sectional design, prevents assumptions regarding temporality and causality of the associations observed as well as potential residual confounding; (2) our sample include mainly men; so results cannot be extrapolated to women; and (3) the low variability in diet adherence due to homogenous data from FFQ and ties in diet score, which limit the full expression of the effects of diet. Strengths of this study include the large sample size; the use of a MeDi score that has been widely validated in our population based on an FFQ, which refers to dietary behavior over the previous year, designed to measure long-term dietary patterns; and the collection of clinical and state-of-the-art imaging data on multiple territories according to strict protocols and with homogeneous age range in this mainly male population that facilitates to control for the most important confounding factors.

In conclusion, our study is the first large observational epidemiologic study to examine the relationship between MeDi adherence and the presence and extent of subclinical atherosclerosis in different territories. Our results suggest that higher MeDi adherence is directly associated with a reduced burden of atherosclerosis in femoral arteries independent of other risk factors. This effect is mainly observed in current smokers in which MeDi lead to around 60% protection against plaque presence. The MeDi adherence showed a dose-dependent association with the number of plaques in any territory but mostly in femoral arteries. Higher MeDi adherence has a greater impact on smokers in femoral territories, which may suggest that the protective role of this dietary pattern against CVD by preventing oxidation of atherogenic lipoproteins.

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Authors' contributions: R.M.G. analyzed data, performed statistical analysis, wrote the article, and had primary responsibility for its final content; I.U. analyzed data and performed statistical analysis; B.M.F. conducted research and analyzed data; M.L.L. designed and conducted research and analyzed data; J.A.C. designed and conducted research and analyzed data; M.L. analyzed data and performed statistical analysis; J.L.P. and F.C. analyzed data, performed statistical analysis, wrote the article, and had primary responsibility for its final content. All authors have approved the final article.

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Supplemental Table 1 Association between aMED score and presence of plaque in different territories in non-smoker AWHs participants

		OR (95% CI)				P for trend
		Quartile 1	Quartile 2	Quartile 3	Quartile 4	
		0–2 points N = 100	3–4 points N = 228	5 points N = 114	6–9 points N = 142	
Carotid						
Age, gender-adjusted, N = 403/137*	Ref.	0.86 (0.49–1.53)	0.93 (0.48–1.79)	1.03 (0.56–1.91)	.761	
Multivariable-adjusted 1 [†] , N = 403/137*	Ref.	0.91 (0.51–1.63)	1.05 (0.54–2.06)	1.17 (0.61–2.24)	.472	
Multivariable-adjusted 2 [‡] , N = 383/127*	Ref.	1.01 (0.55–1.87)	1.01 (0.49–2.08)	1.18 (0.59–2.35)	.606	
Femoral						
Age, gender-adjusted, N = 356/174*	Ref.	0.99 (0.56–1.75)	1.25 (0.67–2.35)	1.55 (0.85–2.81)	.066	
Multivariable-adjusted 1 [†] , N = 356/174*	Ref.	1.01 (0.57–1.79)	1.30 (0.68–2.49)	1.60 (0.86–2.98)	.062	
Multivariable-adjusted 2 [‡] , N = 339/159*	Ref.	1.02 (0.55–1.87)	1.29 (0.64–2.59)	1.64 (0.84–3.19)	.070	
Aorta						
Age, gender-adjusted, N = 291/215*	Ref.	1.07 (0.64–1.80)	1.11 (0.62–2.00)	0.99 (0.56–1.75)	.930	
Multivariable-adjusted 1 [†] , N = 291/215*	Ref.	1.07 (0.63–1.81)	1.07 (0.58–1.95)	0.95 (0.53–1.71)	.795	
Multivariable-adjusted 2 [‡] , N = 273/205*	Ref.	1.12 (0.64–1.96)	1.11 (0.58–2.13)	1.02 (0.55–1.90)	.951	
Any territory						
Age, gender-adjusted, N = 195/368*	Ref.	1.29 (0.78–2.13)	1.50 (0.83–2.72)	1.34 (0.76–2.37)	.283	
Multivariable-adjusted 1 [†] , N = 195/368*	Ref.	1.29 (0.78–2.13)	1.49 (0.84–2.66)	1.34 (0.78–2.35)	.339	
Multivariable-adjusted 2 [‡] , N = 186/343*	Ref.	1.39 (0.82–2.38)	1.52 (0.81–2.88)	1.42 (0.7–2.61)	.321	

aMED, alternative MEDiterranean; AWHs, Aragon Workers' Health Study; CI, confidence interval; OR, odds ratio.

*N, Number of subjects without/with plaque.

†Adjusted by age, gender, energy intake, education level (less than high school, high school or some college, college degree, and other), tobacco consumption (non-smoker, current smoker, and former smoker), and physical activity.

‡Adjusted by age, gender, energy intake, education level (less than high school, high school or some college, college degree, and other), tobacco consumption (non-smoker, current smoker, and former smoker), physical activity, body mass index, low-density lipoprotein cholesterol, systolic and diastolic blood pressure, diabetes mellitus, C-reactive protein (imputed), and lipoprotein(a) (imputed).

Supplemental Table 2 Association between aMED score and presence of plaque in different territories in former smoker AWHs participants

	OR (95% CI)				<i>P</i> for trend
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
	0–2 points	3–4 points	5 points	6–9 points	
	N = 139	N = 393	N = 220	N = 260	
Carotid					
Age, gender-adjusted, N = 590/342*	Ref.	1.07 (0.68–1.67)	1.13 (0.70–1.84)	1.20 (0.75–1.92)	.388
Multivariable-adjusted 1 [†] , N = 589/342*	Ref.	1.07 (0.69–1.68)	1.17 (0.71–1.91)	1.22 (0.75–1.97)	.364
Multivariable-adjusted 2 [‡] , N = 539/317*	Ref.	1.22 (0.75–1.96)	1.39 (0.82–2.39)	1.43 (0.84–2.42)	.174
Femoral					
Age, gender-adjusted, N = 443/484*	Ref.	0.87 (0.57–1.34)	0.70 (0.44–1.12)	0.62 (0.39–0.97) [§]	.013
Multivariable-adjusted 1 [†] , N = 443/483*	Ref.	0.87 (0.56–1.34)	0.72 (0.45–1.16)	0.61 (0.38–0.97) [§]	.016
Multivariable-adjusted 2 [‡] , N = 407/442*	Ref.	0.96 (0.60–1.53)	0.79 (0.47–1.33)	0.75 (0.45–1.25)	.155
Aorta					
Age, gender-adjusted, N = 393/460*	Ref.	1.15 (0.74–1.76)	0.77 (0.45–1.15)	0.84 (0.54–1.32)	.136
Multivariable-adjusted 1 [†] , N = 393/459*	Ref.	1.20 (0.77–1.85)	0.80 (0.49–1.29)	0.98 (0.62–1.57)	.498
Multivariable-adjusted 2 [‡] , N = 356/433*	Ref.	1.34 (0.85–2.11)	0.90 (0.539–1.49)	1.07 (0.65–1.75)	.698
Any territory					
Age, gender-adjusted, N = 210/778*	Ref.	1.15 (0.70–1.89)	0.74 (0.44–1.26)	0.85 (0.51–1.43)	.215
Multivariable-adjusted 1 [†] , N = 210/777*	Ref.	1.17 (0.70–1.94)	0.80 (0.47–1.38)	0.92 (0.53–1.57)	.391
Multivariable-adjusted 2 [‡] , N = 184/719*	Ref.	1.34 (0.78–2.29)	1.02 (0.57–1.84)	1.15 (0.65–2.06)	.984

aMED, alternative MEDiterranean; AWHs, Aragon Workers' Health Study; CI, confidence interval; OR, odds ratio.

*N, Number of subjects without/with plaque.

†Adjusted by age, gender, energy intake, education level (less than high school, high school or some college, college degree, and other), tobacco consumption (non-smoker, current smoker, and former smoker) and physical activity.

‡Adjusted by age, gender, energy intake, education level (less than high school, high school or some college, college degree, and other), tobacco consumption (non-smoker, current smoker, and former smoker), physical activity, body mass index, low-density lipoprotein cholesterol, systolic and diastolic blood pressure, diabetes mellitus, C-reactive protein (imputed), and lipoprotein(a) (imputed).

§*P* < .050 with respect to first quartile.

Supplemental Table 3 Association of Mediterranean diet-score components (for the comparison of participants who received a point associated with the component with participants who did not) with presence of plaque in each territory in AWHs participants*

Presence of plaque	Fruits	Vegetables	Nuts	Legumes	Whole grains	Red meat	Fish	Ratio MFA/SFA	Alcohol
Carotid, N = 1354/751 [†]	0.985 (0.812-1.193)	1.106 (0.915-1.336)	1.050 (0.868-1.269)	1.021 (0.834-1.250)	0.954 (0.783-1.162)	1.309 (1.065-1.609) [‡]	1.038 (0.860-1.254)	1.044 (0.859-1.267)	1.024 (0.851-1.234)
Femoral, N = 1036/1249 [†]	0.754 (0.621-0.915) [‡]	0.938 (0.775-1.135)	0.902 (0.745-1.092)	1.061 (0.864-1.302)	0.869 (0.714-1.059)	0.954 (0.774-1.174)	0.993 (0.821-1.201)	0.824 (0.677-1.004)	0.855 (0.708-1.032)
Aorta, N = 896/1022 [†]	0.952 (0.787-1.152)	1.060 (0.878-1.280)	1.118 (0.927-1.349)	0.952 (0.779-1.163)	0.952 (0.784-1.156)	0.976 (0.797-1.196)	1.029 (0.854-1.241)	0.804 (0.663-0.976) [*]	0.797 (0.662-0.960) [*]
Any territory N = 465/1741 [†]	0.958 (0.767-1.197)	1.118 (0.897-1.393)	1.046 (0.840-1.303)	0.973 (0.772-1.226)	0.894 (0.715-1.119)	0.876 (0.691-1.111)	1.117 (0.898-1.390)	0.806 (0.643-1.011)	0.775 (0.623-0.963) [*]

AWHS, Aragon Workers' Health Study.

*All values are adjusted odds ratio (95% confidence intervals). Adjustment done by age, gender, energy intake, education level (less than high school, high school or some college, college degree, and other), tobacco (non-smoker, current smoker, and former smoker), physical activity, body mass index, low-density lipoprotein cholesterol, systolic and diastolic blood pressure, diabetes mellitus, C-reactive protein (imputed), and lipoprotein(a) (imputed). In women, 1 point was awarded for fruit intake ≥ 1.8 servings/d, vegetable intake ≥ 2.4 servings/d, nut intake ≥ 0.1 serving/d, legume intake ≥ 0.2 servings/d, whole-grain intake ≥ 0.6 servings/d, fish intake ≥ 0.2 servings/d, red meat intake ≤ 0.4 servings/d, ratio of monounsaturated fat to saturated fat ≥ 1.2 , and alcohol intake between 5 and 25 g/d. In men, 1 point was awarded for fruit intake ≥ 1.4 servings/d, vegetable intake ≥ 2.1 servings/d, nut intake ≥ 0.1 serving/d, legume intake ≥ 0.2 servings/d, whole-grain intake ≥ 0.6 servings/d, fish intake ≥ 0.2 servings/d, red meat intake ≤ 0.6 servings/d, ratio of monounsaturated fat to saturated fat ≥ 1.2 , and alcohol intake between 10 and 50 g/d. MFA denotes monounsaturated fatty acids and SFA denotes saturated fatty acids.

[†]N, Number of subjects without/with plaque.

[‡]Significantly different in logistic regression models (reference category is 0), $P < .05$.

Supplemental Table 4 Adjusted mean and maximum IMT and maximum plaque thickness according to aMED quartiles*

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	Adjusted <i>P</i> for trend [†]
	0–2 points	3–4 points	5 points	6–9 points	
	N = 434	N = 1015	N = 535	N = 582	
Carotid artery, N = 2396					
Mean IMT, mm	0.69 ± 0.12	0.69 ± 0.12	0.68 ± 0.12	0.69 ± 0.11	.329
Maximum IMT, mm	0.90 ± 0.29	0.88 ± 0.30	0.87 ± 0.29	0.89 ± 0.25	.650
Maximum plaque thickness, mm	2.08±0.68 [‡]	1.99±0.53 [‡]	1.89±0.51 [‡]	1.90±0.45 [‡]	.027
Femoral artery, N = 2396					
Mean IMT, mm	0.93 ± 0.49	0.90 ± 0.49	0.90 ± 0.48	0.85 ± 0.44	.035
Maximum IMT, mm	1.12 ± 0.66	1.08 ± 0.63	1.07 ± 0.62	1.01 ± 0.59	.025
Maximum plaque thickness, mm	2.32±0.72 [§]	2.24±0.71 [§]	2.28±0.66 [§]	2.21±0.65 [§]	.566

*Data expressed as mean ± standard deviation.

[†]*P* for trend adjusted by age, gender, energy intake, education level (less than high school, high school or some college, college degree, and other), body mass index, tobacco consumption (non-smoker, current smoker, and former smoker), low-density lipoprotein cholesterol, systolic and diastolic blood pressure, diabetes mellitus, physical activity, C-reactive protein (imputed), and lipoprotein(a) (imputed).

[‡]N for these groups are 286 (Quartile 1), 174 (Quartile 2), 181 (Quartile 3), and 202 (Quartile 4).

[§]N for these groups are 508 (Quartile 1), 274 (Quartile 2), 273 (Quartile 3), and 277 (Quartile 4).

4th PUBLICATION:

**The additive effect of adherence to multiple healthy lifestyles on subclinical atherosclerosis:
insights from the AWHs study.
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GENERAL INTRODUCTION / INDIVIDUAL'S CONTRIBUTION

It was seen from the previous article that a higher adherence to MDP is associated with a lower risk of presenting plaque in femoral arteries. In order to further investigate the scope of the benefits of high compliance with MDP and how it interrelates with other lifestyle factors, I aimed to study the association between the highest tertile of adherence to MDP combined with non-smoking, moderate alcohol consumption, no abdominal adiposity, and decreased sedentarism and presence of plaques in femorals, carotids, increased CACs and subclinical atherosclerosis.

Following exactly the same approach as in the previous article I used an “*a-priori*” method of dietary pattern analysis, in particular aMED score to measure the adherence to MDP and have identified the following categories of lifestyle variables: non-drinkers/excess drinkers vs moderate alcohol consumers; current smokers vs non-smokers/former smokers; spending more vs less than 5.5 hours a day sitting; aMED score from 0 to 4 vs aMED score from 5 to 9; and waist circumference (WC) > 102 vs $WC \leq 102$ cm. Various combinations of lifestyle factors with aMED score were used to study a synergetic effect. First, I performed a backward stepwise logistic regression to determine demographic, lifestyle and dietary factors related with subclinical atherosclerosis and its markers.

Taking into account highly prevalent nature of the disease outcome, I estimated the association between the adherence to aMED combined with other lifestyle factors and presence of atherosclerotic plaque in femorals, carotids, increased CACs, and subclinical atherosclerosis using Poisson regression analysis with robust estimates, rather than classic logistic regression which recently has been criticized for overestimating the association in this case (Knol *et al.*, 2012).

Original Article

The additive effect of adherence to multiple healthy lifestyles on subclinical atherosclerosis: Insights from the AWHs

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KEYWORDS:

Healthy lifestyle;
Atherosclerosis;
Coronary artery calcium;
Atherosclerotic plaque

BACKGROUND: Public health strategies targeting multiple healthy behaviors, rather than individual factors, have been proposed as more efficient strategies to promote cardiovascular health. However, the additive effect of multiple targets on primary prevention has not been fully characterized.

OBJECTIVE: To examine how adherence to multiple healthy behaviors is associated with the presence of subclinical atherosclerosis, a measure of early cardiovascular disease.

METHODS: Analysis of a baseline data from 1798 middle-aged men from the Aragon Workers Health Study conducted between 2009 and 2010. Healthy behaviors were defined according to American Heart Association recommendations, aligned with Spanish Nutritional recommendations and included moderate alcohol consumption, smoking abstinence, no abdominal adiposity, decreased sedentarism, and adherence to Alternate Mediterranean Dietary Index. Presence of coronary artery calcium and plaques in femoral and carotid was quantified by a 16-slice computed tomography scanner and 2D ultrasound.

RESULTS: Moderate alcohol consumption, as well as adherence to Mediterranean diet is independently associated with a 6% lower risk of having subclinical atherosclerosis. Smoking abstinence is associated with a 11% lower risk of subclinical atherosclerosis. Those who follow 3 lifestyle behaviors (Mediterranean diet, nonsmoking, and moderate alcohol intake) have 18% lower odds of

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presenting subclinical atherosclerosis compared with those who do not follow these protective lifestyle habits.

CONCLUSION: Adoption of multiple healthy lifestyle behaviors early in life could be a key strategy to tackle the onset of atherosclerosis and reduce cardiovascular disease burden.

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Introduction

Atherosclerosis is directly related to coronary heart disease (CHD) and affects a relatively young population.¹ American Heart Association considered CHD preventable through the lifestyle modifications such as limitation in alcohol consumption, smoking cessation, and physical activity, adherence to healthy diet, healthy weight maintenance, reduced stress, and control of blood cholesterol, blood pressure, and glucose levels.²

Studies have shown that unhealthy behaviors are prevalent, with most adults exhibiting more than 1 at a time in what is called a cluster of unhealthy lifestyles.³ The US National Health Survey of 16,818 adults revealed that a combination of at least 2 of the following lifestyle behaviors—excessive alcohol, cigarette smoking, overweight or physical inactivity—was prevalent among half of the US population.⁴ Thus, by addressing not only individual behavioral risk factors, but rather their combined effect, intervention strategies for cardiovascular health promotion could enhance their effectiveness.

Few studies have investigated the combined effect of adherence to multiple lifestyle factors on cardiovascular disease (CVD) mortality. Thus, Odegaard et al reported that those having at least 5 protective lifestyle factors (dietary pattern, physical activity, alcohol intake, usual sleep, smoking status, and body mass index) had one-fourth of the risk of CVD mortality compared to those with no protective lifestyle factors.⁵ In another study by Knuops et al, the authors estimated that lack of adherence to healthy lifestyle consisted of Mediterranean dietary pattern, moderate alcohol consumption, physical activity, and being nonsmoker accounted for a 64% of deaths from CHD and 61% from CVD.⁶ In terms of assessing how those lifestyle factors are associate with the early stage of the CHD most previous studies investigated the relationship between the combination of lifestyle behaviors and coronary artery calcification (CAC),^{7–9} or intima-media thickness (IMT)^{10,11} as markers of subclinical atherosclerosis.¹² CAC score (CACS) is considered a major predictor of CHD,¹³ a score of 0 being associated with only a 1% probability of having a cardiac event,¹⁴ whereas an elevated score, above 0, has been shown to be associated with a 10-fold increase in the risk of a cardiac event.¹³ Noteworthy, the limitation of CACS as a marker of atherosclerosis is that it can only be identified at late stages of disease progression, which makes it inadequate for its use in asymptomatic individuals. With respect to IMT, some studies have suggested a weak correlation with coronary atherosclerosis.¹⁵ To date, there are a limited number of studies exploring the

association between direct indicators of atherosclerosis, such as the presence of atherosclerotic plaques, and adherence to lifestyle behaviors. Moreover, most of them focus on atherosclerotic plaques located in carotid arteries. Assessment of femoral plaque compared with carotid might potentially become a useful tool in predicting CHD as it has been shown to be better correlated with traditional CVD risk factors.¹⁶ Therefore, in this study we aim to explore the relationship between adherence to multiple healthy lifestyle behaviors and presence of plaques in the femoral artery as well as other markers of subclinical atherosclerosis.

Methods

Study participants

The Aragon Workers Health Study (AWHS) is a longitudinal cohort study aiming to investigate the determinants of the development and progression of metabolic abnormalities and subclinical atherosclerosis among CVD-free middle-aged population. The participants were recruited between February 2009 and December 2010. The details of the study are described in detail elsewhere.¹⁷ The present study was performed on a sample of 1933 participants aged 40–55 y with complete baseline dietary, lifestyle and imaging data. Owing to the low percentage of females in AWHS and recognizing the differential effect of gender on health behaviors, we excluded female participants (N = 135, 0.5% of the sample). The final sample available for analysis was of 1798. The Central Institutional Review Board of Aragón approved the study, and written informed consent was obtained from all study participants.

Assessment of lifestyle behaviors

Information on lifestyle behaviors were collected using food frequency questionnaire and physical activity questionnaires previously validated for Spain.^{18,19} Dietary energy, macronutrient and micronutrient intakes were derived using the Spanish food composition tables.^{20,21} The variables used in the analyses were defined as follows: (1) alcohol: taking into account that there is no universal definition for moderate alcohol consumption, we have examined the evidence from several studies,^{22–24} dietary guidelines for Spanish population, which includes optional moderate alcohol intake in the form of wine or beer,²⁵ recommendations given by American Heart Association,²⁶ and considered the following categories for alcohol

consumption: nondrinkers or excess drinkers (more than 30 grams of ethanol/d) vs moderate drinkers (no more than 30 grams and not less than 10 grams of ethanol/d); (2) smoking: nonsmokers combined with ex-smokers vs current smokers; (3) physical activity: as a proxy for a lack of physical activity we used the sitting time (h/d). Two categories were used—spending less vs more than 5.5 hours a day sitting. Cutoff was based on the First tertile of sitting time as well as previous work by the previously published work by Dr Leon-Latre within AWHs cohort, where it was observed that those who on average spend 5.5 h/d and less sitting had lower glucose, and c-reactive protein, whereas those who is being sedentary for 5.5 h/d and more had increased body mass index (BMI), waist circumference (WC), and insulin level²⁷; (4) diet: we used the Alternate Mediterranean Dietary Index (aMED) score.²⁸ Two categories were used: aMED score from 0 to 4 vs aMED score from 5 to 9 (cutoff based on the median of the sample). Healthy lifestyle behaviors were defined as follows²⁶: moderate alcohol consumption, nonsmoking, normal WC (≤ 102 cm),²⁹ sitting time less than 5.5 h/d, and aMED score higher than 5. The variables for combined lifestyle analysis were considered as follows: (1) aMED score more than 5 and moderate alcohol consumption vs aMED score less than 5 and nondrinkers/excess drinkers (category used as a reference); (2) aMED score more than 5, moderate alcohol consumption, and nonsmoking vs aMED score less than 5, nondrinkers or excess drinkers, and current smoking (category used as a reference); (3) aMED score more than 5, moderate alcohol consumption, nonsmoking, and no abdominal adiposity vs aMED score less than 5, nondrinkers or excess drinkers, current smoking, and abdominal adiposity (category used as a reference); (4) aMED score more than 5, moderate alcohol consumption, nonsmoking, no abdominal adiposity, and sitting time less than 5.5 h/d vs aMED score less than 5, nondrinkers or excess drinkers, current smoking, abdominal adiposity, and sitting time more than 5.5 h/d (category used as a reference).

Assessment of subclinical atherosclerosis

A 16-slice computed tomography scanner (Philips Brilliance, Philips Healthcare, Andover, MA) with noncontract prospective electrocardiography-gated acquisition and Agatston equation was used to quantify CACS.³⁰ The following categories of CACS were computed: CACS = 0 and CACS >0.³¹ The assessment of atherosclerotic plaques was performed in bilateral carotid and femoral arteries using 2D ultrasound (Philips iU22 ultrasound, Philips Healthcare, Bothell, Washington) by a cross-sectional sweep of aforementioned territories. Plaque was defined as a “focal structure that protrudes into the lumen of the carotid artery at least 0.5 mm or $\geq 50\%$ thicker than the surrounding IMT”.³² Presence of subclinical atherosclerosis was defined as the presence of at least 1 plaque in any of the territories and/or CACS >0.

Clinical, anthropometric, and laboratory data and other variables

Protocols for data collection are described in detail elsewhere.¹⁷ The data on medication use, hospitalization occurred over a past year, anthropometric measurements, blood pressure, and heart rate were collected at the clinical examination. Samples of fasting blood and urine were collected and stored for laboratory analysis. Data on sociodemographic and lifestyle variables were self-reported and included age, level of complete education, working shifts, smoking status, and sitting time. Traditional CVD risk factors were defined according to European guidelines as follows: blood pressure $\geq 140/90$ mm Hg (130/80 in participants with diabetes) or current use of antihypertensive medication determined the presence of hypertension³³; total cholesterol ≥ 190 mg/dL (4.9 mmol/L) or use of medication determined a presence of hypercholesterolemia³⁴; a fasting serum glucose ≥ 126 mg/dL (7.0 mmol/L), or a HbA1c $\geq 6.5\%$ or use of medication determined the presence of diabetes.³⁵

Statistical analysis

T-test for continuous variables and chi-square test for categorical variables were used to compare the data between categories. The major demographic, lifestyle, and dietary factors related to subclinical atherosclerosis and its markers were identified by backward stepwise logistic regression. Each block was run independently and the results of the model were unadjusted. Multivariate adjusted Poisson regression models with robust estimates were used to assess the association between lifestyle behaviors (assessing its individual as well as additive effects) and presence of plaques, CACS, and subclinical atherosclerosis. The inclusion of covariates in the model was based on the assessment of sociodemographic, clinical, and lifestyle variables. Those variables that significantly differed between the groups in the descriptive analysis ($P < .05$) as well as significantly contributed to the presence of the atherosclerosis and its markers identified by backward stepwise logistic regression model were included in the model. The fully adjusted model included age (years), education (incomplete high school degree/high school degree/college degree), hypertension (yes/no), hypercholesterolemia (yes/no), and diabetes (yes/no). The number of lifestyle behaviors (cont.) and presence of subclinical atherosclerosis was assessed by fully adjusted Poisson regression models with robust estimates. All statistical analyses were undertaken using SPSS v. 23 (IBM Corp., Armonk, NY).

Results

Participants presenting at least one plaques in carotid or femoral territories and increased CACS were more likely to be older, have lower educational level, being current smokers, present hypercholesterolemia, diabetes,

hypertension, as well as increased BMI and WC. Accordingly, these participants tended to have higher levels of total cholesterol, low-density lipoprotein cholesterol, triglycerides, apolipoprotein B, HbA1c, HOMA-IR, glucose and insulin level, as well as lower apolipoprotein A1 and high-density lipoprotein cholesterol levels (Table 1). Moreover, they were less likely to engage in moderate to vigorous physical activity, more likely to have increased alcohol consumption, and smoke (16–17 cigarettes a day on average). However, those participants presenting plaque in the carotids had a higher adherence to Mediterranean dietary pattern, whereas those with plaques in the femorals, or CACS >0 were more likely to score lower on aMED scale.

The main factors related to the presence of subclinical atherosclerosis were mainly increased age, and traditional CVD risk factors, such as hypertension, hypercholesterolemia, and diabetes (Table 2). An age increase was associated with significant increase in the risk of having plaque in carotids, femorals, CACS >0, and subclinical atherosclerosis ($P < .001$). Higher education status was significantly associated with 24% ($P = .013$) lower risk of plaque in femorals ($P < .001$). Among traditional CVD risk factors, smoking, and hypertension were the main factors related to the presence of subclinical atherosclerosis (odds ratio [OR] = 3.41, 95% confidence interval [CI]: 2.29; 5.06; OR = 3.62, 95% CI: 2.32; 5.63). Hypertension was the main factor related to the presence of plaque in carotids and CACS >0 (OR = 1.94, 95% CI: 1.57; 2.41; OR = 2.08, 95% CI: 1.68; 2.58), whereas smoking determined the presence of plaque in femorals (OR = 3.13, 95% CI: 2.53; 3.88). Assessment of the dietary factors showed that presence of plaque in femorals is less likely to be related to increased fruits (OR = 0.63, 95% CI: 0.52, 0.77), whole grains consumption (OR = 0.72, 95% CI: 0.60, 0.86), and higher MUFA/SFA ratio (OR = 0.79, 95% CI: 0.65, 0.96). Excessive alcohol consumption was the main determinant of subclinical atherosclerosis (OR = 1.63, 95% CI: 1.18; 2.25) and its markers (OR_{femorals} = 1.47, 95% CI: 1.21; 1.78; OR_{carotids} = 1.39, 95% CI: 1.14; 1.70; OR_{CACS >0} = 1.48, 95% CI: 1.22; 1.80).

The analysis of the individual lifestyle factors (Table 3) showed that being nonsmoker significantly associated with a lower risk of having atherosclerosis in all territories (prevalence ratio [PR]_{femorals}: 0.64; 95% CI: 0.60; 0.69; PR_{carotids}: 0.81; 95% CI: 0.71; 0.91; PR_{CACS >0}: 0.82; 95% CI: 0.73; 0.92; PR_{subatherosclerosis}: 0.89; 95% CI: 0.86; 0.93). Adherence to Mediterranean diet is associated with lower risk of having plaque in femorals (PR: 0.84; 95% CI: 0.78; 0.92) and decreased odds of presenting subclinical atherosclerosis (PR: 0.94; 95% CI: 0.91; 0.99); while moderate alcohol consumption is associated with lower risk of having atherosclerosis in all territories (PR_{femorals}: 0.90; 95% CI: 0.83; 0.98; PR_{CACS >0}: 0.84; 95% CI: 0.74; 0.95; PR_{subatherosclerosis}: PR: 0.94; 95% CI: 0.89; 0.98), except for having a plaque in carotid.

The analysis of the association between potential synergic effects of lifestyle factors and disease revealed that

the combination of an adherence to Mediterranean dietary pattern, moderate alcohol intake, and being nonsmoker is associated with 47% ($P < .001$), 35% ($P .01$), and 18% ($P < .01$) lower risk of having femoral plaque, CACS >0, and subclinical atherosclerosis. Complying with any 3 out of 5 healthy lifestyle behaviors is associated with 16% lower prevalence of subclinical atherosclerosis (PR = 0.84, 95% CI = 0.73, 0.99, $P < .05$) (Fig. 1).

Discussion

In this analysis, we demonstrated that adherence to multiple healthy lifestyles has an additive effect on subclinical atherosclerosis measured by direct quantification of the presence of plaques. Consumption of alcohol in moderation, being nonsmoker, and an adherence to a Mediterranean dietary pattern, was associated with a 47% lower prevalence of plaques in the femoral territory, 35% lower risk of CACS >0, and overall to an 18% lower prevalence of subclinical atherosclerosis.

In our study, the moderate alcohol consumption was observed to be associated with lower risk of having plaque in femorals, CACS, and subclinical atherosclerosis. It has been shown that moderate alcohol intake is associated with a decline in fibrinogen, and lipoprotein (a) and increase in apolipoprotein A1 and high-density lipoprotein concentrations,³⁶ which together might promote an antiatherogenic effect. Prospective study reported a J-shaped relationship between alcohol intake and CVD mortality and showing that moderate alcohol consumers had lower risk than either abstainers or heavy drinkers.³⁷ Our findings also support these results to some extent. Once abstainers were included in the category of moderate drinking behavior we observed that the risk of having CACS >0 increased by 4%, whereas the risk of subclinical atherosclerosis increased by 3% and the results became nonsignificant (Supplemental Table 1). Therefore, we could assume that alcohol abstainers might have a slightly higher risk of subclinical atherosclerosis compared with moderate alcohol consumers. Cultural aspects as well as the type of alcohol typically consumed in the area where the study was conducted might be an explanation for the observed protective effect of alcohol. In Spain, one of the major types of alcohol typically consumed is wine, which is known for its cardiovascular health-promoting benefits. Another aspect is that its consumption traditionally takes place mostly during the meal, which in some studies has been observed to offer more protection against atherosclerotic disease.³⁸ Surprisingly, the risk of having a plaque in the carotid arteries significantly decreased by 16% in the fully adjusted model, once alcohol abstainers were included in the category of moderate drinkers. These findings could be partially explained by the dietary behavior of alcohol abstainers, as they were observed to have the lowest intake of sodium, processed meat, together with the highest intakes of fiber and fruits. Although the cardioprotective effect of higher fiber and

Table 1 Baseline characteristics of AWHS male population presented by the presence of plaque in femoral and carotid territories and CACS

	Plaque in femorals		Plaque in carotids		CACS		Plaque and CACS	
	No plaque N = 834	≥1 plaque N = 1115	No plaque N = 1238	≥1 plaque N = 709	CACS = 0 N = 1190	CACS >0 N = 759	No plaque and CACS = 0 N = 232	≥1 plaque and CACS >0 N = 556
Baseline characteristics								
Sociodemographics								
Age (y)	50.8 ± 3.98	52.2 ± 3.41*	51.0 ± 3.92	52.6 ± 3.13*	50.9 ± 3.95	52.7 ± 3.04*	50.0 ± 4.14	52.6 ± 3.07*
Education level								
< High school degree	405 (48.6)	617 (55.4)	619 (50.0)	403 (56.8)	592 (49.8)	430 (56.7)	99 (42.7)	315 (56.7)
High school degree	76 (9.10)	130 (11.7)	141 (11.4)	65 (9.20)*	126 (10.6)	80 (10.5)	28 (12.1)	56 (10.1)
College degree	43 (5.20)	34 (3.10)*	56 (4.50)	21 (3.00)*	50 (4.20)	27 (3.60)	15 (6.50)	20 (3.60)*
Working shift								
Day shift	67 (8.00)	62 (5.60)	83 (6.70)	46 (6.50)	72 (6.10)	57 (7.50)	20 (8.60)	41 (7.40)
Rotation shift	691 (82.9)	927 (83.1)	1038 (83.8)	578 (81.5)	1002 (84.2)	616 (81.2)	191 (82.4)	450 (80.9)
Night shift	76 (9.10)	126 (11.3)	117 (9.50)	85 (12.0)	116 (9.70)	86 (11.3)	21 (9.10)	65 (11.7)
Traditional CVD factors								
Hypercholesterolemia, n (%)	633 (82.3)	909 (88.3)*	951 (83.5)	590 (89.8)*	919 (84.4)	623 (87.9)*	172 (80.0)	458 (88.1)*
Total cholesterol (mg/dL)	220 ± 36.0	223 ± 35.6*	220 ± 35.6	225 ± 36.0*	221 ± 35.6	223 ± 36.1	216 ± 35.0	224 ± 35.9*
LDL cholesterol (mg/dL)	137 ± 31.5	139 ± 32.7	137 ± 31.7	141 ± 32.9*	138 ± 31.3	139 ± 33.6	133 ± 31.0	140 ± 33.8*
HDL cholesterol (mg/dL)	54.4 ± 11.5	51.2 ± 10.8*	53.3 ± 11.7	51.3 ± 10.2*	53.2 ± 11.7	51.6 ± 10.4*	54.3 ± 10.9	51.6 ± 10.2*
Triglycerides (mg/dL)	142 ± 88.3	164 ± 106*	150 ± 99.4	164 ± 99.0*	150 ± 97.7	162 ± 102*	142 ± 93.6	161 ± 102*
Apolipoprotein A1	147 ± 19.4	142 ± 18.2*	145 ± 19.3	143 ± 17.9*	145 ± 19.0	142 ± 18.5*	146 ± 17.4	142 ± 18.4*
Apolipoprotein B	103 ± 22.6	108 ± 22.0*	104 ± 22.3	109 ± 22.3*	104 ± 22.2	108 ± 22.4*	99.0 ± 23.3	110 ± 22.6*
Apolipoprotein A1/ apolipoprotein B	0.71 ± 0.19	0.77 ± 0.19*	0.73 ± 0.19	0.77 ± 0.19*	0.73 ± 0.19	0.77 ± 0.19*	0.69 ± 0.19	0.79 ± 0.19*
Hypertension, n (%)	176 (22.9)	333 (32.4)*	263 (23.1)	246 (37.4)*	239 (21.9)	270 (38.1)*	29 (13.5)	177 (34.0)*
SBP (mm Hg)	123 ± 12.9	128 ± 14.7*	124 ± 13.7	128 ± 14.5*	124 ± 13.3	128 ± 15.0*	121 ± 11.6	127 ± 14.5*
DBP (mm Hg)	81.9 ± 9.09	83.9 ± 9.52*	82.4 ± 9.37	84.2 ± 9.33*	82.5 ± 9.24	83.9 ± 9.55*	80.7 ± 8.45	83.7 ± 9.20*
Diabetes, n (%)	27 (3.50)	77 (7.50)*	49 (4.30)	55 (8.40)*	38 (3.50)	66 (9.30)*	7 (3.30)	41 (7.90)*
Glucose (mg/dL)	97.9 ± 16.1	98.8 ± 18.4	97.5 ± 16.0	100 ± 19.6*	97.4 ± 15.6	100 ± 19.9*	98.6 ± 20.7	98.9 ± 17.6
Insulin (µU/mL)	7.38 ± 5.44	8.25 ± 5.96*	7.60 ± 5.68	8.35 ± 5.88*	7.66 ± 5.54	8.20 ± 6.06	7.20 ± 5.37	7.74 ± 5.75
HOMA_IR	1.86 ± 1.59	2.09 ± 1.82*	1.90 ± 1.69	2.14 ± 1.78*	1.90 ± 1.59	2.13 ± 1.91*	1.84 ± 1.65	1.99 ± 1.76
HOMA_B	81.8 ± 69.9	93.5 ± 80.0*	86.4 ± 74.5	92.1 ± 78.5	87.4 ± 70.4	90.0 ± 83.7	76.2 ± 48.4	83.3 ± 65.4
HbA1c	5.49 ± 0.49	5.61 ± 0.60*	5.52 ± 0.52	5.61 ± 0.62*	5.52 ± 0.53	5.61 ± 0.59*	5.45 ± 0.59	5.59 ± 0.57*
Abdominal obesity, n (%)	227 (28.3)	356 (32.8)*	341 (28.7)	242 (34.8)*	329 (28.7)	254 (34.3)*	54 (24.3)	158 (29.2)
Waist circumference (cm)	96.9 ± 9.12	98.3 ± 8.82*	97.1 ± 8.84	98.7 ± 9.12*	97.1 ± 8.90	98.6 ± 9.00*	95.5 ± 9.07	97.6 ± 8.61*
Body mass index (BMI) (kg/m ²)	27.6 ± 3.41	27.9 ± 3.33	27.6 ± 3.25	28.1 ± 3.55*	27.6 ± 3.34	28.1 ± 3.38*	27.1 ± 3.11	27.8 ± 3.26*
30 > BMI ≥ 25	464 (56.0)	640 (57.5)	700 (56.8)	404 (57.1)	657 (55.4)	447 (59.0)*	128 (55.4)	341 (61.4)*
BMI ≥ 30	186 (22.4)	264 (23.7)	273 (22.2)	176 (24.9)	263 (22.2)	187 (24.7)*	43 (18.6)	118 (21.3)*
Lifestyle								
MPA (h/wk)	3.76 ± 5.23	2.84 ± 4.67*	3.45 ± 5.12	2.86 ± 4.58*	3.35 ± 4.96	3.06 ± 4.90	3.86 ± 5.28	3.03 ± 4.85*
VPA (h/wk)	1.11 ± 2.89	0.84 ± 2.55*	1.05 ± 2.84	0.79 ± 2.44*	1.00 ± 2.75	0.90 ± 2.63	1.36 ± 3.10	0.93 ± 2.68*

(continued on next page)

Table 1 (continued)

Baseline characteristics	Plaque in femorals		Plaque in carotids		CACS		Plaque and CACS	
	No plaque N = 834	≥1 plaque N = 1115	No plaque N = 1238	≥1 plaque N = 709	CACS = 0 N = 1190	CACS >0 N = 759	No plaque and CACS = 0 N = 232	≥1 plaque and CACS >0 N = 556
Sitting time (h/d)	6.07 ± 1.59	6.29 ± 1.61*	6.10 ± 1.58	6.36 ± 1.64*	6.13 ± 1.59	6.29 ± 1.62*	5.94 ± 1.48	6.31 ± 1.62*
Nonsmokers, n (%)	270 (33.8)	144 (13.6)*	307 (25.9)	107 (16.0)*	281 (24.9)	133 (18.3)*	84 (37.5)	92 (17.2)*
Active smokers, n (%)	178 (22.3)	489 (46.2)*	394 (33.2)	272 (40.6)*	379 (33.6)	288 (39.6)*	41 (18.3)	221 (41.3)*
Former smokers, n (%)	350 (43.9)	426 (40.2)*	484 (40.8)	291 (43.4)*	469 (41.5)	307 (42.2)*	99 (44.2)	222 (41.5)*
Cigarettes per day	13.2 ± 10.8	16.3 ± 11.5*	14.1 ± 10.6	17.1 ± 12.2*	14.4 ± 10.9	16.6 ± 11.9*	13.8 ± 11.1	17.0 ± 11.8*
Alcohol intake (g/d)	20.1 ± 19.4	23.5 ± 21.6*	20.6 ± 19.6	24.6 ± 22.3*	20.7 ± 19.6	24.2 ± 22.3*	20.2 ± 18.1	23.7 ± 22.0*
aMED score	4.41 ± 1.72	4.03 ± 1.69*	4.19 ± 1.71	4.21 ± 1.72	4.26 ± 1.71	4.10 ± 1.70*	4.45 ± 1.82	4.11 ± 1.75*

VPA, vigorous physical activity; SBP, systolic blood pressure; DBP, diastolic blood pressure; MPA, moderate physical activity; aMED, alternate Mediterranean diet index; CACS, coronary artery calcification score; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Abdominal obesity is defined as waist circumference > 102 cm.

Data is presented as n (%) or mean ± SD.

*Significant difference between categories ($P < .05$; P value was obtained using t-test for continuous variables and chi-square test for categorical variables).

fruit intakes and salt restriction is well known, processed meat consumption on a regular basis was recently shown to be associated with the slightly higher risk of developing heart disease.³⁹ Not only healthier dietary behavior, but lower plasma concentration of triglycerides together with decreased systolic and diastolic blood pressure observed among this group of participants might have resulted in a beneficial effect of alcohol restriction on the presence of an atherosclerotic plaque in carotid arteries.

Adherence to a Mediterranean dietary pattern in our study was significantly associated with a lower prevalence of plaques in the femoral arteries and subclinical atherosclerosis, but not with CACS, which was rather unexpected taking into account the fact that there is extensive evidence demonstrating the beneficial role of a Mediterranean diet in prevention of CVD.⁴⁰ We hypothesized that a lack of an association with CACS be due to the homogeneity of the dietary habits of our sample, as it was observed in our previous research.⁴¹ Interestingly, participants with at least 1 plaque in the carotid arteries were observed to have better adherence to a Mediterranean dietary pattern as well as higher total cholesterol concentration, low-density lipoprotein cholesterol, increased presence of abdominal obesity, excessive alcohol consumption, and being current smokers. Thus, we could hypothesize that these participants have changed their diet during the past year to address the health-related issues, and therefore, we see the lack of the association with respect to diet, or those factors mentioned previously have overcome a cardioprotective effect of an adherence to a Mediterranean dietary pattern.

Participants presenting at least 3 of 5 cardioprotective behaviors including moderate alcohol consumption, smoking abstinence, and adherence to a Mediterranean diet had an 18% lower odds of presenting subclinical atherosclerosis. Our findings are in line with MESA study, which reported a 46% lower risk of having increased CAC in those who never smoked, maintained a healthy body weight, performed >150 min/wk of moderate or >75 min/wk of vigorous physical activity, and followed a Mediterranean dietary pattern.⁴² Another prospective cohort showed that an overall healthy behavior including moderate alcohol consumption along with smoking abstinence, presence of WC <95 cm, daily physical activity (walking/bicycling ≥40 min/d and exercising ≥1 h/wk), and presenting higher Recommended Food Score is responsible for the prevention of 79% of the myocardial infarction events.⁴³ A point to consider is that the adherence to this healthy lifestyle pattern in our study was not prevalent accounting for only 5.4% of the population presenting “ideal healthy lifestyle”. Supposedly this might reflect either the lack of successful public health interventions targeting middle-age men or the existence of barriers to adopt this ideal lifestyle. Taking into account the amount of time spent at the workplace, these findings are essential for workplace health promotion professionals and might serve as an important key message for public health interventions.

Table 3 Adjusted prevalence ratios PR (95% CI)[†] for the presence of plaques, CACS>0, and subclinical atherosclerosis by individual and combined lifestyle behaviors and adiposity

Lifestyle behaviors and adiposity	Plaque femoral	Plaque carotid	CACS >0	Atherosclerosis
Individual behaviors				
↑ Diet	0.84(0.78;0.92)*	1.03 (0.91; 1.16)	0.92 (0.82; 1.03)	0.94(0.91;0.99)**
↓ Alcohol	0.90 (0.83;0.98)***	1.04 (0.92; 1.18)	0.84(0.74;0.95)**	0.94(0.89;0.98)**
↓ Smoking	0.64 (0.6;0.69)*	0.81(0.71;0.91)*	0.82(0.73;0.92)*	0.89(0.86;0.93)*
↓ Adiposity	0.97 (0.89; 1.06)	0.93 (0.82; 1.06)	1.00 (0.88; 1.12)	1.02 (0.97; 1.06)
↓ Sedentarism	0.93 (0.86; 1.01)	0.92 (0.82; 1.04)	1.02 (0.91; 1.14)	0.97 (0.93; 1.02)
Multiple behaviors				
↑ Diet	0.84 (0.78;0.92)*	1.03 (0.91; 1.16)	0.92 (0.82; 1.03)	0.93(0.89;0.98)**
↑ Diet + ↓ Alcohol	0.77 (0.68; 0.87)*	1.04 (0.87; 1.23)	0.79 (0.66; 0.93)**	0.89 (0.84; 0.95)**
↑ Diet + ↓ Alcohol + ↓ Smoking	0.53 (0.46; 0.62)*	0.95 (0.76; 1.18)	0.65 (0.52; 0.81)*	0.82 (0.75; 0.88)*
↑ Diet + ↓ Alcohol + ↓ Smoking + ↓ Adiposity	0.51 (0.42; 0.62)*	0.94 (0.68; 1.29)	0.63 (0.46; 0.87)**	0.83 (0.75; 0.92)**
↑ Diet + ↓ Alcohol + ↓ Smoking + ↓ Adiposity + ↓ Sedentarism	0.43 (0.33; 0.58)*	0.86 (0.57; 1.29)	0.69 (0.45; 1.04)	0.82 (0.71; 0.95)**

CACS, coronary artery calcification score; PR, prevalence ratio; CI, confidence interval.

↑ Diet, having aMED score 5 or more; ↓ Alcohol, moderate alcohol consumption; ↓ Smoking, nonsmoking; ↓ Adiposity, low WC; ↓ Sedentarism, sitting time less than 5.5 h/d.

* $P < .001$; ** $P < .01$; *** $P < .05$.

†Multivariable (age, education level, and presence of diabetes, hypertension, or hypercholesterolemia) adjusted Poisson regression model with robust estimate.

Our study has several worth mentioning strengths and limitations. One of the major strength of our study is the possibility to evaluate the effect of a healthy lifestyle on atherosclerosis in middle-aged asymptomatic individuals, who would be the ideal candidates for primary prevention as they have not developed the advance stage of the disease yet. It is worth mentioning the choice of the statistical model in our analysis. Recently the criticism has been

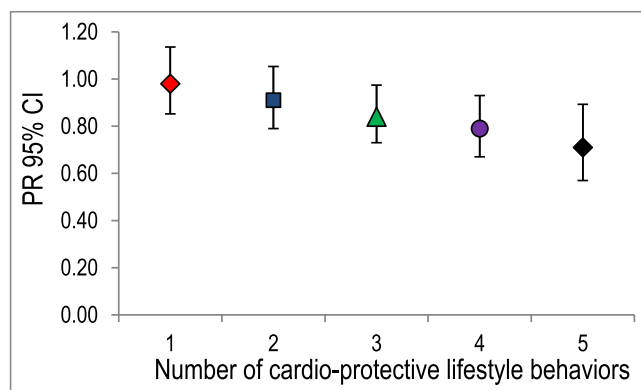


Figure 1 Prevalence ratio (95% CI) for the presence of subclinical atherosclerosis according to the number of cardioprotective lifestyle behaviors. Description of the variable “number if cardio-protective lifestyle behaviors”: (1) 1 of 5 protective lifestyle behaviors are present: either having low WC, or moderate alcohol consumption, or nonsmoking, or sitting time less than 5.5 h/d or having aMED score 5 or more; (2) random combination of 2 of 5 protective behaviors are present etc.; * Poisson regression model with robust estimate is adjusted for age, education, diabetes, hypertension, and hypercholesterolemia. PR, prevalence ratio; CI, confidence interval.

voiced regarding the appropriate assessment of risk in cross-sectional studies showing that the estimates obtained by logistic regression in studies with a highly prevalent disease outcome would greatly overestimate the association.⁴⁴ Therefore, we have chosen to use Poisson regression to estimate the prevalence ratio of adherence to lifestyle behaviors with disease outcome. A major limitation of our study is its cross-sectional design and inability to eliminate the presence of confounding factors, which might explain the reason we see the different degree of the effect in the relationship among lifestyle habits and atherosclerosis in different territories as well as the lack of the contribution of sedentarism and adiposity to the disease risk. Even though multivariable adjustment was applied, still due to the nature of the study the effect of residual confounding factors could not be ruled out. The use of food frequency questionnaire as the assessment method of dietary intake, which relied on the participants memory to recall typical food intake over the past year is another limitation, which might have introduced the error in dietary data. The elimination of female participants from the study limited the generalizability of our results to only male population. Further studies should replicate our analysis including both sexes. Finally, the restricted number of participants with “ideal healthy lifestyle” is another limitation, which could have affected the power of the true association.

From a public health perspective, our findings are of particular importance for health professionals in the clinical settings working with primary prevention and aiming to tackle CVD at its earliest development. Formulating a simple message highlighting the importance to avoid

smoking, follow healthy eating pattern, and consume alcohol in moderation could be an easy solution to prevent coronary artery disease at an early stage.

Conclusion

Consumption of alcohol in moderation, being nonsmoker, and an adherence to a Mediterranean dietary pattern, accounts for the 18% lower prevalence of subclinical atherosclerosis. Highlighting the importance of a healthy lifestyle promotion and introducing this message by health professionals could be a key strategy to tackle the atherosclerosis burden at its earliest development.

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Appendix

Supplemental Table 1 Adjusted prevalence ratio (95% CI)[†] for the presence of plaques in femorals and carotids, CACs > 0 and presence of noncoronary and subclinical atherosclerosis by alcohol consumption (nondrinkers combined with moderate alcohol consumers)

Individual behaviors	Plaque femoral	Plaque carotid	Noncoronary atherosclerosis	CAC>0	Atherosclerosis
↓ Alcohol	0.90 (0.83;0.98)**	0.87 (0.77;0.99)**	0.97 (0.93; 1.02)	0.88 (0.78;0.98)**	0.97 (0.93; 1.01)

** $P < .01$. 1 Model is adjusted for age, education, diabetes, hypertension, and hypercholesterolemia.

↓ Alcohol, 30 grams of ethanol/d and less vs more than 30 grams of ethanol/d.

†Poisson regression model with robust estimate.

5th PUBLICATION:

**Generic meal patterns prevalent among Irish population identified by Latent
Class Analysis: Insights from NANS (National Adult Nutrition Survey).**

Nutrients. 2018; 10(3):310.

GENERAL INTRODUCTION / INDIVIDUAL'S CONTRIBUTION

As it was seen from the first 4 manuscripts, the traditional methods of nutritional epidemiology, namely “*a-posteriori*” and “*a-priori*” analysis proved to be useful methods of dietary patterns identification. However, an important limitation of this approach is the inability to capture individual's food intakes at the meal level, such as breakfast, lunch, or snacks. Moreover, taking into account that a number of studies have shown that meal time and frequency of food consumption has an effect on metabolic profile (Munsters *et al.*, 2012; Asher *et al.*, 2015), it was proposed to introduce the concept of meals in the dietary pattern analysis.

For this study I used the dietary data collected from the NANS dietary survey, which offered the unique opportunity to identify the time of the day when the food was typically consumed, and therefore could be used to aggregate the complex food consumption data into generic meals within eating occasions.

None of the previous “*a-priori*” or “*a-posteriori*” methods of dietary analysis were found to be appropriate to derive meal pattern, due to categorical nature of the meal variables. Therefore, I proposed to use novel data reduction approach, Latent Class Analysis (LCA), commonly used in social science to study the patterns of social behaviour. Previously derived generic meals (Woolhead *et al.*, 2015) were further aggregated and the following meal categories were entered into the LCA model: 4 generic breakfast meal categories (skipped breakfast, cereal and toast or cereal or toast, cooked breakfast, fruits or fruit juice, other); 6 generic light meal categories (skipped light meal, meat fish or dairy sandwich, dairy sandwich, meat or fish sandwich, soups or salads, rice or potato or pasta, other), and 4 generic main meals (skipped main meal, protein and carbohydrate based, protein based, carbohydrates based main meal). I performed LCA separately for weekends and weekdays, and derived the total number of 7 distinctive meal patterns. Further, I have investigated how the meal pattern differ on weekends and whether there is any consistency in adhering to the same meal pattern for most days of the survey. Further, ANOVA analysis were performed to study the association between adherence to meal patterns and consumption of the food groups and CVD biomarkers.



Article

Generic Meal Patterns Identified by Latent Class Analysis: Insights from NANS (National Adult Nutrition Survey)

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Abstract: Nutritional data reduction methods are widely applied in nutrition epidemiology in order to classify individuals into meaningful groups with similar dietary patterns. To date, none of the existing studies have applied latent class analysis to examine dietary patterns which include meal types consumed throughout a day. We investigated main meal patterns followed on weekend and weekdays, and evaluated their associations with cardio-metabolic biomarkers. The analyses were performed within the NANS (National Adult Nutrition Survey) a cross-sectional national food consumption survey of 1500 nationally representative Irish adults. A total number of seven dietary patterns were identified using latent class analysis. The typical meal pattern followed by the majority of the population was characterized by consumption of cereal or toast for breakfast, skipping or consuming a sandwich for light meal, and meat or fish with potatoes, pasta or vegetables for the main meal. Eating patterns differed on weekends, and those participants who consumed meat and eggs for breakfast instead of breakfast cereal and skipped light meal were more likely to have an unhealthier dietary pattern, a higher diastolic blood pressure, and increased serum ferritin. The application of data reduction techniques to simplify the multifaceted nature of dietary data is a useful approach to derive patterns, which might shed further light on the typical dietary patterns followed by populations.

Keywords: latent class analysis; dietary patterns; generic meals; breakfast; light meal; main meal

1. Introduction

The concept of dietary intake data reduction applied to dietary data has been widely used to classify individuals within a population into meaningful groups with similar diets [1,2]. These diets can be defined “a priori” by using established dietary indices [3] and fitting the data into pre-defined dietary patterns or “a posteriori” by means of statistical classification techniques such as factor analysis (FA), or principal component analysis (PCA) that will result in data-driven dietary patterns [4]. Adherence to “a priori” and “a-posteriori” defined dietary patterns has been extensively investigated for the associations with varied disease outcomes. For example, Mediterranean dietary pattern measured by adherence to “a priori” defined dietary indices was associated with reduced cardiovascular, cancer, and all-cause mortality [5], while a Western dietary pattern derived by Principal component (PCA) and Factor

analysis (FA) is linked to type II diabetes, cancer, and biomarkers of obesity and cardiovascular disease (CVD) risk [6–8]. Challenges in accurate determination of dietary patterns still exist, primarily in the need for strategies to reduce the complex multidimensional nutritional data down to an interpretable set of observed patterns.

Latent class analysis (LCA) has been widely used in social and behavioral sciences and also applied to relevant qualitative dietary intake data [9,10], however, few studies have used LCA to characterize diets by classifying individuals into categories (or classes) of similar dietary behaviors. Sotres-Alvarez et al. in their investigations concluded that LCA is a useful approach to classify individuals into exclusive classes based on the similarity in dietary behavior, compared to FA, which could be mainly used identify the combinations of foods typically consumed [11]. Furthermore, since LCA is considered a data driven approach, it may show a more realistic picture of what people eat in daily life and provide interesting insights into dietary behavior [12]. For example, in the work by Padmadas et al. The authors used LCA to derive 5 main dietary patterns prevalent among Indian women revealing a heterogeneity of dietary behaviors across the country which none of the previous studies could detect [12].

To date, none of the existing studies have applied LCA to understand the patterns of meal types consumed throughout a day (for breakfast, lunch, dinner, or snacks). Meal analysis has recently received considerable public health interest following arguments that foods are usually consumed in combination within meals, and studying meals instead of focusing on food groups might have an important contribution towards more effective, meal-based, dietary guidance [13–15]. However, challenges still exist with the fact that not every dietary assessment tool provides the information on meals consumed. In order to address this limitation, Woolhead et al. developed a generic meal coding system, which allowed the aggregation of complex population food consumption data into generic meals within eating occasions. Those included breakfast, light meal, main meal, and snacks specific for the population considered, and in doing so reduced the complexity of data, allowing for the application of PCA to derive meal patterns [13]. However, the heterogeneity of diet in the studied population still hindered full capture of generic meal patterns with as many as twelve components (meal patterns) explaining only 29% of the total variance. Thus, further investigation is required, and therefore the aims of the present study were to use the novel approach of generic meal coding and apply LCA to derive meal consumption patterns among the Irish population, to study how meal patterns differ between weekdays and weekends, and to evaluate the association of these patterns with cardio-metabolic biomarkers.

2. Materials and Methods

2.1. Study Overview

The National Adult Nutrition Survey (NANS) was a cross-sectional national food consumption survey carried out between 2008 and 2010 and collected data on habitual dietary intakes, lifestyle, health related indicators, and attitudes towards food and health among a total of 1500 healthy, free-living adults in Ireland [16]. Male and female participants aged 18 years and over residing in the Republic of Ireland were included in the study. A detailed description of the study design and procedures of data collection are described in detail elsewhere (16). The study protocol was approved by the Human Ethics Research Committee of University College Dublin and the University College Cork Research Ethics Committee of the Cork Teaching Hospitals (ECM 3(p) 04/11/08), and all participants provided written informed consent [16].

2.2. Demographic, Anthropometric, and Clinical Variables

Demographic information from NANS was used in this analysis including sex, age (years), marital status, and social class. Lifestyle-related variables included smoking habits, alcohol consumption assessing how often the participant consumed alcoholic drinks, supplement use as well as self-identification of being responsible for groceries or cooking. Anthropometry included

measurements of height, weight, waist, and hip circumference measured by researchers during the data collection period and were obtained through standardized procedures [16]. Data on serum blood lipids, serum glucose, and C-reactive protein (CRP) was used. Serum triglycerides were analyzed with colorimetric method (Randox Daytona, Randox Laboratories Ltd., Crumlin, UK), serum total cholesterol was analyzed with cholesterol oxidase-enzymatic endpoint method (Randox Daytona), and HDL-c was analyzed with direct clearance method (Randox Daytona). LDL-c was calculated using Friedewald equation. CRP and serum ferritin were analyzed with immunoturbidimetric immunoassay method (Randox Daytona) [16]. Those participants with serum ferritin > 200 mg/L for men and 150 mg/L for women were considered to have increased serum ferritin levels [17] and hypertension if average blood pressure was greater than 140/90 mmHg [18]. Missing values on body mass index (BMI) ($n = 146$), weight ($n = 87$), waist-to-hip ratio ($n = 226$), body fat ($n = 181$), systolic blood pressure ($n = 185$), diastolic blood pressure ($n = 185$), total cholesterol ($n = 369$), triglycerides ($n = 370$), high-density lipoprotein cholesterol (HDL-c) ($n = 377$), low-density lipoprotein cholesterol (LDL-c) ($n = 387$), serum glucose ($n = 373$), and CRP ($n = 652$) were excluded from the analysis. There were no statistically significant differences in sociodemographic characteristics between those who were excluded from and included in the final analysis.

2.3. Dietary Assessment and Generic Meals Determination

Four-day semi-weighed food diaries were used to collect participants' dietary data [16]. Subjects were asked to record and weigh any food, beverage, or supplements at the time of consumption and provide the description of the food, as well as detailed information regarding the preparation method, manufacturer, and time of consumption. Participants were encouraged to include at least one weekend in their dietary record. Weighed Intake Software Program WISP[®] (Tinuviel Software, Anglesey, UK) version 3.0 used "The Composition of Foods" to generate nutrient intake data [19,20].

The dietary data collected within NANS contained the information on the particular meal types corresponding to the food ingested. For example, the cereals were consumed as a part of breakfast, the participant would indicate "breakfast" as a meal type. There were 11 original meal types collected: breakfast; light meal as a part of the lunch, light meal as a part of the evening meal; main meal as a part of the lunch, main meal as a part of the evening meal; morning, afternoon, evening, and night snacks; alcohol and non-alcoholic beverages. Those meal types were reduced to 5: breakfast, light meal, main meal, snacks and beverages and only first three were used in the analysis.

The methodology applied to create generic meals, from the dietary intake data of NANS is described in detail in Woolhead et al. [13]. In brief, the dietary data from 4 days record contained nutritional information on 2552 food items, which were further reduced into 20 food groups based on their nutritional profile. For example, 78 cereal food items were assigned to the food group "cereals", and 21 milk food items were assigned to the group "milks". The 20 food groups included: breads, cereals, milks, fats, fruits, fruit juice, tea/coffee, sugar/jam, cheese, yoghurt, eggs, nuts, vegetables, rice/potatoes/savouries, meat/fish, confectionery/desserts, alcohol, and beverages. The combination of these food groups consumed by one person at a single eating occasion was identified as individual meal. The examples of the individual meals for breakfast are "cereals and milk" "bread and juice" or "bread and fruit", recorded for the analysis as "cereal and toast or cereal or toast". In particular, within Woolhead et al. paper food group combinations were determined based on estimates of prevalence of common combinations followed by the visual inspection to combine similar groupings if required [13] and the total number of 15 generic breakfast meals, 19 light meals, and 15 main meals were previously used. Before applying the reduction of the initial list of generic meals proposed by Woolhead et al., to the ones used in our final analysis, we attempted to fit all original generic meals into LCA. However, based on the assessment of the model fit no interpretable classes were derived, and as such further aggregation of the generic meals was required and similar to Woolhead et al. approach was applied. For example, for a light meal, if the generic meal contained bread, cheese, or meat/fish it was assigned to the group meat/fish/dairy sandwich (MFD sandwich), if the generic meal contained only bread

and meat/fish it was assigned to the group meat/fish sandwich (MF sandwich). Thus, our analyses included 4 generic breakfast meal categories (skipped breakfast, cereal and toast or cereal or toast, cooked breakfast, fruits or fruit juice, other); 6 generic light meal categories (skipped light meal, meat fish of dairy sandwich, dairy sandwich, meat or fish sandwich, soups or salads, rice or potato or pasta, other), and 4 generic main meals (skipped main meal, protein and carbohydrate based, protein based, carbohydrates based main meal) (Tables S1 and S2).

2.4. Dietary Pattern Identification by Using Latent Class Analysis

Patterns of generic meal consumption were identified by LCA. Three main domains (meal types), each containing several categories were used as described above (Tables S1–S3). The dietary data collected from all 1500 NANS participants across all 4 days was used, resulting in a total number of 6000 observations being included in the LCA. Number of classes were chosen based on the assessment of the model fit, which was based on the combinations of the following parameters: (1) smaller model fit indices, including the Akaike information criterion (AIC) and Bayesian information criterion (BIC); (2) the Bootstrap Likelihood ratio test (BS-LRT) comparing k classes vs. $k - 1$ class models; and (3) pattern interpretability [21]. Two-class model was first fit to the data and additional classes (up to ten classes) were added until the optimal number of latent classes was identified. In order to account for weekdays/weekends variation, as we have observed that 1.5% ($N = 22$), 51.4% ($N = 771$), and 47.1% ($N = 707$) participants reported the dietary intake for all 4 weekdays, 3 weekdays/1 weekends, and 2 weekdays/2 weekends, respectively, LCA was performed separately for weekdays and weekends. The total number of 3815 and 2185 dietary records were included in the LCA for weekdays and weekends, respectively. The models which fit the data best according to above mentioned criteria included 4 classes for weekdays and 3 classes for weekends data. Using an inclusive maximum-probability approach, dietary records were assigned to the class based on item response probability, which indicates the chance of a particular meal type to be consumed in the particular Latent Class. Latent Classes were used as the predictors for further analyses.

2.5. Determination of Dominant Classes

As the Latent Classes were not assigned to the individuals themselves but each day of their dietary record, implying that every individual in our study might fall into different Latent Classes depending on the day of the survey, the reduction from the meal level data ($N = 6000$) back to the individual data ($n = 1500$) was needed in order to study the dietary and phenotypic profile of the meal pattern (Figure 1). Once the Classes were derived for weekdays and weekends, the variables reflecting the Classes which the participant belongs to were pooled together to examine how the Classes differed across all 4 days of record for each participant, and the dominant Classes for each individual were determined. Class was considered dominant if the participant belonged to this Class most of the days (weekdays and weekends classes were treated separately). The group “varied” was used when the weekday/weekend patterns were combined together, and there was no constant meal pattern followed by the participants over 4 days. If the participants adhered to Class 1 at least 2 days on the weekdays, and different Classes during the weekends, they were assigned to the group “Class 1 weekdays/varied”. If the participants adhered to Class 1 at least 2 days on the weekdays and Class 1 at the weekend, they were assigned the group “Class 1 weekdays/Class 1 weekends” etc. Number of participants in each group was identified, and all the groups where the percent of participants didn’t exceed 5% of total population were grouped together with the group “varied”. Variation in clinical cardio-metabolic risk factors was examined across the Dominant Latent Class groups.

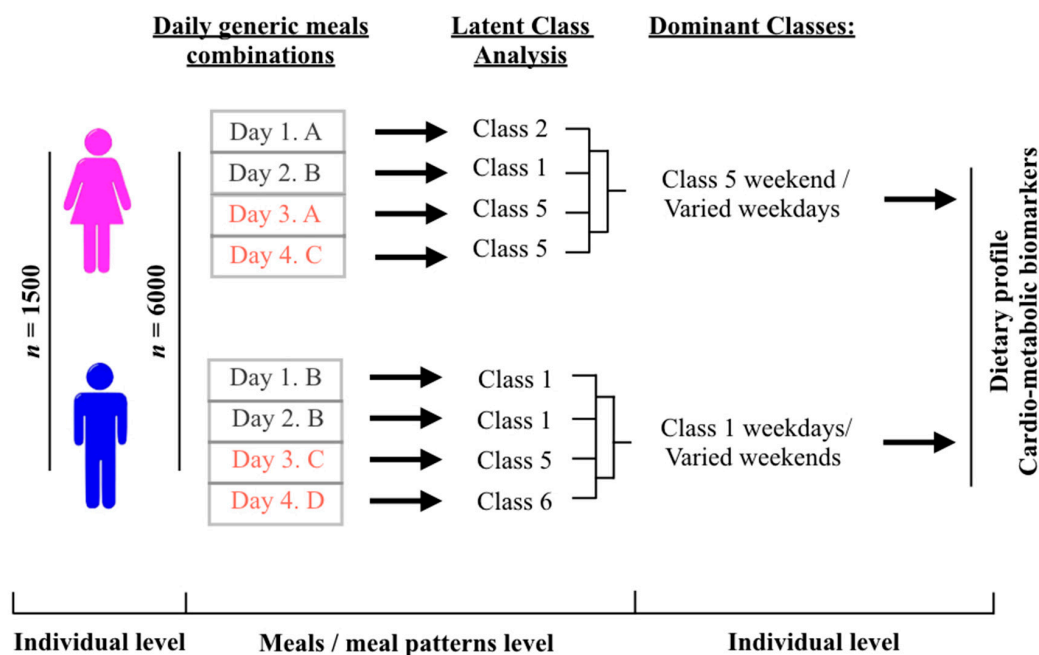


Figure 1. Flow diagram of method to determine meal patterns. The analysis is based on data collected from 1500 male and female participants over 4 days of dietary survey resulting in the total number of 6000 dietary records. A,B,C, and D correspond to the combination of meals types consumed during one day of the dietary survey by a single individual. Day 3 and Day 4 of the dietary survey are weekends. Latent Classes were assigned to each of 6000 dietary records. Separately for weekdays and weekends the dominant Classes were determined based on the adherence to the same latent class over time. Class Varied included the dominant classes representing less than 5% of the total population and the group with no defined dominant classes over 4 days of the survey.

2.6. Statistical Analysis

The data was normally distributed. Analysis of variance (ANOVA) with Bonferroni post-hoc comparison method was used to compare the daily food, macro- and micronutrients intakes, and clinical variables between the classes of meal patterns. *P* values were adjusted for age (years), sex (male/female), social class (professional/manager, non-manual skilled, manual skilled, and semi-skilled/unskilled), and energy intake (kcal). Chi-square was used to compare the demographic and lifestyle-related characteristics between the classes of meal patterns. The multivariate adjusted logistic regression analyses were used to study the association between most dominant latent classes and serum ferritin and diastolic blood pressure (DBP). The model was adjusted for age (years), sex (male/female), social class (professional/manager, non-manual skilled, manual skilled, and semi-skilled/unskilled), and energy intake (kcal). LCA was performed using LatentGold 5.1 (Statistical Innovations Inc., Belmont, MA, USA). All other statistical analyses were performed with the Statistical Package for Social Sciences IBM SPSS Statistics for Windows, version 24 (IBM Corp., Armonk, NY, USA). *p* values < 0.05 (two-sided) were considered statistically significant.

3. Results

Demographic characteristics of participants are presented in a Table S4. A total number of 3815 and 2185 dietary records represented participants dietary intakes during weekdays and weekends, respectively. Of 3815 weekday dietary records, 60%, 26%, 9%, and 5% fell into Class 1, 2, 3, and 4, respectively (Table 1). The intakes classified as Class 1 had 88% chance to have cereal or toast or both for breakfast, 23% or 28% chance to skip light meal or consume meat or fish (MF) sandwich for light meal and 72% chance to have a protein- and carbohydrate-based main meal. Intakes classified as Class

2 with 65% probability consumed cereal or toast or both for breakfast, a slightly higher chance to have MF sandwich (35%) than meat, fish or dairy (MFD) sandwich (23%) for light meal, and 61% and 23% chance to have a protein- and carbohydrate-based dish for main meal or to skip it, respectively. Intakes at Class 3 were cooked breakfast with a 44% probability, skip light meal with a 57% probability, and a 64% chance to consume a protein- and carbohydrate-based dish for main meal. The intakes that were classified as Class 4 had a 33% probability to consume cereal or toast or both, 27% probability to have fruit or fruit juice for breakfast, a 26% probability to consume soups and salad for light meal, and a 39% chance to have protein and carbohydrates based dish for main meal.

Of 2185 weekend dietary records (Table 1), 57%, 22%, and 21% fell into Class 5, 6, and 7, respectively. Intakes from Class 5 would be 88% more likely to have cereal and toast or cereal or toast for breakfast, 36% more likely to skip light meal, and had 86% probability to have a protein- and carbohydrate-based dish for main meal. Those classified into Class 6 were 70% more likely to have cereal and toast or cereal or toast for breakfast, 24% probability of having MF sandwich for light meal, and protein- and carbohydrate-based meal or just protein-based meal as a main meal with a probability of 39% and 21%, respectively. Class 7 was more likely to be characterized by consumption of cooked breakfast with 45% of probability, skip light meal with 69% of probability, and with 76% probability have protein and carbohydrates based dish for main meal.

Comparing the weekdays Classes between each other, Class 1 presented the highest probability to have cereal or toast or both for breakfast, and protein and carbohydrates based main meal; while Class 2 had zero probability to have fruit or fruit juice for breakfast, and the highest chance to consume meat/fish/dairy (MFD) or meat/fish (MF) sandwich for light meal. Class 3 compared to other 3 classes had the highest probability to have cooked breakfast, and skip light meal. Class 4 had higher probability to have fruits or fruit juice, confectionary, and other foods for breakfast, the highest probability to consume soups or salad as a light meal and carbohydrates based main meal. With respect to weekend classes, Class 5 would have the highest probability to consume cereals or toast or both for breakfast, and protein and carbohydrate based main meal. Class 6 comparing to other 2 Classes had the highest probability to have MFD sandwich or soups and salads for light meal, and protein based main meal; while Class 7 had the highest probability to consume cooked breakfast, and skip light meal.

Results of an overall daily intakes of food groups during weekdays showed that Class 1 was characterized by the highest intakes of breakfast cereals, fruits and fruit dishes, and lowest intakes of eggs and egg dishes. Class 2 was observed to be the highest in grains, rice, pasta, and savories, as well as potatoes and potato dishes. Class 3 was characterized by the highest consumption of soups, sauces and miscellaneous. The consumption of vegetables and vegetable dishes was observed to the lowest for Class 4. As for the overall daily intakes of food groups during weekends, the highest intakes of breakfast cereals, vegetables and vegetable dishes, ice cream and dessert have been observed among Class 5 participants. Highest intakes of grains, rice, pasta, and savories together with nuts and herbs tend to be highest among participants adhering to Class 6, who also were observed to have the lowest consumption meat and potatoes. Those who belongs to Class 7 were characterized by the lowest consumption of breakfast cereal, fruit/fruit dishes, milk and yogurt, as well as bread and rolls (Table 2).

In terms of nutritional quality for weekdays Classes, Class 1 was categorized as the highest in dietary fibre. Class 2 was the lowest on sodium and calcium. Class 3 tend to be the lowest in total energy intake and starch. Regarding the weekends, Class 5 tend to have the highest protein and vitamin C intake and the lowest intakes of Vitamin A. Class 6 had the highest intakes of calcium. Class 7 was observed to have the highest intakes of total fat, including monounsaturated fatty acids (MUFA) as well as the lowest intakes of carbohydrates, starch, sugar, dietary fibre, calcium, and iron (Table 3). Food group intakes within each of the specific meal types (breakfast, light meal, main meal, snack) across the Classes are presented in Tables S5–S8.

Table 1. Latent Classes of generic meal intakes.

Meal type	Latent Classes	Weekdays					Weekends		
		Class 1	Class 2	Class 3	Class 4	Class 5	Class 6	Class 7	
N (%)		2288 (60.0)	988 (25.9)	360 (9.4)	179 (4.7)	1249 (57.2)	469 (21.5)	467 (21.4)	
Breakfast	Generic meal	Conditional probabilities ¹							
	No breakfast	0.044	0.087	0.139	0.030	0.045	0.096	0.153	
	Cereal and toast or cereal or toast	0.877	0.648	0.129	0.327	0.883	0.703	0.142	
	Cooked breakfast	0.008	0.105	0.444	0.001	0.029	0.056	0.450	
	Fruit/fruit juice	0.039	0.000	0.034	0.266	0.005	0.037	0.089	
	Other	0.029	0.157	0.251	0.374	0.037	0.106	0.164	
	Light meal	Skip light meal	0.229	0.011	0.569	0.172	0.364	0.157	0.688
		MFD sandwich	0.113	0.233	0.051	0.038	0.098	0.137	0.067
		Dairy sandwich	0.063	0.080	0.014	0.062	0.068	0.034	0.041
		MF sandwich	0.280	0.348	0.175	0.060	0.204	0.244	0.084
Soups and salads		0.081	0.008	0.084	0.264	0.057	0.109	0.052	
Rice potato pasta		0.013	0.148	0.000	0.148	0.004	0.101	0.007	
Other		0.220	0.168	0.103	0.253	0.201	0.215	0.059	
Main meal	Skip main meal	0.023	0.231	0.049	0.051	0.000	0.169	0.060	
	Protein and carbohydrates	0.720	0.607	0.635	0.387	0.857	0.391	0.755	
	Protein	0.144	0.150	0.194	0.183	0.105	0.210	0.087	
	Carbohydrates	0.081	0.009	0.087	0.216	0.031	0.166	0.069	
	Other	0.029	0.000	0.031	0.160	0.005	0.061	0.026	

¹ Values are conditional probabilities to consume one of the listed generic meals given that the participant is classified in one of the following latent classes; N (%) is the number of dietary records of 1500 participants over 4 days across all latent classes. MFD—meat/fish/dairy; MF—meat/fish.

Table 2. Overall daily intakes ¹ (g/day or mL/day) of selected food groups by latent classes.

Latent Classes	Weekdays					Weekends	
	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6	Class 7
N (%)	2288 (60.0)	988 (25.9)	360 (9.4)	179 (4.7)	1249 (57.2)	469 (21.5)	467 (21.4)
Grains, rice, pasta & savories	55.5 ± 11.1 ^{b,c}	86.6 ± 13.6 ^{a,c,d}	30.8 ± 9.3 ^{a,b}	44.1 ± 9.6 ³	42.8 ± 9.3 ⁹	94.4 ± 16.3 ⁸	62.0 ± 12.3 ^{e,f}
Breakfast cereals	76.4 ± 99.6 ^{b,c,d}	45.8 ± 81.3 ^a	53.7 ± 106 ^a	39.4 ± 68.9 ^a	77.2 ± 101 ^{f,g}	58.4 ± 88.5 ^{e,g}	2.90 ± 16.7 ^{e,f}
Potatoes/potato dishes	116 ± 124 ^{b,c,d}	163 ± 146 ^{a,c,d}	17.3 ± 57.8 ^{a,b,d}	47.6 ± 91.2 ^{a,b,c}	148 ± 128 ^f	64.0 ± 121 ^{e,g}	140 ± 138 ^f
Vegetables/vegetable dishes	120 ± 117 ^{b,d}	103 ± 113 ^{a,d}	116 ± 167 ^d	70.3 ± 93.5 ^{a,b,c}	123 ± 114 ^{f,g}	106 ± 141 ^e	103 ± 113 ^e
Fruits/fruit dishes	175 ± 191 ^{b,c,d}	119 ± 172 ^a	141 ± 193 ^a	128 ± 189 ^a	137 ± 178 ^g	145 ± 196 ^g	94.0 ± 170 ^{e,f}
Nuts, seeds, herbs	3.0 ± 14.3	2.5 ± 11.9	3.2 ± 13.5	1.0 ± 6.3	2.98 ± 13.0 ^f	4.85 ± 18.0 ^{e,g}	1.51 ± 9.37 ^f
Milk and yogurt	269 ± 238 ^{b,c}	241 ± 246 ^a	219 ± 252 ^a	246 ± 240	238 ± 213 ^g	231 ± 253 ^g	150 ± 209 ^{e,f}
Meat and meat products	176 ± 155 ^d	191 ± 156 ^{c,d}	159 ± 181 ^b	138 ± 170 ^{a,b}	193 ± 144 ^f	130 ± 154 ^{e,g}	204 ± 149 ^f
Fish/fish dishes	35.0 ± 78.2 ^c	31.7 ± 78.8 ^a	22.1 ± 60.6	22.0 ± 56.5	25.3 ± 68.1	20.0 ± 64.8	17.9 ± 58.6
Cheeses	15.0 ± 27.3 ^{b,d}	10.4 ± 24.3 ^{a,d}	12.3 ± 27.3 ^d	22.5 ± 32.9 ^{a,b,c}	12.7 ± 26.9	15.3 ± 30.3 ^g	10.7 ± 26.9 ^f
Eggs/eggs dishes	12.2 ± 36.2 ^{b,c,d}	16.7 ± 38.7 ^{a,c}	23.2 ± 54.8 ^{a,b}	20.4 ± 55.3 ^a	12.9 ± 35.1 ^{f,g}	24.0 ± 53.2 ^{e,g}	35.3 ± 50.9 ^{e,f}
Biscuits, cakes and pastries	29.9 ± 49.2	26.8 ± 47.9	27.3 ± 48.1	29.5 ± 52.0	32.6 ± 54.0	29.4 ± 59.7	26.8 ± 53.3
Cream, ice-cream and dessert	19.2 ± 55.6	17.7 ± 55.9	13.0 ± 46.4	20.3 ± 61.6	30.8 ± 65.7 ^{f,g}	16.6 ± 53.0 ^e	20.4 ± 56.1 ^e
Soups and sauces	61.0 ± 117 ^c	50.8 ± 91.9 ^c	84.1 ± 151 ^{a,b,d}	48.4 ± 108 ^c	50.4 ± 95.5	53.6 ± 109	48.8 ± 99.2
Breads and rolls	129 ± 83.9 ^{b,c}	96.7 ± 84.7 ^{a,d}	106 ± 97.2 ^{a,d}	147 ± 91.8 ^{b,c}	111 ± 78.0 ^g	119 ± 91.2 ^g	90.0 ± 82.2 ^{e,f}
Sugar, confectionary, snacks	28.6 ± 36.7 ^d	30.0 ± 39.2	29.5 ± 39.7	37.0 ± 48.0 ^a	28.3 ± 40.6	33.2 ± 44.9	28.7 ± 40.7
Beverages including water	1421 ± 938	1443 ± 1093	1399 ± 1022	1546 ± 1100	1529 ± 1264 ^g	1700 ± 1454	1789 ± 1653 ^e

¹ Values are mean (±SD). ANOVA with Bonferroni correction was applied. Multiple comparison was performed for Classes 1–4 and Classes 5–7 separately. Classes definition: Weekdays: Class 1–88% cereal or toast for breakfast, 23% skip light meal/28% MF sandwich/22% other for light meal, 72% protein and carbohydrates based for main meal; Class 2–64% cereal or toast for breakfast, 23% MFD sandwich/35% MF sandwich for light meal, 23% skip main meal/60% protein and carbohydrates based for main meal; Class 3–13% cereal or toast/44% cooked breakfast/25% other for breakfast, 57% skip light meal, 64% protein and carbohydrates based for main meal; Class 4–33% cereal and toast/27% fruit/37% other, 26% soups and salads/25 other, 39% protein and carbohydrates based for main meal; ^a significant difference from Class 1 ($p < 0.05$); ^b significant difference from Class 2 ($p < 0.001$); ^c significant difference from Class 3 ($p < 0.001$); ^d significant difference from Class 4 ($p < 0.001$). Weekends: Class 5–88% cereal or toast for breakfast, 35% skip light meal/20% MF sandwich/20% other for light meal, 85% protein and carbohydrates based for main meal; Class 6–70% cereal or toast for breakfast, 24% MF sandwich/22% other for light meal, 39% protein and carbohydrates based for main meal; Class 7–45% cooked breakfast, 69% skip light meal, 76% protein and carbohydrates based for main meal; ^e significant difference from Class 5 ($p < 0.05$); ^f significant difference from Class 6 ($p < 0.001$); ^g significant difference from Class 7 ($p < 0.001$).

Table 3. Mean daily macro- and micronutrients intake ¹ by latent classes.

Latent Classes	Weekdays					Weekends	
	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6	Class 7
N (%)	2288 (60.0)	988 (25.9)	360 (9.4)	179 (4.7)	1249 (57.2)	469 (21.5)	467 (21.4)
Energy intake, kcal	1993 ± 749 ^c	2052 ± 816 ^c	1678 ± 775 ^{ab,d}	1917 ± 840 ^c	2074 ± 829	2075 ± 955	2108 ± 1043
Fat, %TE	33.5 ± 8.73 ^c	34.0 ± 9.66	35.2 ± 12.1 ^a	34.2 ± 9.20	33.0 ± 9.30 ^g	33.3 ± 10.3 ^g	35.6 ± 11.6 ^{ef}
SFA, %TE	13.1 ± 4.65 ^c	13.2 ± 4.92 ^c	14.2 ± 6.71 ^{ab}	13.5 ± 4.94	13.0 ± 4.86	13.3 ± 5.41	13.7 ± 5.93
MUFA, %TE	12.0 ± 3.82 ^b	12.5 ± 4.16 ^a	12.5 ± 4.97	12.1 ± 3.97	12.0 ± 4.03 ^g	12.0 ± 4.37 ^g	13.5 ± 4.78 ^{ef}
PUFA, %TE	6.08 ± 3.54	6.03 ± 3.33	6.42 ± 4.23	6.19 ± 3.17	5.68 ± 2.94 ^g	5.8 ± 3.55	6.22 ± 3.60 ^e
Protein, %TE	17.8 ± 5.17 ^{cd}	17.8 ± 5.77 ^{cd}	16.3 ± 5.86 ^{ab}	16.0 ± 5.83 ^{ab}	17.5 ± 5.17 ^{fg}	15.1 ± 5.30 ^{efg}	16.6 ± 5.70 ^{ef}
Carbohydrates, %TE	47.9 ± 9.26 ^b	46.1 ± 10.1 ^a	46.6 ± 12.4	47.1 ± 11.9	45.8 ± 9.76 ^g	46.5 ± 11.9	40.1 ± 12.1 ^{ef}
Starch, g	139 ± 58.0 ^{cd}	138 ± 59.1 ^c	102 ± 60.8 ^{ab,d}	127 ± 59.5 ^{ac}	136 ± 56.3 ^g	135 ± 73.0 ^g	118 ± 61.7 ^{ef}
Sugars, g	92.1 ± 50.0	88.3 ± 55.5	86.0 ± 52.4	89.4 ± 61.8	92.5 ± 55.3 ^g	93.3 ± 60.1 ^g	81.7 ± 63.4 ^{ef}
Dietary fiber, g	20.8 ± 9.46 ^{b,c,d}	18.3 ± 8.97 ^{a,c}	16.4 ± 11.6 ^{ab}	16.2 ± 9.62 ^{ab}	19.6 ± 9.18 ^g	19.0 ± 11.2 ^g	15.4 ± 8.74 ^{ef}
Calcium, mg/10 MJ/d	1227 ± 531 ^b	1070 ± 520 ^{a,c,d}	1311 ± 851 ^b	1247 ± 491 ^b	1102 ± 496 ^{fg}	1273 ± 639 ^{efg}	944 ± 569 ^{ef}
Iron, mg/10 MJ/d	18.4 ± 22.7	17.9 ± 29	18.8 ± 29.4	16.2 ± 20.7	18.7 ± 22.4 ^g	19.6 ± 34.7 ^g	14.7 ± 23.9 ^{ef}
Sodium, mg/10 MJ/d	3072 ± 1113 ^{b,c,d}	2837 ± 1164 ^{a,c,d}	3420 ± 1535 ^{ab}	3363 ± 1176 ^{ab}	1654 ± 401 ^g	1617 ± 477	1552 ± 438 ^e
Vitamin A, mcg/10 MJ/d	1549 ± 2302	1504 ± 4687	1495 ± 1769	1090 ± 1142	2849 ± 1103 ^{fg}	3182 ± 1199 ^e	3078 ± 1269 ^e
Vitamin C, mg/10 MJ/d	177 ± 404 ^b	132 ± 311 ^a	178 ± 369	115 ± 250	1587 ± 2656 ^{fg}	1211 ± 1387 ^e	1172 ± 1277 ^e
Vitamin D, mcg/10 MJ/d	6.45 ± 9.49 ^b	5.3 ± 8.32 ^{a,c}	6.82 ± 10.91 ^b	4.92 ± 6.41	159 ± 298	149 ± 258	148 ± 355
Vitamin E, mcg/10 MJ/d	16.9 ± 30.3	14.9 ± 35.1	19.4 ± 41.8	17.4 ± 46.5	5.93 ± 9.13	5.11 ± 6.97	4.88 ± 6.86
Folate, mcg/10 MJ/d	491 ± 791 ^c	441 ± 544 ^c	508 ± 1082 ^{ab}	408 ± 253	15.7 ± 34.0	18.1 ± 45.1 ^g	11.8 ± 12.1 ^f
Vitamin B-12, mcg/10 MJ/d	8.41 ± 28.5	8.32 ± 39.7	7.16 ± 10.8	7.03 ± 13.7	4.78 ± 46.7	5.37 ± 24.22	39.4 ± 435
Vitamin B2, mg/10 MJ per d	3.82 ± 9.77	3.2 ± 7.21	4.12 ± 9.2	4.59 ± 16.1	7.55 ± 15.1	13.3 ± 103	7.19 ± 17.7

¹ Values are mean (±SD). ANOVA with Bonferroni correction was applied. Multiple comparison was performed for Classes 1–4 and Classes 5–7 separately. Classes definition: Weekdays: Class 1—88% cereal or toast for breakfast, 23% skip light meal/28% MF sandwich/22% other for light meal, 72% protein and carbohydrates based for main meal; Class 2—64% cereal or toast for breakfast, 23% MFD sandwich/35% MF sandwich for light meal, 23% skip main meal/60% protein and carbohydrates based for main meal; Class 3—13% cereal or toast/44% cooked breakfast/25% other for breakfast, 57% skip light meal, 64% protein and carbohydrates based for main meal; Class 4—33% cereal and toast/27% fruit/37% other, 26% soups and salads/25 other, 39% protein and carbohydrates based for main meal. a significant difference from Class 1 ($p < 0.05$), b significant difference from Class 2 ($p < 0.05$), c significant difference from Class 3 ($p < 0.05$); d significant difference from Class 4 ($p < 0.05$). Weekends: Class 5—88% cereal or toast for breakfast, 35% skip light meal/20% MF sandwich/20% other for light meal, 85% protein and carbohydrates based for main meal; Class 6—70% cereal or toast for breakfast, 24% MF sandwich/22% other for light meal, 39% protein and carbohydrates based for main meal; Class 7—45% cooked breakfast, 69% skip light meal, 76% protein and carbohydrates based for main meal. e significant difference from Class 5 ($p < 0.05$), f significant difference from Class 6 ($p < 0.05$); g significant difference from Class 7 ($p < 0.05$). SFA—saturated fatty acids, MUFA—monounsaturated fatty acids, PUFA—polyunsaturated fatty acids.

Once the participants' adherence to latent classes for weekends and weekdays were pooled together, 20 groups with the most dominant classes were identified (Table 4). Among all possible combinations, approximately 50% of the sample showed a predominant dietary behavior (dominant class) on weekdays and on weekends, falling into 4 possible combinations with distinctive food intakes (Table 5). Participants who followed Class 1 during weekdays and Class 5 during weekends had significantly ($p < 0.05$) lower intakes of grains, rice and pasta compared to those falling into Class 1 weekdays/Class 6 weekends, and the highest intakes of breakfast cereals compared to the rest of the Classes. They also were observed to have significantly higher intakes of fruits, bread and rolls and lower intakes of meat and meat dishes compared to participants from Class 2 weekdays/Class 5 weekends pattern. In turn, participants from Class 1 weekdays/Class 7 weekends pattern were observed to have the lowest intakes of breakfast cereals among all the Classes and significantly higher intakes of eggs and egg dishes, compared to Class 1 weekdays/Class 5 weekends pattern. As for the Class 2 weekdays/Class 5 weekends pattern, participants who adhered to that Class had the highest intakes of potatoes and potato dishes compared to other three Classes, significantly lower intakes of fruits and higher intakes of meat compared to Class 1 weekdays/Class 5 weekends and Class 1 weekdays/Class 6 weekends.

The analysis of clinical variables with multivariable adjustment across most Dominant Latent Classes (Table 6) showed that participants who followed Class 1 weekdays/Class 7 weekends pattern had significantly ($p < 0.05$) higher DBP compared to those falling into Class 1 weekdays/Class 6 weekends, as well as significantly higher risk of presenting increased serum ferritin (Table S9) comparing to those from Class 1 weekdays/Class 5 weekends pattern (OR: 3.14; 95%CI: 1.63; 6.03).

Table 4. Identification of dominant classes of NANS participants ($n = 1500$) over 4 days of food diary assessment.

Dominant Class Weekdays	Dominant Class Weekends	N (%)
Class 1	-	148 (9.90)
Class 2	-	47 (3.10)
Class 3	-	12 (0.80)
Class 4	-	2 (0.10)
-	Class 5	135 (9.00)
-	Class 6	37 (2.50)
-	Class 7	60 (4.00)
Class 1	Class 5	439 (29.3)
Class 1	Class 6	114 (7.60)
Class 1	Class 7	87 (5.80)
Class 2	Class 5	100 (6.70)
Class 2	Class 6	28 (1.90)
Class 2	Class 7	60 (4.00)
Class 3	Class 5	13 (0.90)
Class 3	Class 6	12 (0.80)
Class 3	Class 7	17 (1.10)
Class 4	Class 5	6 (0.40)
Class 4	Class 6	4 (0.30)
Class 4	Class 7	5 (0.30)
-	-	174 (11.6)

Classes definition: Weekdays: Class 1–88% cereal or toast for breakfast, 23% skip light meal/28% MF sandwich/22% other for light meal, 72% protein and carbohydrates based for main meal; Class 2–64% cereal or toast for breakfast, 23% MFD sandwich/35% MF sandwich for light meal, 23% skip main meal/60% protein and carbohydrates based for main meal; Class 3–13% cereal or toast/44% cooked breakfast/25% other for breakfast, 57% skip light meal, 64% protein and carbohydrates based for main meal; Class 4–33% cereal and toast/27% fruit/37% other, 26% soups and salads/25 other, 39% protein and carbohydrates based for main meal. Weekends: Class 5–88% cereal or toast for breakfast, 35% skip light meal/20% MF sandwich/20% other for light meal, 85% protein and carbohydrates based for main meal; Class 6–70% cereal or toast for breakfast, 24% MF sandwich/22% other for light meal, 39% protein and carbohydrates based for main meal; Class 7–45% cooked breakfast, 69% skip light meal, 76% protein and carbohydrates based for main meal.

Table 5. Daily food intakes (g/day)¹ by most Dominant Classes.

	Class 1	Class 1	Class 1	Class 2	Class 1	Class 5	Varied (n = 477)
	Weekdays/Class 5 Weekends (n = 439)	Weekdays/Class 6 Weekends (n = 114)	Weekdays/Class 7 Weekends (n = 87)	Weekdays/Class 5 Weekends (n = 100)	Weekdays (n = 148)	Weekends (n = 135)	
Grains, rice, pasta & savories	48.8 ± 57.2 ^g	67.7 ± 69.9	60.8 ± 72.4	62.7 ± 73.2	64.1 ± 72.7	45.8 ± 58.7 ^g	70.3 ± 83.2 ^{a,f}
Breakfast cereals	84.1 ± 90.5 ^{b,c,d,g}	52.9 ± 67.5 ^a	41.7 ± 57.4 ^{a,e}	51.1 ± 63.8 ^a	78.9 ± 92.9 ^{c,g}	71 ± 80.8 ^g	41.7 ± 71.5 ^{a,e,f}
Potatoes/potato dishes	133 ± 84.4 ^{b,e,g}	93.8 ± 73.4 ^{a,d,f}	114 ± 78.8 ^d	160 ± 90.2 ^{b,c,e,g}	102.7 ± 80 ^{a,d}	131 ± 87.6 ^b	110 ± 85.6 ^{a,d}
Vegetables/vegetable dishes	124 ± 79.9 ^g	110 ± 92.2	123 ± 78.3	107 ± 71.7	120 ± 78.5	113 ± 84.3	103 ± 85.8 ^a
Fruits/fruit dishes	168 ± 147 ^{d,g}	168 ± 162 ^d	144 ± 143	107 ± 131 ^{a,b}	158 ± 132.7	139 ± 151	127 ± 143 ^a
Nuts, seeds, herbs	2.80 ± 9.20	3.50 ± 10.0	3.00 ± 8.50	2.80 ± 10.3	3.13 ± 8.46	2.11 ± 6.65	2.88 ± 9.99
Milk and yogurt	283 ± 206	252 ± 196	215 ± 164	270 ± 257	228 ± 167	235 ± 185	208 ± 184
Meat/meat products	176 ± 90.0 ^d	152 ± 100 ^d	175 ± 85.2	209 ± 105 ^{a,b}	184 ± 107	176 ± 81.2	181 ± 108
Fish/fish dishes	33.1 ± 39.1 ^a	30.6 ± 44.2	36.9 ± 42.5	24.3 ± 38.1	27.7 ± 41.2	28.5 ± 40.1	24.1 ± 40.8 ^g
Cheeses	13.2 ± 17.1	17.5 ± 21.5	11.0 ± 13.7	14.8 ± 22.8	12.7 ± 41.2	12.9 ± 14.5	13.7 ± 18.5
Eggs/eggs dishes	13.3 ± 20.8 ^g	12.3 ± 18.2 ^g	20.7 ± 25.1	12.9 ± 18.8 ^g	19.2 ± 24.3	14.0 ± 20.2	20.8 ± 27.9 ^{a,b,d}
Cream, ice-cream and dessert	26.3 ± 39.6 ^g	18.2 ± 38.1	15.2 ± 25.5	22.2 ± 44.8	18.2 ± 32.5	26.8 ± 43	16.4 ± 29.3 ^a
Soups and sauces	56.4 ± 66.9	53.8 ± 69.3	65.2 ± 67.4	45.6 ± 59.5	54.7 ± 64.9	59.6 ± 66.1	58.0 ± 73.4
Sugar, confectionary, snacks	29.0 ± 27.4	30.5 ± 26.6	23.4 ± 22.3	30.5 ± 31.8	27.8 ± 26.0	26.6 ± 28.5	32.0 ± 29.5
Breads and rolls	128 ± 62.3 ^{c,d,g}	121 ± 59.2	109 ± 56.8	103 ± 63.0 ^a	120 ± 57.9	118 ± 66.0	104 ± 63.9 ^a
Beverages	1393 ± 749 ^g	1437 ± 1007	1631 ± 809	1499 ± 893	1466 ± 805	1508 ± 841	1600 ± 919 ^a
Nutritional supplements	59.1 ± 115	36.4 ± 124	69.2 ± 174	56.0 ± 111	52.9 ± 108	48.7 ± 112	50.0 ± 118
Biscuits and cakes	33.8 ± 36.5	25.8 ± 35.5	31.0 ± 28.7	24.0 ± 29.7	29.6 ± 33.6	29.1 ± 31.3	27.6 ± 36.5
Butter and oils	18.0 ± 17.7	14.8 ± 18.3	14.3 ± 12.2	15.0 ± 13.8	14.8 ± 18.3	15.2 ± 16.4	13.3 ± 14.7

¹ Values are mean (±SD). ANOVA with Bonferroni correction was applied; ^a significant difference from Class 5 weekends and Class 1 weekdays ($p < 0.05$); ^b significant difference from Class 6 weekends and Class 1 weekdays ($p < 0.05$); ^c significant difference from Class 7 weekends and Class 1 weekdays; ^d significant difference from Class 5 weekends and Class 2 weekdays ($p < 0.05$); ^e significant difference from Class 1 weekdays; ^f significant difference from Class 5 weekends; ^g significant difference from Class “Varied”. Classes definition: Weekdays: Class 1–88% cereal or toast for breakfast, 23% skip light meal/28% MF sandwich/22% other for light meal, 72% protein and carbohydrates based for main meal; Class 2–64% cereal or toast for breakfast, 23% MFD sandwich/35% MF sandwich for light meal, 23% skip main meal/60% protein and carbohydrates based for main meal; Weekends: Class 5–88% cereal or toast for breakfast, 35% skip light meal/20% MF sandwich/20% other for light meal, 85% protein and carbohydrates based for main meal; Class 6–70% cereal or toast for breakfast, 24% MF sandwich/22% other for light meal, 39% protein and carbohydrates based for main meal; Class 7–45% cooked breakfast, 69% skip light meal, 76% protein and carbohydrates based for main meal. Varied—included the dominant classes representing less than 5% of the total population and the group with no defined dominant classes over 4 days of the record.

Table 6. Clinical variables by most dominant latent classes computed over 4 days records (weekdays and weekends).

	Class 1 Weekdays/Class 5 Weekends (n = 439)	Class 1 Weekdays/Class 6 Weekends (n = 114)	Class 1 Weekdays/Class 7 Weekends (n = 87)	Class 2 Weekdays/Class 5 Weekends (n = 100)	Class 1 Weekdays (n = 148)	Class 5 Weekends (n = 153)	Varied (n = 459)
BMI, kg/m ²	26.9 ± 4.71	27.0 ± 6.03	27.3 ± 5.04	27.2 ± 4.98	27.4 ± 4.83	27.2 ± 4.39	27.0 ± 5.13
Weight, kg	76.4 ± 14.8	76.8 ± 17.6	77.5 ± 16.8	78.7 ± 16.2	78.8 ± 17.3	78.9 ± 15.4	77.8 ± 16.6
Waist-to-hip ratio	0.88 ± 0.08	0.86 ± 0.08	0.87 ± 0.09	0.89 ± 0.09	0.87 ± 0.08	0.89 ± 0.09	0.88 ± 0.09
Body fat, %	29.7 ± 8.81	28.6 ± 9.55	30.1 ± 7.79	29.0 ± 9.26	28.9 ± 9.05	29.6 ± 8.25	28.7 ± 9.51
SBP, mmHg	126 ± 18.4	122 ± 15.4	125 ± 15.8	125 ± 17.2	127.9 ± 19	124.2 ± 17.4	123.8 ± 17.9
DBP, mmHg	78.2 ± 10.7	75.6 ± 9.51 ^c	79.6 ± 10.5 ^b	78.3 ± 9.94	79.3 ± 11.5	77.4 ± 11	78.2 ± 10.7
Total cholesterol, mmol/L	4.99 ± 1.07	4.91 ± 0.83	4.89 ± 0.98	5.16 ± 0.94	4.81 ± 1.1	4.89 ± 0.94	4.91 ± 0.98
Triglycerides, mmol/L	1.31 ± 0.79	1.26 ± 0.68	1.26 ± 0.77	1.47 ± 0.94	1.26 ± 0.84	1.37 ± 0.77	1.29 ± 0.74
HDL-c, mmol/L	1.58 ± 0.43	1.53 ± 0.41	1.60 ± 0.5	1.57 ± 0.37	1.51 ± 0.4	1.5 ± 0.36	1.57 ± 0.46
LDL-c, mmol/L	2.81 ± 0.90	2.81 ± 0.74	2.72 ± 0.85	2.96 ± 0.87	2.73 ± 0.9	2.77 ± 0.84	2.75 ± 0.85
Glucose, mmol/L	5.42 ± 1.17	5.17 ± 0.85	5.50 ± 1.63	5.41 ± 0.96	5.23 ± 0.89	5.33 ± 0.89	5.26 ± 1.29
CRP, mg/L	2.72 ± 2.82	2.6 ± 2.57	3.13 ± 2.96	3.16 ± 4.23	2.46 ± 2.73	2.77 ± 2.53	2.66 ± 2.93
Serum ferritin, ng/mL	121 ± 117 ^c	105 ± 100	148 ± 152 ^a	144 ± 130	123 ± 123.4	112 ± 97.9	109 ± 94.9

¹ Values are mean and standard deviation. *P* values are adjusted for age, gender, social class, and energy intake. ^a significant difference from Class 5 weekends and Class 1 weekdays (*p* < 0.05); ^b significant difference from Class 6 weekends and Class 1 weekdays (*p* < 0.05); ^c significant difference from Class 7 weekends and Class 1 weekdays. Classes definition: Weekdays: Class 1–88% cereal or toast for breakfast, 23% skip light meal/28% MF sandwich/22% other for light meal, 72% protein and carbohydrates based for main meal, Class 2–64% cereal or toast for breakfast, 23% MFD sandwich/35% MF sandwich for light meal, 23% skip main meal/60% protein and carbohydrates based for main meal. Weekends: Class 5–88% cereal or toast for breakfast, 35% skip light meal/20% MF sandwich/20% other for light meal, 85% protein and carbohydrates based for main meal, Class 6–70% cereal or toast for breakfast, 24% MF sandwich/22% other for light meal, 39% protein and carbohydrates based for main meal, Class 7–45% cooked breakfast, 69% skip light meal, 76% protein and carbohydrates based for main meal. Varied–included the dominant classes representing less than 5% of the total population and the group with no defined dominant classes over 4 days of the record.

4. Discussion

In this study, we identified the most common meal patterns in Ireland. We used a novel technique, of using generic meal data within LCA, that applied to NANS data allows for identification of food/food group combinations at different eating occasion during the day. Accounting also for difference in patterns between weekdays and weekends, we were able to organize people into meaningful groups with similar dietary behaviour. Thus, a total of 7 distinctive meal patterns were characterized. The majority of Irish adults followed a dietary lifestyle characterised by cereal or toasts for breakfast, skipped or consumed a sandwich as a light meal, and meat or fish with potatoes, pasta or vegetables for the main meal.

A number of methods have been described to elucidate dietary patterns in different populations. Data-driven approaches such as PCA and FA are widely used in nutritional epidemiology [2], whereas LCA has been used mostly in social studies [9] and with limited application to study dietary behaviours. Padmadas et al. applied LCA to understand dietary intake pattern from Indian National Family Health Survey [12]. Seven food groups each with four categories of frequency of intake were used for the analyses, and derived 5 mutually exclusive classes, which reflected the heterogeneity of dietary behaviour among Indian women population. Another study by Sotres-Alvarez et al. also used LCA to derive dietary patterns based on the dietary data from the 3rd cohort of the Pregnancy, Infection and Nutrition (PIN) Study of women [11]. In particular, they used the data from reported intakes of 105 food groups on which individuals were categorized into non-consumers (0 g/day), low consumers and high consumers (below and higher than median), respectively [11]. Three main dietary patterns were derived: Prudent, which was high in fibre, folate, and vitamins; Health Conscious Western—greater intakes of fast food, salty snacks, and sweets, fruits and vegetables; and Hard-Core Western—decreased intakes of fruits and vegetables, nuts and beans, and increased consumption of fried meat, fish, white bread, and sugar sweetened beverages [14]. However, to date most of the studies in the literature that have applied LCA to derive dietary patterns used food-based models which significantly limit the assessment of complexity of the diet, as it only gives the estimation of food types consumed within the identified dietary pattern and does not reflect the timing when the food is consumed or the combination of foods consumed at a single occasion, e.g., breakfast, light meal, or snack. Nor they can be used to explore the sequence of patterns and estimate to what extent a specific type of meal might impact the subsequent food intakes throughout the day. An interesting attempt to circumvent the food based approach and study dietary groups was performed by Wang et al. in the study of Australian men and women [22]. In this particular study, LCA was based on the diets followed such as low-fat, low-fat/low sugar, low-salt, and glycaemic index (GI) diets, prescription diets, gluten-free, vegetarian, vegan, high protein, or lactose free to derived dietary patterns which would determine participants preferences towards these types of diets [22]. The only study, to date, which attempted to examine dietary patterns by meal type was conducted based on Main Meals Repertoire Survey which captured the information on 81 dishes prepared for main meals, for example, beef burger, vegetable soup, fish steak, sausages, roasted lamb, lasagne, etc. without considering side dishes or starters [23].

Although in nutritional epidemiology some studies have attempted to tackle the lack of research involving meal patterns, the application of LCA to study meal intakes remains largely unexplored. The data driven approach using LCA has been utilized previously in Irish adult population in order to determine typical dietary patterns [24], however, it makes it difficult to compare with the meal patterns derived in our study, as the models were food-based and determined only “extreme” patterns: “Healthy”, characterized by high intakes of fruits and vegetables, low fat dairy, and high compliance with DASH dietary index; “Western”—characterised by higher intakes of cereals, breads and potatoes, processed meat and foods from the upper corner of the food pyramid; and “low energy”—included lower intake sweets, red meat, and energy intake overall.

Within the current analysis, meal patterns derived by LCA were determined separately for weekdays and weekends, taking into account the variability that exists in dietary intakes between weekdays and weekends [25]. Alignment of derived meal patterns with actual intake was performed

by comparing the actual foods and nutrients intakes across different LCA classes. For example, participants in dietary patterns with highest probability to have breakfast cereal for meal type “breakfast”, bread and rolls for “light meal”, and protein based “main meal” were shown to have the highest actual intake of these food groups. However, there were some discrepancies. The intake of some food items, such as eggs, fish, or meat, which might be consumed as a sandwich, cooked breakfast, or salad, were not in agreement with the actual meat intake when compared. Furthermore, food serving size was not available, and generic meals capture only the types of foods consumed assuming an average portion size [13]. Therefore, there is a possibility that for a group of participants which would consume a small amount of given food but more frequently, this would result in the highest probability of consuming this particular food, but the actual dietary intake would be low. As such, further refinement in generic meal definition including food weight is needed in order to reduce such error.

Linking meal patterns to phenotypes and disease risks, in our study, we observed that meal patterns differed between weekdays and weekends, with results showing that number of participants were more likely to consume cooked breakfast consisted of meat and eggs or roll instead of breakfast cereal on weekends. Those opting to follow this meal pattern on weekends had on average an unhealthier overall diet characterized by lower intakes of vegetables and fruits, milk and dairy, and higher intake of meat and meat dishes, as well as 3.14-fold higher odds for increased serum ferritin. The most studied meal in the scientific literature is breakfast, which shows that breakfast consist of cereals or toast is associated with satiety, overall daily energy intake, and appetite regulation [26,27], as well CVD risk factors [28] and atherosclerosis [29]. The role of other meals, in particular light meal, remained largely unexplored, and requires further research.

There are strengths and limitations to the work presented here. Strengths of this study include the large, nationally representative sample of Irish men and women and the comprehensive nutritional assessment which captured variation in population’s dietary intake over 4 days. The ability to capture overall dietary intake in the identification of our dietary classes by using frequency of consumption instead of incorporating the mean daily food or nutrients intakes in the model, as it has been done for studies using PCA, is another strength. By analysing dietary patterns followed during weekdays and weekends we were able to understand how stable the dietary patterns were, and whether those who follow a particular pattern during the weekdays keep adhering to the same dietary behaviours during weekends. A novel feature of this work was the application of the innovative approach to generate unique generic meals using recently published meal coding system [13,30], that could be translated into other data sets and the use of LCA to examine meal based eating patterns while taking into account meal occasion across the day. The number of classes were identified using standardised criteria which minimized researchers’ involvement into the patterns determination and therefore interpretability of the findings. In the previous study [13] where the meal aggregation method was first developed and applied in principal component analysis in order to identify meal patterns, the main limitation which affected the findings obtained, was a high inter-individual variation in diet across the population. In this work, by reducing the large variation in the meal types, we were able to derive smaller number of meal patterns and categorise all the participants into one of them. On the other hand, a major limitation is the person-centred, data-driven approach which makes the findings non-generalizable to other populations. In addition, within the present study as the population had relatively similar dietary behaviours, and it would be interesting to conduct future analysis with more heterogeneous populations to determine more extreme meal patterns.

5. Conclusions

LCA was observed to be a useful exploration tool, which in combination with generic meal-coding system could simplify the complexity of dietary data and derive interpretable meal based dietary patterns. Further work will be directed at refining the generic-meal coding system to include serving size in the meal definition and explore snacking and beverages consumption. These findings could

be applied to tackle the chronic diseases by translating the message into public health guidelines and recommendations, complementing current dietary advice to assist the population in achieving the recommended daily intakes of foods and nutrients. Moreover, meal-based dietary guidelines may be easier translate to the population and for the individuals to follow.

Supplementary Materials: The following are available online at www.mdpi.com/2072-6643/10/3/310/s1, Table S1: Recoding of generic meal codes and descriptions for the meal type “breakfast”, Table S2: Recoding of generic meal codes and descriptions for the meal type “light meal”, Table S3: Recoding of generic meal codes and descriptions for the meal type “main meal”, Table S4: Demographic and lifestyle-related characteristics of National Adult Nutrition Survey (NANS) population presented by gender, Table S5: Intakes (g/day or mL/day) of selected foods by latent classes during breakfast, Table S6: Intakes (g/day or mL/day) of selected foods by latent classes during light meal, Table S7: Intakes (g/day or mL/day) of selected foods by latent classes during main meal, Table S8: Intakes (g/day or mL/day) of selected foods by latent classes during snacking occasions, Table S9: Association between most dominant latent classes computed over 4 days records (weekdays and weekdays) and serum ferritin and DBP.

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Supplemental Material

Generic Meal Patterns Identified by Latent Class Analysis: Insights from NANS (National Adult Nutrition Survey)

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Supplemental Table 1. Recoding of generic meal codes and descriptions for the meal type “breakfast”

New Generic Meal Code and Description		Consumption Frequency, <i>n</i> (%)	Previous Generic Meal Code and Description		Consumption Frequency, <i>n</i> (%)
0	Skip breakfast	412 (6.9)	0	No breakfast	412 (6.9)
		4287 (71.5)	1.1	Cereals and milk and bread and juice	330 (5.5)
1	Cereal and toast or cereal or toast		1.2	Cereals and milk and bread and fruit	147 (2.5)
			1.3	Cereals and milk and bread and other	559 (9.3)
			2.1	Cereals and milk and juice	480 (8.0)
			2.2	Cereals and milk and fruit	305 (5.1)
			2.3	Cereals and milk and other	1383 (23.1)
			3.3	Bread and fruit	170 (2.8)
			3.4	Bread and juice	88 (1.5)
			3.5	Bread and other	825 (13.8)
			3.1	Bread and cooked breakfast	359 (6.0)
			3.2	Bread and egg	190 (3.2)
3	Fruit/fruit juice	236 (3.9)	4.2	Fruit/juice	236 (3.9)
4	Other	516 (8.6)	4.1	Confectionery	265 (4.4)
			4.3	Other	251 (4.2)
Total		6000 (100)	Total		6000 (100)

Supplemental Table 2. Recoding of generic meal codes and descriptions for the meal type “light meal”.

New Generic Meal Code and Description		Consumption Frequency, <i>n</i> (%)	Previous Generic Meal Code and Description		Consumption Frequency, <i>n</i> (%)
0	Skip light meal	1722 (28.7)	0	Skip light meal	1722 (28.7)
		678 (11.3)	1.1	Bread cheese meat/fish vegetables	177 (3.0)
			1.2	Bread cheese meat/fish fat	185 (3.1)
1	MFD sandwich		1.3	Bread cheese meat/fish fat vegetables	150 (2.5)
			1.4	Bread cheese meat/fish and other	166 (2.8)
		343 (5.7)	2.1	Bread cheese vegetables	72 (1.2)
			2.2	Bread cheese fat	120 (2.0)
2	Dairy sandwich		2.3	Bread cheese vegetables fat	81 (1.4)
			2.4	Bread cheese and other	70 (1.2)
		1438 (24.0)	3.1	Bread meat/fish vegetables	351 (5.9)
			3.2	Bread meat/fish fat	491 (8.2)
3	MF sandwich		3.3	Bread meat/fish vegetables fat	309 (5.2)
			3.4	Bread meat/fish and other	287 (4.8)
4	Soup and salad	460 (7.7)	4	Soups	342 (5.7)
			5	Salads	118 (2.0)
5	Rice potato pasta	207 (3.5)	8	Rice potato pasta	207 (3.5)
			6	Egg based	231 (3.9)
6	Other	1162 (19.2)	7	Bread and other	380 (6.3)
			9	Other	541 (9.0)
	Total	6000 (100)		Total	6000 (100)

MFD – meat/fish/dairy

Supplemental Table 3. Recoding of generic meal codes and descriptions for the meal type “main meal”.

New Generic Meal Code and Description		Consumption Frequency, <i>n</i> (%)	Previous Generic Meal Code and Description		Consumption Frequency, <i>n</i> (%)
0	Skip main meal	347 (5.8)	0	Skip main meal	347 (5.8)
		4130 (68.8)	1.1	Meat/fish and rice/potato/pasta and vegetable and soups/sauces and confectionery	399 (6.7)
1	Protein and carbohydrates	874 (14.6)	1.3	Meat/fish and rice/potato/pasta and vegetable and confectionery	519 (8.7)
			2.1	Meat/fish and rice/potato/pasta and soups/sauces and confectionery	283 (4.7)
			1.2	Meat/fish and rice/potato/pasta and vegetable and soups/sauces	779 (13.0)
			1.4	Meat/fish and rice/potato/pasta and vegetable and other	1247 (20.8)
			2.2	Meat/fish and rice/potato/pasta and soups/sauces	243 (4.1)
			2.3	Meat/fish and rice/potato/pasta and other	660 (11.0)
			3.1	Meat/fish and vegetable and confectionery	153 (2.6)
			5	Meat/fish and confectionery	145 (2.4)
2	Protein	472 (7.9)	3.2	Meat/fish and vegetable	287 (4.8)
			6	Meat/fish no vegetables and other	289 (4.8)
3	Carbohydrates	472 (7.9)	4	Rice/potato/pasta and vegetable	218 (3.6)
			7	Rice/potato/pasta and no vegetables	254 (4.2)
4	Other	177 (3.0)	8	Other	177 (3.0)
Total		6000 (100)	Total		6000 (100)

Supplemental Table 4. Demographic and lifestyle-related characteristics of National Adult Nutrition Survey (NANS) population presented by gender.

	Male (n=740)	Female (n=760)	Total (n=1500)
Age, years	43.8±17.2	45.2±16.8	44.5±17.0
Energy intake	2237±663	1698±471	2013±656
Social class			
Professional/manager	322 (44.5)	348 (48.9) *	670 (46.7)
Non-manual skilled	113 (15.6)	154 (21.7)	267 (18.6)
Manual skilled	131 (18.1)	82 (11.5)	213 (14.8)
Semi-skilled /unskilled	158 (21.8)	127 (17.9)	285 (19.9)
Supplement use			
Yes	194 (26.5)	268 (35.8) *	462 (31.2)
No	439 (60.1)	363 (48.5)	802 (54.2)
Sometimes	98 (13.4)	118 (15.8)	216 (14.6)
Smoking			
Current smoker	147 (20.2)	156 (20.8)	303 (20.5)
Former smoker	202 (27.7)	302 (27.0)	405 (27.4)
Never smoker	380 (52.1)	392 (52.2)	722 (52.2)
Alcohol consumption			
Almost every day	322 (44.1)	237 (31.6) *	559 (37.8)
Once a week	170 (23.3)	170 (22.7)	340 (23.0)
2 times per / month	64 (8.80)	86 (11.5)	150 (10.1)
Once a month or less	99 (13.6)	131 (17.5)	230 (15.5)
Never	75 (10.3)	126 (16.8)	201 (13.6)
Responsible for grocery			
Myself	211 (28.5)	555 (73.0) *	766 (51.1)
Others	529 (71.5)	205 (27.0)	734 (48.9)
Responsible for cooking			
Myself	239 (32.3)	585 (77.0) *	824 (54.9)
Others	501 (67.7)	175 (23.0)	676 (45.1)

Values are presented as N (%) or mean (±SD). * significant difference between males and females (chi-square analysis is performed).

Supplemental Table 5. Intakes¹ (g/day or ml/day) of selected foods by latent classes during breakfast.

Latent Classes	Weekdays				Weekends		
	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6	Class 7
N (%)	2288 (60.0)	988 (25.9)	360 (9.4)	179 (4.7)	1249 (57.2)	469 (21.5)	467 (21.4)
Breakfast cereals	72.5±96.8 ^{bcd}	41.3±77.4 ^a	45.9±97.2 ^a	34.1±61.1 ^a	73.5±98.2 ^{fg}	55.6±86.5 ^{eg}	0.00±0.00 ^{ef}
Fruits/fruit dishes	64.6±107 ^{bd}	45.8±99.6 ^a	59.1±107 ^d	32.3±82.6 ^{ac}	51.8±94.5	63.4±120	51.1±113
Milk and yogurt	112±111 ^{bc}	97.4±115 ^{ac}	78.0±108 ^{ab}	95.0±121	111±110 ^g	104±115 ^g	43.9±80.2 ^{ef}
Meat/meat products	0.37±5.65 ^{bcd}	11.2±35.1 ^{ac}	7.39±27.1 ^{ab}	10.7±32.9 ^a	1.93±21.8 ^g	6.10±25.7 ^g	41.4±65.2 ^{ef}
Eggs/eggs dishes	0.85±9.63 ^{bcd}	12.3±32.4 ^{ac}	8.18±25.7 ^{ab}	9.89±34.2 ^a	2.18±13.5 ^{fg}	6.85±27.2 ^{eg}	29.4±44.5 ^{ef}
Sugar, confectionary, snacks	6.21±11.0	6.69±12.8	5.01±9.62	6.02±10.8	7.02±11.9 ^g	5.84±10.5	4.24±11.0 ^e
Breads and rolls	26.7±37.1 ^b	40.3±48.2 ^{acd}	30.5±42.0 ^b	26.0±39.1 ^b	34.5±39.8 ^g	30.5±41.7 ^g	42.6±47.8 ^{ef}
Beverages (incl. water)	251±233	263±246	243±246	242±216	263±241	256±258	278±317

¹ Values are mean (±SD). ANOVA with Bonferroni correction was applied. Multiple comparison was performed for Classes 1-4 and Classes 5-7 separately. Classes definition: Weekdays: Class 1—88% cereal or toast for breakfast, 23% skip light meal / 28% MF sandwich/ 22% other for light meal, 72% protein and carbohydrates based for main meal; Class 2—64% cereal or toast for breakfast, 23% MFD sandwich / 35% MF sandwich for light meal, 23% skip main meal / 60% protein and carbohydrates based for main meal; Class 3—13% cereal or toast / 44% cooked breakfast / 25% other for breakfast, 57% skip light meal, 64% protein and carbohydrates based for main meal; Class 4—33% cereal and toast / 27% fruit / 37% other, 26% soups and salads / 25 other, 39% protein and carbohydrates based for main meal. ^a significant difference from Class 1 ($p < 0.05$); ^b significant difference from Class 2 ($p < 0.001$); ^c significant difference from Class 3 ($p < 0.001$); ^d significant difference from Class 4 ($p < 0.001$). Weekends: Class 5—88% cereal or toast for breakfast, 35% skip light meal / 20% MF sandwich/ 20% other for light meal, 85% protein and carbohydrates based for main meal; Class 6—70% cereal or toast for breakfast 24% MF sandwich / 22% other for light meal, 39% protein and carbohydrates based for main meal; Class 7—45% cooked breakfast, 69% skip light meal, 76% protein and carbohydrates based for main meal. ^e significant difference from Class 5 ($p < 0.05$); ^f significant difference from Class 6 ($p < 0.001$); ^g significant difference from Class 7 ($p < 0.001$).

Supplemental Table 6. Intakes¹ (g/day or ml/day) of selected foods by latent classes during light meal.

Latent Classes	Weekdays				Weekends		
	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6	Class 7
N (%)	2288 (60.0)	988 (25.9)	360 (9.4)	179 (4.7)	1249 (57.2)	469 (21.5)	467 (21.4)
Grains, rice, pasta & savouries	2.16±18.9 bcd	9.5±46.2 ad	13.0±57.5 ad	28.3±84.3 abc	2.12±22.5 f	24.2±79.0 eg	1.62±22.3 f
Potatoes/potato dishes	3.26±22.6 bcd	9.62±45.0 ad	9.06±41.2 ad	33.5±76.5 abc	3.01±21.8 f	21.3±65.6 eg	1.43±13.1 f
Vegetables/vegetable dishes	33.7±60.6 bcd	8.08±30.3 acd	22.4±79.2 abd	47.2±64.4 abc	20.5±54.5 fg	36.7±73.3 eg	8.34±33.4 ef
Fruits/fruit dishes	41.4±88.7 bc	7.19±36.4 ad	6.97±33.7 ad	34.0±99.3 bc	21.0±68.3 g	24.6±73.7 e	6.92±34.9 ef
Milk and yogurt	8.11±24.3 bc	0.90±7.99 ad	2.59±13.9 ad	6.67±25.4 bc	31.2±69.2 g	36.7±74.8 e	9.97±46.2 ef
Meat and meat products	34.5±58.5 bcd	13.6±45.6 ad	10.3±43.1 ad	59.5±69.3 abc	22.6±53.3 fg	53.0±87.7 eg	7.25±27.7 ef
Fish and fish dishes	8.84±33.5 bc	2.92±19.0 ad	4.28±24.8 ad	11.6±37.1 bc	22.6±53.3 f	53.0±87.7 eg	7.25±27.7 f
Cheeses	10.8±22.0 bcd	2.32±10.4 ad	1.23±8.60 ad	15.4±26.2 abc	7.23±19.3 g	8.19±20.0 g	3.66±12.6 ef
Eggs and eggs dishes	7.17±27.0 bc	0.89±9.00 ad	1.52±11.8 a	6.91±32.8 b	7.18±27.9 g	9.16±30.1 g	1.50±13.2 ef
Biscuits, cakes and pastries	8.11±24.3 bc	0.90±7.99 ad	2.59±13.9 a	6.67±25.4 b	6.49±22.3 g	8.71±34.9 g	2.75±16.9 ef
Cream, ice-cream and dessert	2.31±22.2 d	0.62±10.2 d	0.93±15.7 d	7.63±38.1 abc	2.46±18.8	4.42±23.3 g	0.53±7.68 f
Soups and sauces	31.7±90.4 b	13.1±59.6 acd	42.1±105 b	43.0±102 b	17.3±64.2 f	30.4±86.6 eg	14.2±63.8 f
Sugar, confectionary, snacks	6.22±14.69 bc	2.22±9.59 ad	1.92±8.43 ad	7.27±16.2 bc	3.90±12.5 f	6.66±22.2 eg	2.16±12.2 f
Breads and rolls	73.3±50.1 bcd	15.4±37.1 ad	12.5±36.6 ad	82.7±52.9 abc	46.3±50.9 fg	53.6±58.1 eg	16.5±39.1 ef
Beverages (incl. water)	305±285 bcd	87.7±196 ad	124±252 ad	361±270 abc	189±251 fg	324±355 eg	77.5±201 ef

¹ Values are mean (±SD). ANOVA with Bonferroni correction was applied. Multiple comparison was performed for Classes 1-4 and Classes 5-7 separately. Classes definition: Weekdays: Class 1—88% cereal or toast for breakfast, 23% skip light meal / 28% MF sandwich/ 22% other for light meal, 72% protein and carbohydrates based for main meal; Class 2—64% cereal or toast for breakfast, 23% MFD sandwich / 35% MF sandwich for light meal, 23% skip main meal / 60% protein and carbohydrates based for main meal; Class 3—13% cereal or toast / 44% cooked breakfast / 25% other for breakfast, 57% skip light meal, 64% protein and carbohydrates based for main meal; Class 4—33% cereal and toast / 27% fruit / 37% other, 26% soups and salads / 25 other, 39% protein and carbohydrates based for main meal. ^a significant difference from Class 1 ($p < 0.05$); ^b significant difference from Class 2 ($p < 0.001$); ^c significant difference from Class 3 ($p < 0.001$); ^d significant difference from Class 4 ($p < 0.001$). Weekends: Class 5—88% cereal or toast for breakfast, 35% skip light meal / 20% MF sandwich/ 20% other for light meal, 85% protein and carbohydrates based for main meal; Class 6—70% cereal or toast for breakfast 24% MF sandwich / 22% other for light meal, 39% protein and carbohydrates based for main meal; Class 7—45% cooked breakfast, 69% skip light meal, 76% protein and carbohydrates based for main meal. ^e significant difference from Class 5 ($p < 0.05$); ^f significant difference from Class 6 ($p < 0.001$); ^g significant difference from Class 7 ($p < 0.001$).

Supplemental Table 7. Intakes¹ (g/day or ml/day) of selected foods by latent classes during main meal.

Latent Classes	Weekdays				Weekends		
	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6	Class 7
N (%)	2288 (60.0)	988 (25.9)	360 (9.4)	179 (4.7)	1249 (57.2)	469 (21.5)	467 (21.4)
Grains, rice, pasta & savouries	51.1±105 ^{bcd}	72.2±124 ^{acd}	11.8±55.8 ^{ab}	0.00±0.00 ^{ab}	37.7±84.5 ^{fg}	63.0±139 ^e	54.0±117 ^e
Potatoes/potato dishes	112±121 ^{bcd}	150±134 ^{acd}	2.00±20.8 ^{ab}	0.00±0.00 ^{ab}	141±120 ^{fg}	34.1±84.9 ^{eg}	126±121 ^{ef}
Vegetables/vegetable dishes	84.0±96.1 ^d	88.1±104 ^d	80.6±134 ^d	10.7±49.0 ^{abc}	98.4±92.8 ^{fg}	64.2±120 ^{eg}	80.5±96.0 ^{ef}
Fruits/fruit dishes	21.1±66.2 ^d	18.0±69.3 ^d	21.7±73.1 ^d	1.30±10.0 ^{abc}	20.8±70.5 ^g	15.0±59	10.0±49.0 ^e
Milk and yogurt	51.7±117 ^d	57.2±131 ^{cd}	36.9±95.6 ^{bd}	7.80±49.2 ^{abc}	44.6±114 ^f	27.2±81.2 ^e	43.9±122
Meat and meat products	135±135 ^{bd}	155±138 ^{acd}	127±170 ^{bd}	51.0±134 ^{abc}	160±125 ^{fg}	59.0±115 ^{eg}	142±123 ^{ef}
Fish and fish dishes	25.3±68.3 ^d	26.4±73.2 ^d	16.2±53.2	5.90±32.8 ^{ab}	20.2±62.5 ^f	7.40±42.8 ^e	13.9±49.9
Cheeses	2.30±11.1 ^c	2.10±8.80 ^c	7.20±22.1 ^{abd}	0.80±4.60 ^c	2.64±13.0	4.04±15.5	3.31±15.1
Eggs and eggs dishes	3.60±22.1 ^c	2.70±16.1 ^c	11.4±46.4 ^{abd}	0.80±8.30 ^c	2.72±16.1 ^f	7.43±35.8 ^{eg}	3.06±21.3 ^f
Biscuits, cakes and pastries	7.00±24.0 ^d	6.70±24.2 ^d	6.60±26.5 ^d	0.70±6.59 ^{abc}	10.4±30.2 ^{fg}	4.87±20.9 ^e	4.74±18.7 ^e
Cream, ice-cream and dessert	11.2±40.9 ^d	10.4±44.7 ^d	5.80±34.1	0.30±4.50 ^{ab}	20.4±54.5 ^{fg}	7.40±40.3 ^e	11.0±36.0 ^e
Soups and sauces	25.6±65.5 ^d	30.6±65.5 ^d	34.6±100 ^d	0.60±3.80 ^{abc}	28.9±60.9 ^f	16.5±50.0 ^{eg}	28.4±65.2 ^f
Sugar, confectionary, snacks	3.08±11.1 ^d	2.77±8.60 ^c	4.61±13.1 ^{bd}	0.53±3.80 ^{ac}	2.67±9.67 ^f	5.18±23.5 ^{eg}	2.84±12.9 ^f
Breads and rolls	11.5±35.5 ^c	11.9±33.4 ^c	35.9±55.5 ^{abd}	7.10±27.5 ^c	13.0±34.7 ^f	21.3±41.9 ^{eg}	13.6±36.3 ^f
Beverages (incl. water)	316±345 ^{bd}	378±424 ^{acd}	307±379 ^{bd}	72.0±205 ^{abc}	406±563 ^f	315±509 ^e	386±595

¹ Values are mean (±SD). ANOVA with Bonferroni correction was applied. Multiple comparison was performed for Classes 1-4 and Classes 5-7 separately. Classes definition: Weekdays: Class 1—88% cereal or toast for breakfast, 23% skip light meal / 28% MF sandwich/ 22% other for light meal, 72% protein and carbohydrates based for main meal; Class 2—64% cereal or toast for breakfast, 23% MFD sandwich / 35% MF sandwich for light meal, 23% skip main meal / 60% protein and carbohydrates based for main meal; Class 3—13% cereal or toast / 44% cooked breakfast / 25% other for breakfast, 57% skip light meal, 64% protein and carbohydrates based for main meal; Class 4—33% cereal and toast / 27% fruit / 37% other, 26% soups and salads / 25 other, 39% protein and carbohydrates based for main meal. ^a significant difference from Class 1 ($p < 0.05$); ^b significant difference from Class 2 ($p < 0.001$); ^c significant difference from Class 3 ($p < 0.001$); ^d significant difference from Class 4 ($p < 0.001$). Weekends: Class 5—88% cereal or toast for breakfast, 35% skip light meal / 20% MF sandwich/ 20% other for light meal, 85% protein and carbohydrates based for main meal; Class 6—70% cereal or toast for breakfast 24% MF sandwich / 22% other for light meal, 39% protein and carbohydrates based for main meal; Class 7—45% cooked breakfast, 69% skip light meal, 76% protein and carbohydrates based for main meal. ^e significant difference from Class 5 ($p < 0.05$); ^f significant difference from Class 6 ($p < 0.001$); ^g significant difference from Class 7 ($p < 0.001$).

Supplemental Table 8. Intakes¹ (g/day or ml/day) of selected foods by latent classes during snacking occasions.

Latent Classes	Weekdays				Weekends		
	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6	Class 7
N (%)	2288 (60.0)	988 (25.9)	360 (9.4)	179 (4.7)	1249 (57.2)	469 (21.5)	467 (21.4)
Potatoes/potato dishes	1.15±12.9 ^{cd}	2.60±22.9 ^d	5.46±35.6 ^{ad}	11.9±55.4 ^{abc}	2.96±27.5 ^g	7.17±40.3	11.2±55.0 ^e
Fruits/fruit dishes	46.1±95.4	44.9±106	51.1±122	57.1±116	38.9±90.9 ^g	34.8±90.1	24.4±80.1 ^e
Milk and yogurt	49.4±95.2 ^d	57.7±110	59.3±122	75.0±135 ^a	43.1±85.8	47.4±103	45.9±114
Meat and meat products	5.68±28.4 ^{bcd}	11.7±44.9 ^a	13.6±43.2 ^a	16.9±61.7 ^a	7.67±32.4 ^g	11.9±46.0	15.4±52.5 ^e
Biscuits, cakes and pastries	14.1±31.2	16.8±37.4	16.6±37.9	17.6±42.2	15.3±36.3	15.2±42.7	15.5±37.9
Cream, ice-cream and dessert	5.21±30.9 ^d	4.59±24.9 ^d	5.30±24.9	12.0±50.1 ^{ab}	7.47±33.5 ^f	3.37±21.0 ^e	5.59±28.0
Soups, sauces, & miscellaneous	3.52±29.5	5.37±32.7	6.87±50.3	3.25±17.4	3.35±27.3	5.82±43.8	4.68±36.1
Sugar, confectionary, savoury snacks	12.4±26.7 ^{bcd}	17.1±32.0 ^a	16.9±33.9 ^a	22.2±39.1 ^a	13.5±31.7 ^g	14.8±31.1	19.0±35.0 ^e
Breads and rolls	17.9±38.6 ^{bcd}	28.8±54.9 ^a	26.5±54.1 ^a	30.9±58.3 ^a	17.4±39.2	13.2±35.5	17.3±38.4
Beverages (incl. water)	316±422 ^{bd}	364±457 ^a	370±499	439±489 ^a	334±500	308±491	357±513

¹ Values are mean (±SD). ANOVA with Bonferroni correction was applied. Multiple comparison was performed for Classes 1-4 and Classes 5-7 separately. Classes definition: Weekdays: Class 1—88% cereal or toast for breakfast, 23% skip light meal / 28% MF sandwich/ 22% other for light meal, 72% protein and carbohydrates based for main meal; Class 2—64% cereal or toast for breakfast, 23% MFD sandwich / 35% MF sandwich for light meal, 23% skip main meal / 60% protein and carbohydrates based for main meal; Class 3—13% cereal or toast / 44% cooked breakfast / 25% other for breakfast, 57% skip light meal, 64% protein and carbohydrates based for main meal; Class 4—33% cereal and toast / 27% fruit / 37% other, 26% soups and salads / 25 other, 39% protein and carbohydrates based for main meal. ^a significant difference from Class 1 ($p < 0.05$); ^b significant difference from Class 2 ($p < 0.001$); ^c significant difference from Class 3 ($p < 0.001$); ^d significant difference from Class 4 ($p < 0.001$). Weekends: Class 5—88% cereal or toast for breakfast, 35% skip light meal / 20% MF sandwich/ 20% other for light meal, 85% protein and carbohydrates based for main meal; Class 6—70% cereal or toast for breakfast 24% MF sandwich / 22% other for light meal, 39% protein and carbohydrates based for main meal; Class 7—45% cooked breakfast, 69% skip light meal, 76% protein and carbohydrates based for main meal. ^e significant difference from Class 5 ($p < 0.05$); ^f significant difference from Class 6 ($p < 0.001$); ^g significant difference from Class 7 ($p < 0.001$).

Supplemental Table 9. Association between most dominant latent classes computed over 4 days records (weekdays and weekends) and serum ferritin and DBP.

	Class 1 weekdays / Class 5 weekends (n = 493)	Class 1 weekdays/ Class 6 weekends (n = 114)	Class 1 weekdays / Class 7 weekends (n = 87)	Class 2 weekdays / Class 5 weekends (n = 100)
Adjusted OR (95% CI)				
Increased serum ferritin ^a				
Model 1	1 (ref)	1.25 (0.67; 2.33)	2.75 (1.47; 5.16) [†]	2.11 (1.14; 3.92) [*]
Model 2	1 (ref)	1.48 (0.78; 2.79)	3.14 (1.63; 6.03) [†]	1.91 (1.00; 3.65)
Hypertension				
Model 1	1 (ref)	0.32 (0.09; 1.11)	1.31 (0.53; 3.21)	0.86 (0.36; 2.09)
Model 2	1 (ref)	0.36 (0.10; 1.26)	1.46 (0.59; 3.63)	0.60 (0.22; 1.68)

* $p < 0.05$, $† p < 0.01$, $†† p < 0.0001$. Classes definition: Weekdays: Class 1—88% cereal or toast for breakfast, 23% skip light meal / 28% MF sandwich/ 22% other for light meal, 72% protein and carbohydrates based for main meal. Weekends: Class 5—88% cereal or toast for breakfast, 35% skip light meal / 20% MF sandwich/ 20% other for light meal, 85% protein and carbohydrates based for main meal; Class 6—70% cereal or toast for breakfast, 24% MF sandwich / 22% other for light meal, 39% protein and carbohydrates based for main meal; Class 7—45% cooked breakfast, 69% skip light meal, 76% protein and carbohydrates based for main meal. Models were adjusted as follows: Model 1: age, sex. Model 2: social class, energy intake. ^aModel 1 was only adjusted for age.

6th PUBLICATION:

The importance of breakfast in atherosclerosis disease:

Insights from the PESA study.

J Am Coll Cardiol. 2017;70(15):1833-1842.

GENERAL INTRODUCTION / INDIVIDUAL'S CONTRIBUTION

LCA approach applied to derive meal patterns has proven to be a promising technique in dietary pattern analysis; however, limited results were observed with regards to the cardio-metabolic outcomes. Moreover, while working with meal patterns it was observed that there is a lack of universal definition with regard to meals, indicating that more research in this area is needed.

Therefore, it was proposed to study the eating occasions individually, and in particular breakfast consumption, by identifying different breakfast patterns and how they relate to presence of CVD risk factors, distribution, and extension of subclinical atherosclerosis.

Using the eating occasions dietary data from PESA study I identified three breakfast patterns: high energy breakfast (HBF) identified as “the first meal of the day eaten before 10:00 in the morning, and of an energy level between 20 and 35% of total daily energy need” and “consisted of at least one food group, and not limited to only coffee, water or nonalcohol beverage”; low energy breakfast (LBF), was applied to breakfast types for which the energy level ranged between 5% and 20% of total energy intake (TEI), and breakfast skipping (SBF), was defined as energy consumed at breakfast lower than 5% of TEI.

I further analysed breakfast patterns with relation to demographic and lifestyle characteristics by performing ANOVA and chi-square. The results of the disease frequencies showed that subclinical, non-coronary and generalized atherosclerosis is more likely to be seen among SBF participants. In order to investigate the association between breakfast patterns and direct indicators of atherosclerosis I performed a logistic regression adjusted for demographic, lifestyle, and dietary variables, as well as traditional CVD risk factors.

ORIGINAL INVESTIGATIONS

The Importance of Breakfast in Atherosclerosis Disease

Insights From the PESA Study



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ABSTRACT

BACKGROUND Daily habits, including the number and quality of eating occasions, are potential targets for primary prevention strategies with large health impacts. Skipping breakfast is considered a frequent and unhealthy habit associated with an increased cardiovascular (CV) risk.

OBJECTIVES The study sought to explore the association between different breakfast patterns and CV risk factors and the presence, distribution, and extension of subclinical atherosclerosis.

METHODS Cross-sectional analysis was performed within the PESA (Progression of Early Subclinical Atherosclerosis) study, a prospective cohort of asymptomatic (free of CV events at baseline) adults 40 to 54 years of age. Lifestyle and multivascular imaging data along with clinical covariates were collected from 4,052 participants. Multivariate logistic regression models were used in the analysis.

RESULTS Three patterns of breakfast consumption were studied: high-energy breakfast, when contributing to >20% of total daily energy intake (27% of the population); low-energy breakfast, when contributing between 5% and 20% of total daily energy intake (70% of the population); and skipping breakfast, when consuming <5% of total daily energy (3% of the population). Independent of the presence of traditional and dietary CV risk factors, and compared with high-energy breakfast, habitual skipping breakfast was associated with a higher prevalence of noncoronary (odds ratio: 1.55; 95% confidence interval: 0.97 to 2.46) and generalized (odds ratio: 2.57; 95% confidence interval: 1.54 to 4.31) atherosclerosis.

CONCLUSION Skipping breakfast is associated with an increased odds of prevalent noncoronary and generalized atherosclerosis independently of the presence of conventional CV risk factors. (Progression of Early Subclinical Atherosclerosis [PESA]; [NCT01410318](https://doi.org/10.1016/j.jacc.2017.08.027)) (J Am Coll Cardiol 2017;70:1833–42) © 2017 by the American College of Cardiology Foundation.



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ABBREVIATIONS AND ACRONYMS

BMI	= body mass index
CAC	= coronary artery calcium
CI	= confidence interval
CVD	= cardiovascular disease
EI	= energy intake
HBF	= high-energy breakfast
LBF	= low-energy breakfast
MetS	= metabolic syndrome
OR	= odds ratio
SBF	= skipping breakfast
WC	= waist circumference

Several conditions associated with the development of cardiovascular disease (CVD) such as diabetes (1), obesity (2), hypertension (3), and dyslipidemia (4) are known to be modifiable by changes in lifestyle. Among lifestyle factors, our diet, including both the nutritional quality and our acquired eating patterns, constitutes a major target of CVD prevention strategies.

Eating patterns are highly dependent on cultural, social and psychological determinants, as people integrate them into their daily life routines. A particular habit that might have a significant effect on CV health is breakfast consumption, as it is associated with factors such as satiety (5), daily energy intake (EI) (6), metabolic efficiency of the diet, and appetite regulation (7). A number of studies have reported associations between the habit of omitting breakfast and increased cardiometabolic health markers (8), including obesity (9), diabetes (10), and unfavorable lipid profile (11). Although there are some studies linking skipping breakfast with coronary heart disease risk (12,13), to the best of our knowledge, no studies have investigated the association with this dietary habit on the presence of subclinical atherosclerosis. The aim of our study was to characterize the association between different breakfast patterns and CVD risk factors, and in particular, whether regularly skipping breakfast is associated with subclinical atherosclerosis, by investigating the presence of atherosclerotic plaques in the carotid arteries, aorta, and iliofemoral arteries or coronary artery calcium, in a population with no previous history of CVD.

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METHODS

STUDY OVERVIEW. The PESA (Progression of Early Subclinical Atherosclerosis) study is an ongoing observational prospective cohort of 4,082 employees of the Bank Santander Headquarters in Madrid, Spain, aiming to discover the factors related to the development and progression of atherosclerosis. A detailed description of the study design and procedures of data collection has been reported elsewhere (14). The study protocol was approved by the Ethic committee of Instituto de Salud Carlos III (Madrid, Spain). All participants provided written informed consent (14).

STUDY PARTICIPANTS. Male and female volunteers 40 to 54 years of age were included in the study if at baseline they were free of any CV or chronic kidney disease, were not under active treatment for cancer, did not have previous transplant, did not exceed body mass index (BMI) of 40 kg/m², and did not have any disease that might affect life expectancy and decrease it to <6 years. Of the initial participants, 26 having missing values in some of the variables of interest, and 4 reporting extreme values for daily EI (<800 or >4,200 kcal for men, and <500 or >3,500 kcal for women) (15) were excluded from the analyses. The final sample consisted therefore of 4,052 participants.

DIETARY ASSESSMENT. To estimate usual diet of PESA study participants we used a computerized questionnaire (dietary history of the ENRICA [Estudio de Nutrición y Riesgo Cardiovascular] study) developed and validated for the Spanish population within the ENRICA study (16) containing nutritional information on 861 food items (including 184 typically consumed Spanish meals and dishes). Briefly, subjects were asked to report foods consumed in the past 15 days, taking into consideration eating occasions (waking up, breakfast, midmorning, lunch, mid-afternoon, and dinner). Once a food item was consumed at least once, it was considered “usually consumed.” Conversion factors were used to calculate the annual frequency of consumption (16). Based on these data, the variable “energy consumed during breakfast” was computed and the breakfast patterns in our study was based on the percentage of the daily total EI consumed at breakfast. As a first step, our definition of breakfast was based on quantitative description provided by Timlin and Pereira (7), where it is defined as “the first meal of the day that breaks the fast after the longest period of sleep, eaten before or at the start of daily activities (e.g., errands, travel, work), within 2 h of waking, typically no later than 10:00 in the morning, and of an energy level between 20 and 35% of total daily energy need.” Based on this definition, we identified foods consumed before 10:00 AM in the PESA study database and those participants whose energy intake at breakfast exceeded 20% of total energy intake, were considered breakfast consumers (high-energy breakfast [HBF]). As a second step, we applied the qualitative definition of breakfast provided by O’Neil et al. (17), where

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breakfast is defined as “a food or beverage from at least one food group, and may be consumed at any location. Coffee, water and nonalcoholic beverages are not included in a food group.” Therefore, taking into account only coffee, and nonalcoholic beverages, we estimated that 300 ml of orange juice (typically consumed for breakfast in Spain) would contain 123 kcal, 100 ml of coffee with milk would contain 38 kcal, and 100 ml of coffee with milk and 20 g of added sugar would contain 119 kcal according to Spanish food composition database (18). The mean energy intake in the PESA study population is 2,314 kcal/day, and 123 kcal/day would represent 5% of the total intake. Therefore, we hypothesized that if participants’ morning energy intake would not exceed 123 kcal (5% of total daily energy intake), that could be approximated as skipping breakfast (SBF) as no other food was consumed. Following this rationale, 3 major groups were identified: those having <5% of total EI in the morning as a proxy for having only coffee or coffee with milk, juice, or other nonalcoholic beverages (SBF); those having >20% of total EI in the morning as breakfast consumers (HBF); and those participants in between 5% and 20% were called low-energy breakfast (LBF) consumers. Overall dietary quality was assessed by participants’ adherence to a posteriori defined dietary patterns (Mediterranean, Western, and Social-business), which are described in detail in our previous work (19).

ANTHROPOMETRIC AND CLINICAL MEASUREMENTS.

Anthropometric (height, weight, and waist circumference [WC]) and clinical measurements were collected as previously reported and through standardized procedures according to the PESA study protocol (14). Using this information, CVD risk factors were defined as follows: obesity if BMI was ≥30 kg/m²; hypercholesterolemia if total cholesterol was ≥240 mg/dl or low-density lipoprotein cholesterol was ≥160 mg/dl or high-density lipoprotein cholesterol was <40 mg/dl, or use of lipid-lowering medication (20); hypertension if average blood pressure was >140/90 mm Hg or use of antihypertensive medication (21); diabetes if fasting plasma glucose levels were >126 mg/dl or glycosylated hemoglobin was >6.5 or use of insulin or hypoglycemic medication (22). Metabolic syndrome (MetS) was defined as presenting at least 3 of the following criteria: WC >88 cm for women and WC >102 cm for men, fasting plasma glucose >100 mg/dl or medication treatment, triglycerides >150 mg/dl or medication treatment, high-density lipoprotein cholesterol <40 mg/dl or medication treatment, and blood pressure >140/90 mm Hg or medication

TABLE 1 Demographics and Lifestyle Characteristics

	HBF (n = 1,122)	LBF (n = 2,812)	SBF (n = 118)
Demographics			
Age, yrs	45.41 ± 4.23*†	45.95 ± 4.27‡	46.53 ± 4.27‡
Female	503 (44.8)*†	951 (33.8)‡	34 (28.8)‡
Education			
High school or lower	238 (21.4)*	741 (26.5)‡	34 (28.8)
College degree	160 (14.4)	423 (15.2)	21 (17.8)
University degree or higher	714 (64.2)*	1628 (58.3)‡	63 (53.4)
Marital status			
Married	851 (83.4)	2,139 (83.8)	91 (82.0)
Single	73 (7.2)	219 (8.6)	14 (12.6)
Divorced	90 (8.80)	185 (7.20)	6 (5.40)
Widow	6 (0.60)	11 (0.40)	0 (0.00)
Lifestyle			
Physical activity level (total physical activity counts/day)	3,604 ± 6,071	3,537 ± 5,179	3,668 ± 5,223
Smoking status			
Current smoker	196 (17.5)*†	588 (20.9)†‡	49 (41.5)*‡
Social smoker	103 (9.2)	226 (8.0)	11 (9.3)
Ex-smoker	344 (30.7)	928 (33.0)	33 (28.0)
Nonsmoker	479 (42.7)*†	1070 (38.1)†‡	25 (21.2)*‡
Dieting to lose weight	89 (7.9)*†	367 (13.1)‡	21 (17.8)‡
Time spent on breakfast, min	11.00 ± 5.81*†	8.40 ± 5.84†‡	4.93 ± 7.16*‡
% of daily EI at lunch	38.63 ± 6.25*†	41.97 ± 6.55†‡	47.53 ± 9.25*‡

Values are mean ± SD or n (%). Bonferroni correction was applied for categorical variables (p < 0.017). *p < 0.05 vs. LBF. †p < 0.05 vs. SBF. ‡p < 0.05 vs. HBF.
 EI = energy intake; HBF = high-energy breakfast; LBF = low-energy breakfast; PA = physical activity; SBF = skipping breakfast; WC = waist circumference.

treatment (20). The European Society of Cardiology cardiovascular disease risk assessment tool, the Systematic Coronary Risk Evaluation, was used to assess the fatal cardiovascular risk (23).

OTHER VARIABLES. Other variables including age (years), sex (male or female), marital status (single, married, divorced, widow), highest educational level achieved (high school, college degree, or university and higher), smoking status (current smoker, nonsmoker, social smoker, or ex-smoker), and dieting to lose weight (yes/no) were self-reported. Physical activity was assessed by a triaxial accelerometer placed on the waist for a period of 7 days, providing activity counts per day (Acti Trainer, Actigraph, Pensacola, Florida).

ASSESSMENT OF ATHEROSCLEROSIS. The assessment of atherosclerotic plaques in multiple vascular territories; bilateral carotid, infrarenal abdominal aorta and iliofemoral arteries was performed by 2-dimensional ultrasound (Philips iU22 ultrasound, Philips Healthcare, Bothell, Washington) in the PESA study examination center as previously described (14). The presence of atherosclerotic plaques was

TABLE 2 Overall Dietary Profile of PESA Study Participants According to Breakfast Pattern

	HBF (n = 1,122)	LBF (n = 2,812)	SBF (n = 118)
Macronutrients, g/day or mg/day			
Energy intake, kcal	2,234 ± 450*†	2,345 ± 467‡	2,358 ± 562‡
Total protein	94.3 ± 18.0*†	102.4 ± 20.0‡	105.7 ± 24.0‡
Animal protein	64.8 ± 15.0*†	72.1 ± 17.1‡	76.6 ± 20.7*‡
Vegetable protein	29.08 ± 8.23*	29.84 ± 8.39‡	28.69 ± 9.83
Total fat	103.1 ± 22.9*†	108.3 ± 24.2‡	113.6 ± 30.6‡
Cholesterol	334.4 ± 98.2*†	361.6 ± 94.8‡	385.7 ± 111.0*‡
MUFA	47.0 ± 11.6*†	49.3 ± 11.5‡	52.4 ± 13.8*‡
PUFA	16.62 ± 5.09*†	17.81 ± 5.48‡	19.05 ± 7.06*‡
SFA	29.98 ± 8.62*†	32.05 ± 9.00‡	32.84 ± 10.90‡
Carbohydrates	218.5 ± 58.1†	220.0 ± 58.8†	197.0 ± 63.8*‡
Sugar	94.0 ± 31.8*†	90.9 ± 30.6‡	75.5 ± 34.4*‡
Polysaccharides	119.7 ± 40.6*	125.7 ± 43.2‡	119.2 ± 46.0
Fiber	21.08 ± 6.48†	20.90 ± 5.99†	18.99 ± 6.19*‡
Food group, g/day			
Fruits and vegetables	474 ± 210*†	435 ± 202‡	369 ± 182*‡
Dried fruits	7.30 ± 10.76	7.94 ± 12.26	9.65 ± 16.64
Legumes	25.2 ± 21.2	26.0 ± 22.9	27.4 ± 23.3
Potatoes	20.0 ± 17.1	21.1 ± 17.7	19.3 ± 16.7
Refined grains	216.0 ± 92.8*	234.0 ± 98.7‡	231.0 ± 101.5
Whole grains	14.3 ± 31.9*†	9.1 ± 21.6†	2.5 ± 10.6*‡
Nuts	5.03 ± 5.92	5.41 ± 5.68	5.16 ± 4.91
Olives	4.05 ± 6.30*†	4.65 ± 6.52‡	7.26 ± 15.13*‡
Red meat	93.0 ± 42.2*†	112.9 ± 50.1‡	145.1 ± 68.6*‡
Lean meat	63.3 ± 30.7*	66.9 ± 33.5‡	67.7 ± 32.6
Seafood (fish, shellfish)	75.8 ± 36.2*	79.1 ± 38.9‡	78.1 ± 39.9
Dairy	207 ± 151†	196 ± 137†	141 ± 116*‡
Low-fat dairy	88.3 ± 125.8	90.0 ± 121.8†	61.4 ± 112.7*
Vegetable oil and fat	5.02 ± 5.92	5.40 ± 5.68	5.15 ± 4.91
Butter	5.89 ± 6.72*†	4.26 ± 4.39†	2.30 ± 2.03*‡
Olive oil	31.7 ± 14.1*	29.9 ± 12.2‡	31.0 ± 11.8
Precooked meals, fast food	55.6 ± 34.0*†	66.9 ± 42.1‡	68.6 ± 35.6‡
Chips and snacks	5.02 ± 7.06*†	6.49 ± 9.12‡	8.69 ± 11.73*‡
Commercial bakery	71.4 ± 50.0†	69.6 ± 47.8†	54.3 ± 47.5*‡
Alcohol (distilled spirits, wine, beer)	122 ± 144*†	190 ± 227†	299 ± 328*‡
SSB	132 ± 184*†	157 ± 204†	256 ± 439*‡
Tea, coffee	167 ± 131†	174 ± 128	202 ± 193‡
Dietary quality			
Mediterranean cluster	533 (47.5)*†	1,052 (37.4)‡	30 (25.4)*‡
Western cluster	485 (43.2)†	1,148 (40.8)†	35 (29.7)*‡
Social business cluster	104 (9.3)*†	612 (21.8)‡	53 (44.9)*‡
Values are mean ± SD or n (%). Bonferroni correction was applied for categorical variables (p < 0.017). *p < 0.05 vs. LBF. †p < 0.05 vs. SBF. ‡p < 0.05 vs. HBF.			
MUFA = monounsaturated fatty acids; PESA = Progression of Early Subclinical Atherosclerosis; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids; SSB = sugar-sweetened beverages; other abbreviations as in Table 1.			

assessed by cross-sectional sweep of above mentioned territories. Plaque was defined as a focal protrusion into the arterial lumen of thickness >0.5 mm or >50% of the surrounding intima-media thickness or a diffuse thickness >1.5 mm measured between the media-adventitia and intima-lumen

interfaces (24). Coronary artery calcium (CAC) was assessed by noncontrast electrocardiography-gated prospective acquisition with a 16-slice computed tomography scanner (Philips Brilliance CT, Philips Healthcare, Andover, Massachusetts) and CAC score was calculated using an Agatston equation (25). Sub-clinical atherosclerosis was defined as the presence of plaque in the right carotid, left carotid, aorta, right iliofemoral, or left iliofemoral or as the presence of calcium (CAC score >0) in the coronary arteries; noncoronary atherosclerosis was defined as presence of plaque in the previously mentioned territories and excluding CAC. Depending on the number of sites affected with atherosclerosis (a maximum of 6), if 4 to 6 sites were affected, it was defined as generalized atherosclerosis (26).

STATISTICAL ANALYSIS. Baseline characteristics are presented as count and percentage for categorical variables, and as mean ± SD for continuous variables. Analysis of variance for continuous variables and chi-square for categorical variables were used to compare the data between categories with Bonferroni correction for multiple comparisons. Multivariate logistic regression models were used to assess the association between breakfast patterns: 1) main outcomes: subclinical, noncoronary and generalized atherosclerosis, presence of atherosclerotic plaques in the aorta, carotid and iliofemoral arteries, CAC score >0; and 2) secondary outcomes: obesity (BMI ≥30 kg/m²), abdominal obesity, MetS, low high-density lipoprotein cholesterol, and hypertension. We followed a 2-step approach for the inclusion of covariates in the models. First, sociodemographic, clinical, and lifestyle variables were compared among the 3 breakfast groups. Those variables that significantly differed between breakfast groups were included in the model. Second, the remaining variables were introduced sequentially in the model and kept if the beta coefficient varied more than 10% (27). All statistical analyses were performed with IBM SPSS Statistics for Windows, version 24 (IBM Corporation, Armonk, New York).

RESULTS

Of 4,052 participants, 2.9%, 69.4%, and 27.7% fell into SBF, LBF, and HBF categories, respectively. Compared with HBF and LBF, the SBF group consisted of mostly men, who were currently smokers, reported having changed their diet in the past year to lose weight, and consumed the highest percentage of energy at lunch. Compared with HBF, LBF participants were more likely to be a man with a lower education level, a current smoker, and also consume

a greater proportion of calories at lunch (Table 1). In terms of nutritional quality, SBF participants were more likely to consume more energy, protein (particularly from animal sources), and dietary cholesterol; have the lowest fiber and carbohydrate intakes; and tended to consume more alcoholic and sugar-sweetened beverages, as well as red meat. Compared with HBF, participants in the LBF group had greater daily EI, animal protein intake, and dietary cholesterol intake, and lower intakes of sugar and polysaccharides. This group also had lower intakes of fruits and vegetables, whole grains, and olive oil, and higher intakes of refined grains, red meat, fast food, and precooked meals as well as lean meat and seafood (Table 2). Participants in the HBF group presented significantly greater intakes of carbohydrates and dietary fiber, and tended to consume greater amounts of fruits and vegetables, whole grains, high-fat dairy, and sweets (Table 2).

Morning dietary habits differed significantly across breakfast groups. On average, SBF participants spent no more than 5 min on breakfast, and consumed mostly coffee or orange juice. The most frequent choices among the HBF group were coffee, orange juice, bread toasts with olive oil, tomato, ham, fresh fruit, breakfast cereal, whole grain cookie, or pastries and jam. Regarding LBF participants, they were more likely to have coffee, orange juice, as well as fresh fruit, toast, cookies, or pastries (Online Table 1).

Lunch and dinner intakes also differed significantly between the breakfast patterns groups (Online Tables 2 and 3). Across all the groups SBF participants had the highest intakes of red and processed meat, appetizers, sugar-sweetened beverages, and alcohol at lunch and dinner, as well as the lowest consumption of fruits during lunch. The LBF group compared with HBF had similar intakes of fruits, and higher consumption of appetizers at lunchtime, and also higher intakes of vegetables, lean meat, seafood, and eggs, as well as potatoes, pasta, commercial bakery goods, red and processed meat, sugar-sweetened beverages, and alcohol at dinner.

Compared with the HBF group, cardiometabolic risk markers were more prevalent in the LBF group and even more so in the SBF group, showing the greatest WC and BMI, blood pressure, blood lipids, and fasting glucose levels (Table 3). SBF participants were also more likely to score the highest on the European Society of Cardiology Systematic Coronary Risk Evaluation risk scale (Table 3). The probabilities of presenting obesity, abdominal obesity, MetS, low high-density lipoprotein cholesterol, and hypertension were significantly higher for participants in the SBF group compared with HBF (Figure 1, Online Table 4). Taking

TABLE 3 Distribution of CVD Risk Factors of PESA Study Participants According to Breakfast Pattern

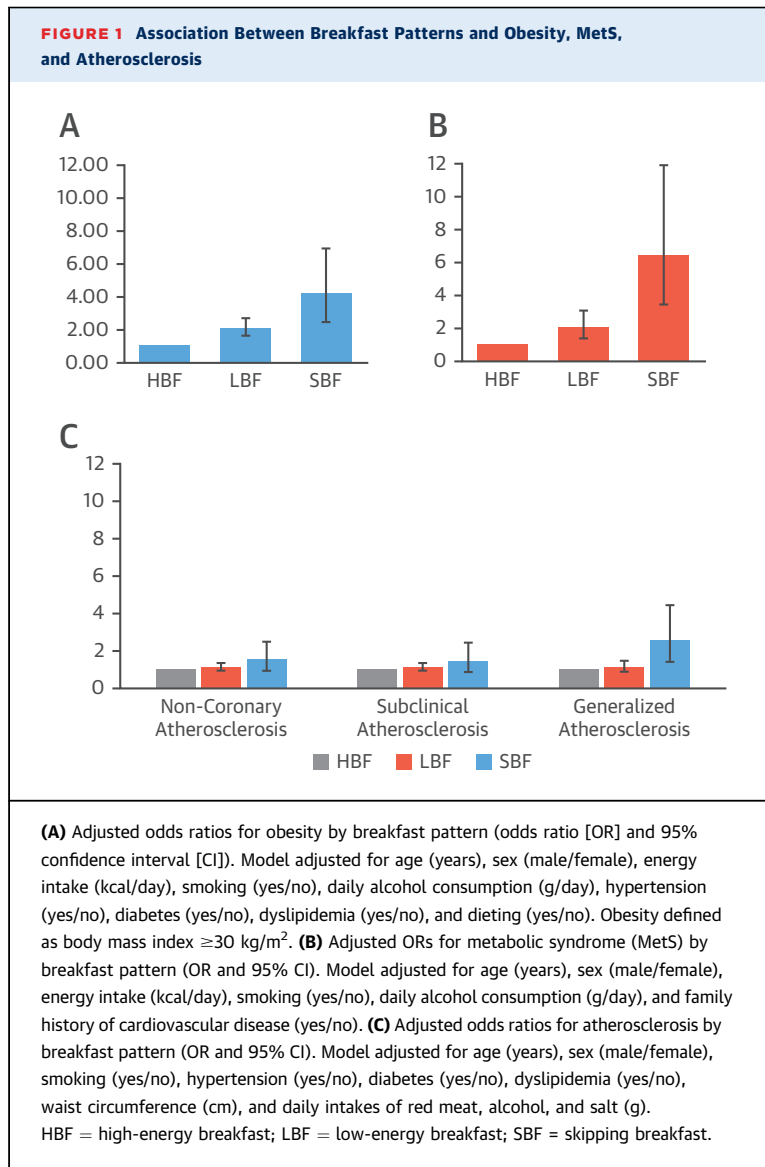
CVD Risk Factors	HBF (n = 1,122)	LBF (n = 2,812)	SBF (n = 118)
Central obesity	136 (12.1)*†	680 (24.2)†‡	45 (38.1)*†‡
Weight, kg	72.5 ± 13.8*†	78.1 ± 14.9†‡	83.3 ± 17.6*†‡
BMI, kg/m ²	25.01 ± 3.39*†	26.55 ± 3.85†‡	28.04 ± 4.66*†‡
Waist circumference, cm	85.6 ± 11.0*†	90.7 ± 12.0†‡	95.5 ± 14.5*†‡
Hypertension, mm Hg	97 (8.6)*†	356 (12.7)†‡	27 (22.9)*†‡
SBP	114.3 ± 12.1*†	117.0 ± 12.6‡	119.0 ± 13.6‡
DBP	70.75 ± 8.88*†	73.12 ± 9.52†‡	75.36 ± 10.20*†‡
Dyslipidemia, mg/dl	374 (33.3)*†	1241 (44.1)†‡	66 (55.9)*†‡
Total cholesterol	196.4 ± 31.4*†	202.0 ± 33.8‡	205.8 ± 35.5‡
LDL-c	128.5 ± 28.0*†	133.7 ± 30.2‡	136.0 ± 30.9‡
HDL-c	50.8 ± 12.1*†	48.4 ± 12.1‡	46.8 ± 13.2‡
Triglycerides	84.5 ± 45.4*†	98.3 ± 59.9†‡	114.0 ± 74.5*†‡
Diabetes	8 (0.7)*†	65 (2.3)‡	6 (5.1)‡
Fasting glucose, mg/dl	87.9 ± 8.8*†	91.4 ± 15.0†‡	94.7 ± 15.9*†‡
CVD risk scores			
SCORE, %	0.40 ± 0.53*†	0.60 ± 0.61†‡	0.85 ± 0.85*†‡
Number of CVD risk factors			
0	594 (52.9)*†	1,158 (41.2)†‡	32 (27.1)*†‡
1	395 (35.2)*	1,135 (40.4)‡	39 (33.1)
2	120 (10.7)*†	445 (15.8)†‡	35 (29.7)*†‡
>2	13 (1.2)*†	74 (2.6)†‡	12 (10.2)*†‡

Values are mean ± SD or n (%). *p < 0.05 vs. LBF. †p < 0.05 vs. SBF. ‡p < 0.05 vs. HBF. BMI = body mass index; CVD = cardiovascular disease; DBP = diastolic blood pressure; HDL-c = high-density lipoprotein cholesterol; LDL-c = low density lipoprotein cholesterol; SBP = systolic blood pressure; SCORE = European Society of Cardiology Systematic Coronary Risk Evaluation; other abbreviations as in Tables 1 and 2.

into consideration the higher proportion of participants reporting to be on a diet to lose weight among SBF participants, the model for the association with obesity (BMI >30 kg/m²) was additionally adjusted for dieting in a sensitivity analysis, resulting in a 4.7% decrease in the association (data not shown).

The prevalence of subclinical, noncoronary and generalized atherosclerosis for PESA participants included in the final analysis were 62.5%, 60.3%, and 13.4%, respectively (Central Illustration). Highest prevalence of atherosclerotic plaques was found in iliofemoral (44.2%) and carotid arteries (31.5%), with the lowest prevalence observed in the aorta (24.6%). CAC score >0 was detected among 18.1% of the total PESA study population.

Subclinical atherosclerosis was observed more frequently among the SBF group (Central Illustration); with higher odds of having plaques in abdominal aorta (odds ratio [OR]: 1.79; 95% confidence interval [CI]: 1.16 to 2.77), carotid atherosclerotic plaques (OR: 1.76; 95% CI: 1.17 to 2.65), and iliofemoral plaques (OR: 1.72; 95% CI: 1.11 to 2.64) (Tables 4 and 5). Regarding the presence of noncoronary and generalized atherosclerosis, the odds were significantly higher for SBF participants, compared with HBF



(OR: 1.55; 95% CI: 0.97 to 2.46; OR: 2.57; 95% CI: 1.54 to 4.31) (Figure 1). Participants in the LBF group had higher risk of presenting carotid or iliofemoral atherosclerotic plaques (OR: 1.21; 95% CI: 1.03 to 1.43; OR: 1.17; 95% CI: 1.00 to 1.37) (Tables 4 and 5).

DISCUSSION

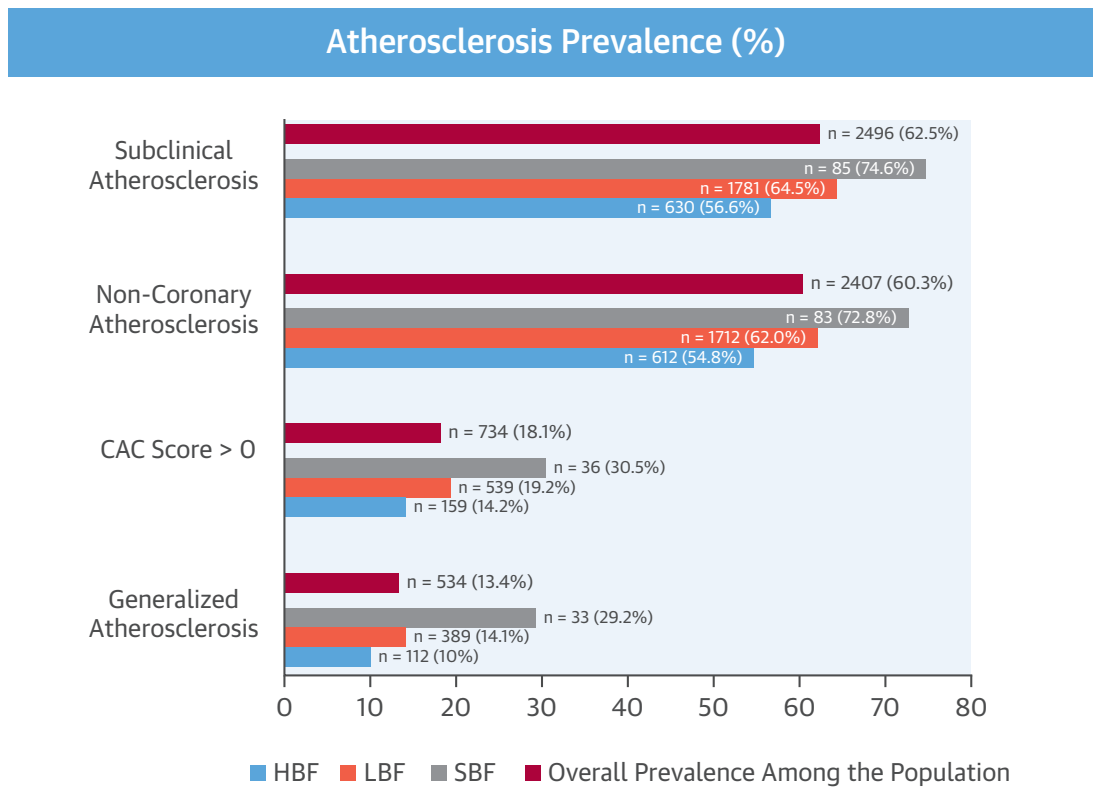
We report here, for the first time, evidence of the association between different breakfast patterns such as SBF as well as LBF and HBF consumption with the risk of atherosclerosis. In our study, regular skipping breakfast was associated with a 1.55- and 2.57-fold higher odds for noncoronary and generalized atherosclerosis, respectively, independently of the presence of conventional CVD risk factors and considering overall diet quality. A modest 3% of the

participants were classified as not consuming breakfast (<5% of total daily EI consumed up to 10:00 AM), and were further characterized by following an overall unhealthy lifestyle, including poor overall diet, frequent alcohol consumption, and smoking. These findings are in agreement with previous reports where skipping breakfast was associated with smoking (28), increased EI (9), and noncompliance with Healthy Eating recommendations (29). The results from our most adjusted models suggest a possible at least partial association between skipping breakfast and prevalence of subclinical atherosclerosis independent of the overall unhealthy lifestyle of these participants in the SBF group. Also, participants in the LBF group, who consumed mostly toasts or pastries and coffee in the morning, had an increased risk of having carotid and iliofemoral atherosclerotic plaques compared with participants in the HBF group.

To date there are only 2 studies evaluating the habit of regularly skipping breakfast in relation to CVD. Results from a cohort study showed a 14%, 18%, and 36% greater risks for total CVD, total stroke, and hemorrhage, respectively, among those skipping breakfast (13). In a second study, participants who reported skipping breakfast had on average 27% higher risk of coronary heart disease; however, the risk was mediated by BMI and health conditions (12). To investigate whether the observed association in our study could be simply explained by the higher prevalence of CVD risk factors among SBF participants, we performed a multivariable analysis controlling for waist circumference, hypertension, diabetes, dyslipidemia, and smoking. After adjusting for these conditions, as well as the exclusion of obese participants in a sensitivity analysis (data not shown), the risk estimates decreased but remained significant, suggesting that indeed skipping breakfast could be 1 of the risk factors clustering around the early onset and development of atherosclerosis.

The overall dietary pattern followed by SBF participants falls predominantly into our previously defined “social-business eating pattern” (19), with 45% of participants following this specific behavior. It is characterized by overall unhealthy food choices, frequent eating out, and busy schedules, which might shed light not only on the factors affecting the association between skipping breakfast and disease outcomes, but on the underlying reasons for this habit. In line with this cluster of behaviors, we hypothesize that aside from a direct association with CV risk factors, and atherosclerosis that deserves further research, SBF might serve as a marker for a general unhealthy diet or lifestyle, which in turn is associated with the development and progression of atherosclerosis.

CENTRAL ILLUSTRATION Overall Prevalence of an Atherosclerosis Among PESA Study Participants and According to the Type of Breakfast Consumed



Uzhova, I. et al. *J Am Coll Cardiol.* 2017;70(15):1833-42.

The prevalence of an atherosclerosis is presented for total population, as well as by breakfast habits categories. The SBF group presents the highest proportion of individuals with subclinical, noncoronary, generalized atherosclerosis and increased coronary artery calcium score. CACs = coronary artery calcium score; HBF = high-energy breakfast; LBF = low-energy breakfast; PESA = Progression of Early Subclinical Atherosclerosis; SBF = skipping breakfast.

In line with the previously mentioned observation regarding overall unhealthy dietary choice, by examining specifically dietary intakes at lunch and dinner, we showed that SBF participants had the highest intakes of red and processed meat, appetizers, SSB and alcohol during the rest of the day. The LBF group compared with HBF was higher in intakes of commercial bakery goods, red and processed meat, sugar-sweetened beverages, and alcohol. However, they still consumed similar or for some food groups higher amounts of cardioprotective food items including fruits, vegetables, lean meat, seafood, and eggs, which might explain the lack of an association observed between LBF and atherosclerosis.

It is worth mentioning that the percentage of participants in our study who were overweight or obese was significantly higher among SBF compared with LBF or HBF participants. It has been shown that adipose tissue

not only serves as body energy storage but also plays an important role in CV inflammation processes. Obesity is a major source of inflammatory factors such as C-reactive protein, interleukin-6, P-selectin, vascular cell adhesion protein-1, fibrinogen, and others, and it is directly related to systemic inflammation and atherosclerosis (30). The results of the association of SBF with obesity seen in our study are in line with the observations of a large prospective cohort of men (9), although in this study the investigators reported that their findings could be partially explained by the higher proportion of sedentary individuals among the SBF group. In our study, SBF participants were not less physically active, and the observed association between breakfast and obesity was not mediated by the level of physical activity. In addition to the higher prevalence of obese individuals among the SBF group, this group was also more likely to engage in dieting, probably in an attempt

TABLE 4 Association Between Breakfast Pattern and Coronary Artery Calcification

	HBF (n = 1,122)	LBF (n = 2,812)	SBF (n = 118)
CAC score >0			
Cases/noncases	159/963	539/2,273	36/82
Model 1	1.00 (reference)	1.19 (0.97-1.47)	2.07 (1.29-3.30)*
Model 2	1.00 (reference)	1.08 (0.87-1.34)	1.62 (1.00-2.63)†
Model 3	1.00 (reference)	1.04 (0.84-1.29)	1.43 (0.87-2.36)
CAC score >100			
Cases/noncases	23/1,099	112/2,700	7/111
Model 1	1.00 (reference)	1.63 (1.02-2.59)†	2.10 (0.85-5.13)
Model 2	1.00 (reference)	1.44 (0.90-2.31)	1.52 (0.60-3.84)
Model 3	1.00 (reference)	1.37 (0.85-2.22)	1.31 (0.51-3.41)
CAC score >300			
Cases/noncases	12/1,122	34/2,778	2/116
CAC score >400			
Cases/noncases	7/1,115	21/2,791	1/117

Values are n/N or odds ratio (95% confidence interval). Model 1: age, sex; Model 2: Model 1 plus waist circumference, hypertension, dyslipidemia, diabetes, and smoking. Model 3: Model 2 plus red meat, alcohol, and salt. Regression for coronary calcium (CAC) score >300 and >400 was not performed due to small number of cases. *p < 0.01. †p < 0.05.
Abbreviations as in Table 1.

to lose weight. Once dieting was included in the model, the risk slightly decreased by 4.7%, modifying the degree of the relationship between SBF and obesity (data not shown).

A recent report from American Heart Association discussed the time of eating occasions with relation

to cardiometabolic risk, suggesting that a greater percentage of energy consumed earlier in a day may beneficially impact risk factors for heart disease and diabetes (8). Clinical studies report that consuming a high-calorie meal in the morning would result in a significant decrease in fasting glucose and insulin (31), as well as reduced plasma ghrelin concentrations, a hormone associated with food perception, leading toward lower energy foods preference (32). Moreover, studies linking breakfast consumption with overall diet quality and regulation of appetite (33,34), reported that not only micronutrient-rich breakfast but the morning meals in general were potentially satiating and had a beneficial effect on appetite regulation, which would help to balance the EI throughout the day and prevent overeating and subsequent obesity.

Considering the importance of regular breakfast consumption for primary CVD prevention, our findings are important for health professionals and might be used as an important key, and simple message for lifestyle-based interventions and public health strategies, as well as informing dietary recommendations and guidelines.

STUDY STRENGTHS AND LIMITATIONS. Our study has some limitations worth considering. Due to the cross-sectional nature of this study we are not able to address a temporal association between breakfast skipping and atherosclerosis. For obesity, reverse causation could not be ruled out and the observed results might be explained by obese participants skipping breakfast to lose weight, rather than skipping breakfast directly influencing the obesity and CVD risk factors associated with this condition. The fact that those participants who skip breakfast were more likely to report having been dieting, we could assume that they might have weight instability (so-called weight fluctuation), which has been reported to be associated with a higher risk of coronary and CVD events (35), and therefore might have served as a mediator between skipping breakfast and atherosclerosis presence in our study. However, taking into account that the nature of our analysis is cross-sectional and there are no available follow-up data, we were unable to address this issue. Additionally, even though our study comprised a large sample size, the participants of the PESA study have a characteristic occupation and lifestyle that might not be representative of the general population. Also, the duration of the overnight fasting was not available in our data and that variable could not be factored into the models; participants who regularly skip breakfast could have a late dinner, and therefore the duration of overnight fasting in this group would have been

TABLE 5 Association Between Breakfast Patterns and Presence of Atherosclerotic Plaques in Several Territories According to Breakfast Habits Categories Among PESA Study Participants

	HBF (n = 1,122)	LBF (n = 2,812)	SBF (n = 118)
Plaque in abdominal aorta			
Case number	244/876	707/2,091	41/75
Model 1	1.00 (reference)	1.14 (0.96-1.35)	1.75 (1.15-2.66)*
Model 2	1.00 (reference)	1.19 (1.01-1.42)†	1.92 (1.25-2.94)*
Model 3	1.00 (reference)	1.17 (0.98-1.40)	1.79 (1.16-2.77)*
Plaque in carotid artery			
Case number	297/825	926/1,884	53/65
Model 1	1.00 (reference)	1.25 (1.06-1.46)*	1.96 (1.32-2.93)*
Model 2	1.00 (reference)	1.23 (1.05-1.45)*	1.86 (1.24-2.79)*
Model 3	1.00 (reference)	1.21 (1.03-1.43)†	1.76 (1.17-2.65)*
Plaque in iliofemoral artery			
Case number	417/702	1,294/1,501	71/64
Model 1	1.00 (reference)	1.27 (1.09-1.48)*	2.17 (1.43-3.30)‡
Model 2	1.00 (reference)	1.23 (1.05-1.43)*	1.95 (1.28-2.99)*
Model 3	1.00 (reference)	1.17 (1.00-1.37)†	1.72 (1.11-2.64)*

Values are n/N or odds ratio (95% confidence interval). Model 1: age, sex; Model 2: Model 1 plus waist circumference, hypertension, dyslipidemia, diabetes, and smoking. Model 3: Model 2 plus red meat, alcohol, and salt. *p < 0.01. †p < 0.05. ‡p < 0.0001.
Abbreviations as in Tables 1 and 2.

equal to the one of the group who consumed breakfast in the morning and had earlier dinner, resulting in a somehow similar metabolic profile. Another limitation of our study is the sample size of the SBF group: only 3% of the population was considered to skip breakfast. However, this rather extreme definition was chosen to allow the comparison with previous studies on skipping breakfast (7,17). It might be interesting for future studies to validate our definition of breakfast and replicate the findings in a different population, as well as to study the association between fasting time and atherosclerosis disease development and progression. Despite the previously mentioned limitations, key advantages of our study are its large sample size, well-characterized diet and lifestyle data, atherosclerosis assessment measured by direct indicators of disease such as presence of plaques, and the possibility to study the association on middle-aged asymptomatic individuals, who would be the ideal candidates for primary prevention.

CONCLUSIONS

Skipping breakfast could serve as a marker of unhealthy dietary and lifestyle behavior and is associated with the presence of noncoronary and generalized atherosclerosis independent of conventional CVD risk factors in a sample of middle-aged

asymptomatic individuals. Our findings highlight the message of the importance of healthy eating, including an energetic breakfast.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Regular breakfast skipping in a middle-aged asymptomatic population without previously diagnosed CVD is associated with increased odds of atherosclerosis independent of the presence of CVD risk factors.

TRANSLATIONAL OUTLOOK: Highlighting the importance of observing a quality breakfast in our daily routines is a simple but important message to be used by health professionals to prevent atherosclerosis disease at its earliest stages.

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KEY WORDS atherosclerosis, atherosclerotic plaque, coronary artery calcification, lifestyle, skipping breakfast

APPENDIX For supplemental tables, please see the online version of this article.

Online Appendix

Table 1: Intakes of foods and nutrients at breakfast according to breakfast-pattern groups of PESA participants

Food intake (g/day)	Breakfast pattern categories		
	HBF N=1122	LBF N=2812	SBF N=118
	Mean ±SD		
Food groups *			
Coffee	83.3±60.9 ^c	79.1±60.3 ^c	102.7±79.1 ^{ab}
Dairy	150±96.6 ^{bc}	122±80.1 ^{ac}	59.8±62.3 ^{ab}
Fresh orange juice	37.7±64.4 ^{bc}	24.9±53.2 ^a	14.9±38.0 ^a
Fresh fruit	40.5±87.8 ^{bc}	19.1±51.2 ^{ac}	4.60±18.1 ^{ab}
Fruit preserves/jam	6.30±11.0 ^{bc}	3.40±6.70 ^{ac}	0.50±2.10 ^{ab}
Bread	35.7±35.1 ^{bc}	18.3±20.9 ^{ac}	1.40±3.50 ^{ab}
Cookies	10.9±20.3 ^{bc}	8.00±12.4 ^{ac}	1.20±4.10 ^{ab}
Pastries	15.5±24.1 ^{bc}	9.80±15.6 ^{ac}	1.10±3.90 ^{ab}
Processed meat	5.00±13.5 ^{bc}	2.80±8.70 ^{ac}	0.50±2.30 ^{ab}
Breakfast cereal	8.60±18.8 ^{bc}	4.10±11.1 ^{ac}	0.40±1.70 ^{ab}
Tomato puree	10.4±17.7 ^{bc}	3.80±8.60 ^{ac}	0.20±0.80 ^{ab}
Olive oil	7.70±9.20 ^{bc}	3.20±4.70 ^{ac}	0.10±0.60 ^{ab}
Nutrients			
Energy intake, kcal	551±148 ^{bc}	323±107 ^{ac}	77.0±36.0 ^{ab}
Total protein, % EI	10.9±3.38 ^{bc}	12.3±4.66 ^{ac}	18.7±11.7 ^{ab}
Animal protein, % EI	5.32±3.54 ^{bc}	6.99±4.62 ^{ac}	12.6±11.4 ^{ab}
Vegetable protein, % EI	5.44±1.96 ^b	5.05±2.46 ^{ac}	5.83±9.19 ^b
Total fat, % EI	36.9±9.37 ^{bc}	33.4±10.4 ^{ac}	20.5±15.1 ^{ab}
Cholesterol, mg	49.0±50.0 ^{bc}	30.2±31.0 ^{ac}	6.40±9.62 ^{ab}
MUFA, % EI	16.8±7.68 ^{bc}	13.9±7.14 ^{ac}	6.69±6.42 ^{ab}
PUFA, % EI	4.76±3.11 ^{bc}	4.06±2.89 ^{ac}	2.17±3.73 ^{ab}
SFA, % EI	12.3±5.14 ^c	12.5±5.53 ^c	9.06±8.07 ^{ab}
Carbohydrates, % EI	51.9±9.30 ^{bc}	54.0±10.2 ^{ac}	60.3±16.8 ^{ab}
Sugar, % EI	25.8±10.9 ^{bc}	29.9±13.5 ^{ac}	46.3±25.8 ^{ab}
Fiber, g	4.44±3.48 ^{bc}	2.37±2.28 ^{ac}	0.26±0.59 ^{ab}

HBF = high-energy breakfast; LBF = low energy breakfast; SBF = skipping breakfast; MUFA=monounsaturated fatty acids. PUFA=polyunsaturated fatty acids. SFA=saturated fatty acids

^a – p<0.05 versus HBF

^b – p<0.05 versus LBF

^c – p<0.05 versus SBF

* Food groups in the table represent typical breakfast foods consumed in Spain

Table 2: Lunch dietary profile of PESA participants according to breakfast pattern

	Breakfast pattern categories		
	HBF N=1122	LBF N=2812	SBF N=118
Macro nutrients g/day			
Energy intake, Kcal	860±212	982±240	1104±285
Total protein, %EI	20.5±3.09 ^{bc}	20.0±2.87 ^a	19.5±3.04 ^a
Animal protein, %EI	24.9±3.57 ^b	13.4±3.33 ^a	14.2±3.58
Vegetable protein, %EI	5.60±1.31 ^c	5.58±1.21 ^c	5.29±3.31 ^{ab}
Total fat, %EI	43.2±5.98 ^b	42.7±5.95 ^a	44.0±6.12
Cholesterol, mg	149±46.0 ^{bc}	166±52.3 ^{ac}	195±60.6 ^{ab}
MUFA, %EI	21.2±3.88	20.6±3.86	21.5±3.98
PUFA, %EI	7.41±1.89 ^b	7.34±1.86 ^{ac}	7.38±.83 ^b
SFA, %EI	10.9±2.31	11.0±2.26	11.4±2.22
Carbohydrates, %EI	33.7±7.35	34.2±7.34 ^c	32.1±7.98 ^b
Sugar, %EI	10.9±4.59 ^c	10.6±4.32 ^c	9.30±4.49 ^{ab}
Fiber, g	9.52±3.21 ^{bc}	10.3±3.29 ^a	10.6±3.60 ^a
Food groups (g/day)			
Fruits	61.5±61.9 ^c	61.1±64.5 ^c	39.8±45.9 ^{ab}
Vegetables	131±58.0	132±62.2	133±64.1
Potatoes	23.2±16.4 ^{bc}	26.1±18.5 ^a	27.6±19.5 ^a
Legumes	28.9±24.4	29.7±24.8	30.9±23.8
Grains and bread	77.0±41.7 ^{bc}	92.5±46.1 ^a	100±53.9 ^a
Pasta	34.5±23.4 ^b	37.8±25.7 ^a	39.2±28.5
Red meat	41.1±22.4 ^{bc}	49.1±27.0 ^{ac}	62.5±30.2 ^{ab}
Processed meat	9.53±9.02 ^{bc}	11.2±10.3 ^{ac}	14.7±15.0 ^{ab}
Lean meat	25.4±14.5	26.5±16.4	26.5±16.4
Seafood	45.4±25.1	45.8±26.5	46.3±28.8
Eggs	6.92±7.75	7.19±7.62	8.55±9.26
Dairy	42.5±41.6 ^b	48.5±45.0 ^{ac}	34.5±39.9 ^b
Commercial bakery	3.70±8.90 ^{bc}	4.52±10.3 ^a	6.6±15.2 ^a
Sweets	0.89±3.48	0.80±2.92	0.93±3.00
Sandwiches, hamburgers, ready to eat food	4.18±7.45	4.76±9.82	3.90±8.78
Appetizers	1.08±2.64 ^c	1.35±3.41 ^c	2.22±4.16 ^{ab}
SSB	41.0±80.1 ^c	45.6±87.5 ^c	72.1±108 ^{ab}
Alcoholic beverages *	50.8±70.0 ^{bc}	76.3±104.1 ^{ac}	124±162 ^{ab}

HBF = high-energy breakfast; LBF = low energy breakfast; SBF = skipping breakfast; MUFA=monounsaturated fatty acids. PUFA=polyunsaturated fatty acids. SFA=saturated fatty acids. * distilled spirits, wine, beer

^a – p<0.05 versus HBF

^b – p<0.05 versus LBF

^c – p<0.05 versus SBF

Table 3: Dinner dietary profile of PESA participants according to breakfast pattern

	Breakfast pattern categories		
	HBF N=1122	LBF N=2812	SBF N=118
Macro nutrients g/day			
Energy intake, Kcal	593±184	711±221	811±278
Total protein, %EI	20.1±3.98	19.9±3.58	19.3±3.38
Animal protein, %EI	15.5±4.75	15.2±4.24	14.7±4.09
Vegetable protein, %EI	4.57±1.48	4.61±1.39	4.59±1.57
Total fat, %EI	45.3±8.08	45.3±7.57	46.0±8.62
Cholesterol, mg	119±49.5 ^{bc}	138±49.2 ^{ac}	150±62.7 ^{ab}
MUFA, %EI	21.2±5.14	20.9±4.66	21.6±4.99
PUFA, %EI	7.13±2.60	7.09±2.39	7.10±2.03
SFA, %EI	13.0±3.76	13.2±3.82	13.4±3.75
Carbohydrates, %EI	31.6±9.74	31.5±8.91	30.0±9.68
Sugar, %EI	12.3±7.13 ^{bc}	11.2±6.04 ^{ac}	9.65±7.06 ^{ab}
Fiber, g	5.18±2.54 ^{bc}	5.87±2.66 ^a	5.97±2.85 ^a
Food groups (g/day)			
Fruits	52.2±63.4	52.3±65.5	40.7±72.5
Vegetables	81.9±59.9 ^b	89.5±63.5 ^a	93.7±67.1
Potatoes	9.95±11.7 ^b	12.1±12.6 ^a	11.6±11.9
Legumes	4.19±11.7	3.76±11.1	2.92±7.30
Grains and bread	43.3±32.2 ^{bc}	56.2±38.6 ^{ac}	64.7±44.9 ^{ab}
Pasta	12.0±17.3 ^b	14.4±21.3 ^a	14.0±21.6
Red meat	10.8±13.9 ^{bc}	13.1±15.7 ^{ac}	20.2±24.2 ^{ab}
Processed meat	17.2±19.1 ^{bc}	22.7±24.0 ^{ac}	31.0±33.1 ^{ab}
Lean meat	11.0±12.4 ^b	12.8±13.9 ^a	12.7±14.8
Seafood	28.8±21.3 ^b	31.0±2.30 ^a	29.7±21.9
Eggs	12.6±9.83 ^b	13.6±9.65 ^a	13.1±11.0
Dairy	61.9±57.1	63.6±58.3	62.0±83.6
Commercial bakery	1.77±5.93 ^b	2.38±6.90 ^a	2.08±7.73
Sweets	0.73±3.87	0.81±4.53	0.36±1.83
Sandwiches, hamburgers, ready to eat food	5.26±8.61 ^{bc}	7.25±10.3 ^a	7.63±11.9 ^a
Appetizers	0.86±4.06 ^c	0.97±4.52	1.97±7.13 ^a
SSB	18.1±57.8 ^{bc}	29.4±78.9 ^{ac}	53.6±134 ^{ab}
Alcoholic beverages *	45.1±80.5 ^{bc}	63.6±112 ^{ac}	102±147 ^{ab}

HBF = high-energy breakfast; LBF = low energy breakfast; SBF = skipping breakfast; MUFA=monounsaturated fatty acids. PUFA=polyunsaturated fatty acids. SFA=saturated fatty acids. * distilled spirits, wine, beer

^a – p<0.05 versus HBF

^b – p<0.05 versus LBF

^c – p<0.05 versus SBF

Table 4: Association between breakfast-patterns and components of the metabolic syndrome among PESA participants.

	Breakfast pattern categories		
	HBF N=1122	LBF N=2812	SBF N=118
	OR (95% CI)		
Central obesity			
Cases/ noncases	136/986	680/2132	45/73
Model 1	1 (ref)	2.14 (1.74; 2.63) ††	4.26 (2.76; 6.58) ††
Model 2	1 (ref)	1.40 (1.03; 1.89) *	1.31 (0.64; 2.66)
Elevated triglycerides			
Cases/ noncases	91/1031	352/2460	21/97
Model 1	1 (ref)	1.34 (1.04; 1.72) *	1.83 (1.07; 3.14) *
Model 2	1 (ref)	1.13 (0.88; 1.46)	1.22 (0.69; 2.14)
Low HDL-c			
Cases/ noncases	327/795	955/1857	45/73
Model 1	1 (ref)	1.29 (1.10; 1.50) *	1.76 (1.18; 2.64) *
Model 2	1 (ref)	1.13 (0.97; 1.32)	1.32 (0.87; 1.99)
Hypertension			
Cases/ noncases	97/1025	356/2456	27/91
Model 1	1 (ref)	1.27 (0.99; 1.62)	2.46 (1.47; 4.09) †
Model 2	1 (ref)	1.03 (0.80; 1.33)	1.52 (0.88; 2.61)

HBF = high-energy breakfast; LBF = low energy breakfast; SBF = skipping breakfast

* p < 0.05, † p < 0.01, †† p < 0.0001

Models were adjusted as follows: Model 1: age, sex, energy intake, daily alcohol consumption, smoking, family history of CVD. Model 2: Model 1 plus BMI.

Central obesity was defined as waist circumference > 102cm for men, and > 88cm for women

Elevated triglycerides were defined as triglycerides ≥ 150 mg/dL. How HDL-c was defined as HDL-c < 40 mg/dL

Due to small sample size high fasting glucose results were not presented

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**Regularity and quality of breakfast consumption among Irish adult of NANS
(National Adult Nutrition Survey)
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GENERAL INTRODUCTION / INDIVIDUAL'S CONTRIBUTION

The investigation of different, based on the energy intake definition, breakfast patterns from the previous study showed that ingestion of fewer than ~120kcal in the morning, which was estimated as an equivalent to a cup of coffee or an orange juice, was associated with an unhealthy overall dietary and lifestyle pattern. Thus the question remained whether it could only be seen among habitual breakfast skipping or it could be applied also for those who consume breakfast irregularly. Another knowledge gap which was aimed to be addressed is the optimal nutritional composition of regular breakfast consumption.

I first determined the breakfast regularity by estimating the number of days in which breakfast was consumed during the 4 days of the dietary survey. Regular and irregular breakfast consumers were identified as consuming breakfast 3-4 out of 4 days and 1-2 out of 4 days, respectively. Second, I assessed how breakfast regularity relates to the types of meals consumed during the day by using the meals derived from the generic meal coding system previously applied in NANS dataset (Woolhead *et al.*, 2015).

In order to estimate the overall dietary pattern followed by regular and irregular breakfast consumers, I have used an “*a-posteriori*” data reduction technique, such as PCA. The final analysis of Eigen values and scree plot supported the final decision to retain a 7 factors solution, which indicated that there were total number of 7 dietary patterns followed among NANS participant: “Sandwich”, “Desserts”, “WDP”, “Potato”, “Breakfast cereal”, “Vegetarian”, and “Fish and vegetables”. The results showed that irregular breakfast consumers were more likely to follow WDP, while regular breakfast consumers were more likely to follow “Breakfast cereal”, “Vegetarian”, “Fish and vegetables” or “Sandwich” dietary pattern.

I also assessed the overall nutritional quality of regular and irregular breakfast consumption using “*a-priori*” approach, in particular, by estimating an adherence to Nutrient-rich food index (NRF) 9.3 (Drewnowski *et al.*, 2014). Regular breakfast consumers presented on average higher NRF9.3 score which indicated the higher nutritional quality of the diet followed by those participants.

I further assessed an optimal nutritional composition of regular breakfast consumption. It was observed that irregular breakfast contributed to higher fat, and sodium intakes, as well lower fiber intakes at breakfast. Moreover, regular breakfast consumption if consisted of breakfast cereal, bread and rolls, and dairy such as milk and yogurt significantly contributed to daily intakes of iron, calcium, folate, and niacin.

Regular breakfast consumption and diet quality among Irish population: National Adult Nutrition Survey

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INTRODUCTION

Breakfast is often referred to as the most important meal of the day and its omission is associated with cardiovascular risk factors including obesity, diabetes, unfavorable lipid profile, coronary heart disease and atherosclerosis [1-2]. Apart from being one of the most important meals of the day, breakfast was reported to be related to overall lifestyle. Thus studies have shown that skipping breakfast is associated with lower dietary quality [3] and unhealthy lifestyle behaviour, including smoking [4], and lack of physical activity [5]. Few studies have evaluated the contribution of breakfast to daily macro- and micronutrients intake showing that breakfast skippers have significantly lower intakes of dietary fibre, niacin, folate, riboflavin, vitamin C and A, Ca, P, Fe, K and Mg [6-7] and were also less likely to comply with Estimate Average Requirements (EAR) for Ca, vitamin C and folate [8].

Moreover, breakfast consumption has been associated with an overall healthier dietary pattern such as higher adherence to Mediterranean type diet [9] and an increase in overall diet quality measured by adherence to a Healthy Eating Index (HEI) [10]. To the best of our knowledge, there are no studies addressing the optimal composition and regularity of breakfast consumption among adults. Therefore, by addressing this knowledge gap we are aiming to characterize the regularity of breakfast consumption among adult population in Ireland, identify the overall dietary patterns associated with it and to evaluate the nutritional quality of regular breakfast intakes measured by the nutrient-rich food index score (NRF9.3) [11].

METHODS

Study population

The NANS (National Adult Nutrition Survey), is a cross sectional food consumption survey conducted among Irish adult participants. Details of the study design and methodology have been previously described [12]. Data on diet, lifestyle, and attitudes towards food were collected for 1500 free living men and women over 18 years of age residing in Republic of Ireland at the time of the survey. Lactating and pregnant women were excluded from the survey. The study protocol has been approved by the Human Ethics Research Committee of University College

Dublin and the University College Cork Research Ethics Committee of the Cork Teaching Hospitals [ECM 3(p) 04/11/08], and all participants provided written informed consent.

Assessment of demographic, dietary, and other lifestyle data

Demographic information included: gender (male/female), age (years), marital status (married / widow / single / divorced) and education level (secondary school / professional training / higher education). Dietary data was collected by means of a 4-day food diary, where participants were requested to record and weigh any food or beverage consumed over 4 days and provide the information regarding the type of the food and meal consumed (e.g. breakfast, snack, lunch main meal etc.), preparation method, manufacturer and time of ingestion. Weighed Intake Software Program (WISP) version 3.0 (Tinuviel Software) was used to analyse nutrient intake data. A total of 2552 food codes (including 233 supplements) were categorized into 19 food groups for data analysis. Lifestyle variables included smoking status (currently smoke / used to smoke / never smoked). The analysis was performed for breakfast consumers only therefore those participant who skipped breakfast during all 4 days of the food diary (n=14) were excluded and the final sample for analysis consisted of 1486 participants. Missing values on weight (n=87) were excluded from the NRF9.3 index computation.

Definition of breakfast and categorisation of regular versus irregular breakfast consumers

Breakfast was defined as “the first meal of the day that breaks the fast after the longest period of sleep, eaten before or at the start of daily activities (e.g. errands, travel, work), within 2 hours of waking, typically no later than 10:00 in the morning, and of an energy level between 20 and 35% of total daily energy need” [13] and comprised of “a food or beverage from at least one food group, and may be consumed at any location. Coffee, water and non-alcoholic beverages are not included in this definition” [14]. In order to determine the regularity of breakfast consumption, the number of days in which breakfast was consumed during the 4-day collection period were classified as follows: 4/4 – breakfast consumed on 4 out of 4 days; 3/4 – breakfast consumed on 3 out of 4

days; 2/4 – breakfast consumed on 2 out of 4 days; 1/4 – breakfast consumed 1 out of 4 days. Regular and irregular breakfast consumers were defined as consuming breakfast 3-4 out of 4 days and 1-2 out of 4 days, respectively.

Food-based patterns, meal intakes assessment and calculation of nutrient-rich food index score NRF9.3

Overall food-based dietary patterns followed by the participants of the study were identified by Principal Component Analysis (PCA). This method is used to identify common underlying dimensions (factors) of food consumption by deriving factor loadings for each pre-defined food group. Factors were subsequently rotated using a Varimax procedure to maintain uncorrelated factors. Analysis of eigenvalues, scree plot, and the interpretability of the factor solution were used to support a final decision on retaining a 7-factor solution, where each factor had an eigenvalue >0.3. A factor score for each participant was calculated by summing the daily intake of each food group weighted by their factor loadings. Further, a factor score of each of the 7 dietary patterns was split according to tertiles, which were used for the analysis.

In order to assess how breakfast regularity relates to the types of meals consumed during the day we used a generic meal coding system previously applied in NANS dataset. The methodology applied is described in detailed elsewhere [15-16], however in brief, the available data on food items were reduced into 20 pre-defined food categories with meal types corresponding to each of those, among them breakfast, light meals (throughout the day), main meals (including lunch and dinner) were used in the analysis. Within each meal type, the combination of all food groups consumed by one person on a single survey day was identified as an individual meal. The individual meals were grouped into generic categories including 5 generic breakfast meals (skipped breakfast, cereal and toast or cereal or toast, cooked breakfast, fruits or fruit juice, other), 6 generic light meal categories (skipped light meal, meat fish or dairy sandwich, dairy sandwich, meat or fish sandwich, soups or salads, rice or potato or pasta, other) and 4 generic main meals (skipped main meal, protein and carbohydrate based, protein based, carbohydrates based main meal) [16].

To assess the overall nutritional quality of the dietary patterns derived, we used the Nutrient-rich food index (NRF) 9.3 [11], which includes 9 nutrients for which intake is “encouraged” namely protein, dietary fibre, vitamin A,

C and E, calcium, iron, potassium, magnesium, and 3 nutrients “to limit”: saturated fat, total sugar, and sodium. The recommended daily values (DV) used in the calculation of the index were set by the European Food Safety Authority (EFSA Panel on Dietetic Products, Nutrition and Allergies) (**Online Appendix Table 2**). The sub-scores for content of nutrient in 100g of selected food group (NRn_100_g) and content of limiting nutrient in 100g of selected food group (LIM_100_g) were calculated per each of the total 18 food groups (nutritional supplements were excluded). The percentage of reference DV for each nutrient was capped at 100% DV to avoid overvaluing food items that provide very large amounts of a single nutrient. NRn_100_g and LIM_100_g were then further converted into the variable “content of nutrient in 100kcal of selected food group (NRn_100_{kcal}) and into the variable “content of limiting nutrient in 100kcal of selected food group (LIM_100_{kcal}), and the NRF9.3 for each food group was calculated by subtracting LIM_100_{kcal} from NRn_100_{kcal}. Followed by the methodology applied by Sluik and colleagues [17] NRF9.3 food scores per food group were converted to individual NRF9.3 index scores: energy consumed from each food group (in 100-kcal units) was multiplied by NRF9.3 score calculated for each food group, the scores obtained were further summed together and divided by the number of 100kcal units of participants total energy intake (**Online Appendix Table 2**). NRF9.3 index was used to determine the nutritional quality of regular breakfast consumption and dietary patterns derived from PCA.

Statistical analysis

ANOVA for continuous variables and chi-square for categorical variables were used to compare the data between categories of breakfast consumers. Characteristics of the study population were presented as counts and percentages for categorical variables and as mean \pm SD for continuous variables. Non-adjusted logistic regression models were used to assess the association between the adherence to PCA derived dietary patterns (3rd tertile vs. 1st tertiles) and regularity of breakfast consumed. Additionally, to assess the relationship between overall dietary quality measured by nutritional index NRF 9.3 and adherence to dietary patterns identified by PCA the Pearson correlation coefficient was used. P values < 0.05 (two-sided) were considered statistically significant. All statistical analyses were performed with the IBM SPSS Statistics for Windows, version 24 (IBM Corp., Armonk, N.Y., USA)

RESULTS

Of 1486 participants of the study 94% of participants consumed breakfast at least 3 days out of the 4-day collection period and were considered regular breakfast consumers. Participants of all age groups were equally represented, with slightly fewer participants being over 50y and 64y of age (21% and 15%, respectively). Forty-six percent of the cohort reported higher level of education, 58% reported to be married and 52% reported to never have smoked. Nearly 40% of the participants preferred to skip light meal and the majority of the population (93%) consumed protein and carbohydrates contained main meal. With respect to regularity of breakfast consumed, significant differences were observed in gender, age, marital status, smoking habits and type of light meal consumed during the day between irregular and regular breakfast consumers. Approximately half (52%) of the regular breakfast consumers were females. Younger adults (18-35y) were more likely to be irregular breakfast consumers, while older population (50y of age and older) were most likely to consume breakfast regularly. There was no difference observed in education level between regular and irregular breakfast consumers. Single participants and those who currently smoke were more likely to be irregular breakfast consumers. Irregular breakfast consumers were more likely to skip the consumption of a light meal, while regular breakfast consumers were more likely to consume a sandwich as a light meal. The assessment of overall dietary quality of regular and irregular breakfast consumers showed that regular breakfast consumers compared to irregular breakfast consumers had significantly higher NRF 9.3 score (**Table 1**).

Assessment of energy and nutrients intakes at breakfast from major food groups showed that breakfast cereals, breads and rolls, dairy, meat and meat dishes accounted for 22.8%, 19.8%, 11.2% and 10.0% of energy, respectively, resulting in a total contribution of 63.8% of energy, 74.5% of protein, 46.2% of fat, 70.5% of carbohydrates and 71.6% of dietary fibre at breakfast (**Online Appendix Table 2**). Fruits, table sugar, confectionary, preserves, savoury snacks, biscuits and cakes and milk and yogurts contributed to 71.1% of total sugar. It was also found that 52.8% of fat was derived from butter, spread and oils, meats and eggs. Nutritional supplements did not contribute significantly to the intake of energy or macronutrients, accounting for less than 1% of energy, fat and carbohydrate and 1.77% of protein, however, they were a major source of micronutrients,

representing a 66.2% of vitamin B1, 58.7% B2, and 58.8% of B12 intakes (**Online Appendix Table 3**). Excluding nutritional supplements from the analysis revealed that breakfast cereals were the main source of micronutrients including iron, folate, B1, B2, and B3, contributing 56.3%, 39.0%, 50.4%, 44.3%, 56.0% to the total intake respectively. Dairy was the major source of calcium (41.7%) and vitamin B12 (30.4%) (**Online Appendix Table 4**). It was observed that breakfast provided 19.9% of total daily energy intake, 24.4% of dietary fibre, 31.6% of iron, 29.8% of calcium, 32.0% of folate, and 36.5% of B2. Irregular breakfast consumers comparing to regular breakfast consumers had significantly ($p<0.05$) lower intakes of dietary fibre, iron, calcium, folate, B2, and vitamin D, and higher intakes of sodium and fat at breakfast (**Table 2**).

PCA identified 7 main dietary patterns prevalent among the Irish population. “Sandwich” pattern was characterised by higher intakes of breads and rolls, butter, oils and cheese and according to the NRF9.3, greater adherence to this dietary pattern was associated with lowest nutritional quality, and was most likely to be followed by regular breakfast consumers (OR: 3.35; 95%CI: 1.77; 6.33, $p<0.001$). “Western” pattern was characterised by higher intakes of grains, pasta, beverages, meat and eggs and had the lowest probability to be followed among regular breakfast consumers (OR: 0.45; 95%CI: 0.26; 0.79, $p=0.006$). “Breakfast cereal” pattern was characterised by higher intakes of breakfast cereals, dairy, nuts and seeds and had 4.62-fold higher probability to be followed among regular breakfast consumers. Higher adherence to “Vegetarian” dietary pattern was correlated with consumption of nuts, seeds, herbs, soups, fruits, vegetables and nutritional supplements. Regular breakfast consumers had 2.59-fold higher odds of following this pattern. Finally, “Fish and vegetables” pattern was observed be strongly correlated with consumption of fish and vegetables and low intakes of meat and showed a moderate positive linear relationship with NRF 9.3. There was 2.88-fold increase in odds of following this dietary pattern among breakfast consumers. (**Table 3.; Figure 1**).

DISCUSSION

Regular breakfast consumption was associated with higher overall dietary quality in our study. Participants who consumed breakfast regularly had the highest adherence to the healthiest dietary patterns, namely

“Vegetarian”, “Fish and vegetables” and “Breakfast cereals” dietary patterns. In contrast to irregular breakfast, regular breakfast consumption, as observed in NANS, contributed significantly to the total micronutrients profile. It was observed that regular breakfast provides on average as much as 24% of dietary fibre, 32% of iron, 30% of calcium, 32% of folate, and 37% of riboflavin to the daily micronutrient intakes.

Regular breakfast participants predominantly reported to choose cereal or toast for breakfast, known to be a good source of iron, and B-vitamins, as well as dairy; a major source of calcium and vitamin B12. These findings are of particular importance as the promotion of regular breakfast consumption could serve as a simple message used by health professionals to address inadequate micronutrient intake and increase the overall adherence to dietary guidelines. Examination of the types of dietary patterns followed by regular breakfast consumers showed that those participants follow a healthier dietary pattern, indicated by the types of foods consumed and the associated NRF9.3 values. These findings are in line with results reported by Hopkins and colleagues [10], where a higher frequency of breakfast consumption was seen to be associated with higher intakes of beans and green vegetables, whole grains and fruits, which are known for their health benefits [18-19]. Studies evaluating the relationship between regular breakfast consumption and overall diet quality and regulation of appetite [20-21], reported that breakfast cereals were potentially satiating and may have a beneficial effect on appetite regulation. In our study regular breakfast consumers were more likely to choose whole grain breakfast cereal for breakfast or toast. This may have a positive impact on their daily meal consumption pattern, as breakfast consumers might choose the latter dietary intakes more carefully and consciously with the preference lined up toward healthier food choices.

On average in our study 10% of the participants consumed breakfast only 1 or 2 days out of 4 days, and as such were considered an irregular breakfast consumers. Participants falling into this group were observed to be more likely to be current smokers and have a poorer overall diet including lower intakes of dietary fibre, iron, calcium, folate, B2 and vitamin D, and higher intakes of sodium and fat at breakfast. These findings are in agreement with previous studies where irregular breakfast intake was associated with smoking [4], increased daily energy intake (EI) [22], and non-compliance with healthy eating recommendations [23]. The overall dietary pattern followed by

irregular breakfast consumers in our study falls predominantly into “Western dietary pattern”, which was characterized by higher intakes of grains, pasta, savories, beverages, meat and meat dishes and previously has been linked to unhealthier lipid profile, overweight and obesity, and metabolic syndrome [24-26]. In line with this cluster of unhealthy behaviors, irregular breakfast consumption might serve as a marker for a general unhealthy diet and lifestyle as it has been observed in previous studies, where skipping breakfast was defined to be an indicator of unhealthy lifestyle and was associated with an increased risk of atherosclerosis [2].

A number of studies showed that regular breakfast consumption is associated with a lower prevalence of obesity. Young adults in the National Health and Nutrition Examination Survey (NHANES) reported to consume read-to-eat cereals for breakfast were 31% less likely to be overweight or obese and 39% less likely to have abdominal obesity comparing with those who skipped breakfast [7]. Additionally, a meta-analysis of 19 studies showed an overall 75% increase in the risk of obesity among the irregular breakfast consumers [27]. The percentage of participants who were overweight or obese did not differ significantly between regular and irregular breakfast consumers which could be due to reverse causation. Therefore, we can't rule out the possibility that those overweight individuals might have engaged in irregular breakfast consumption in order to lose weight.

Our study has some limitations worth considering. Even though our study comprised of a large sample size, the participants of NANS have a very specific dietary patterns and results might not be generalizable to other populations. Suggestions for future direction include validation of our definition of breakfast irregularity and to study the association between regular and irregular breakfast intakes and chronic disease progression.

CONCLUSION

In this large population, a -representative sample of Irish adults, regular breakfast intake was associated with higher overall dietary quality and could serve as an indicator of healthy dietary and lifestyle behavior. Based on the findings obtained it could be suggested that health professionals highlight the importance of regular breakfast consumption among those who skip breakfast as it could be used as simple message to improve compliance with nutritional recommendations and adherence to a healthy lifestyle

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Table 1. Demographic and lifestyle characteristics of regular and irregular breakfast consumers from a 4-day national dietary survey of Irish adults (N=1486).

Baseline characteristics	Total	Breakfast type	
		Irregular n = 97	Regular n = 1403
N (%)			
Sex			
Male	731 (49.2)	51 (61.4)	680 (48.5) *
Female	755 (50.8)	32 (38.6)	723 (51.5) *
Age Group (years)			
18-35	526 (35.4)	58 (69.9)	468 (33.4) *
36-50	431 (29.0)	18 (21.7)	413 (29.4) *
50-64	304 (20.5)	7 (8.40)	297 (21.2) *
>64	225 (15.1)	0 (0.00)	225 (16.0) *
Education			
Secondary school	480 (33.4)	32 (42.7)	448 (32.9)
Professional training	293 (20.4)	10 (13.3)	283 (20.8)
Higher education	663 (46.2)	33 (44.0)	630 (46.3)
Marital status			
Single	496 (32.3)	47 (62.7)	422 (30.6) *
Married	849 (58.4)	27 (36.0)	822 (59.6) *
Widowed	71 (4.90)	0 (0.00)	71 (5.10) *
Separated	65 (4.50)	1 (1.30)	64 (4.60)
Smoking habits			
Current smoker	290 (20.1)	28 (36.8)	262 (19.1) *
Former smoker	397 (27.5)	14 (18.4)	383 (28.0) *
Never smoker	759 (52.5)	34 (44.7)	725 (52.9) *
Light meal types			
Skip light meal	199 (39.3)	23 (79.3)	176 (36.9) *
MFD sandwich	45 (8.90)	3 (10.3)	42 (8.80)
Dairy sandwich	17 (3.40)	0 (0.00)	17 (3.60)
MF sandwich	140 (27.7)	3 (10.3)	137 (28.7) *
Soup and salad	13 (2.60)	0 (0.00)	13 (2.70)
Rice potato pasta	1 (0.20)	0 (0.00)	1 (0.20)
Main meal types			
Skip main meal	10 (1.00)	1 (2.00)	9 (0.90)
Protein and carbohydrates	943 (92.9)	44 (86.3)	899 (93.3)
Protein	43 (4.20)	5 (9.80)	38 (3.90)
Carbohydrate	16 (1.60)	1 (2.00)	15 (1.60)
NRF 9.3	33.8 ± 14.2	28.5 ± 11.1	34.1 ± 14.3 *

Regular breakfast consumers were defined as consuming breakfast 3-4 out of 4 days and irregular breakfast consumers defined as consuming breakfast 1-2 out of 4 days. Values are n (%). Bonferroni correction was applied ($p < 0.025$). * $p < 0.025$ vs. irregular. Type of meal identified as consuming this type of meal at least 3 out of 4 days.

Table 2. Mean intakes at breakfast and overall daily intakes of energy, micro and macronutrients among breakfast consumers only (N=1486) from a national dietary survey of Irish adults

Nutrient	All Breakfast Consumers N=1486		Regular Breakfast Consumers N=1403		Irregular Breakfast Consumers N=83	
	BF intake	Daily intake	BF % daily	BF intake	Daily intake	BF % daily
	Mean ± SD or %					
Energy, Kcal	400 ± 193	2011 ± 657	19.9	399 ± 186	2004 ± 646	19.9
Protein, g	13.7 ± 7.35	83.4 ± 26.9	16.4	13.7 ± 7.10	83.6 ± 26.7	16.4
Fat, g	12.8 ± 9.70	75.8 ± 29.4	16.9	12.6 ± 9.20	75.6 ± 29.2	16.7
Carbohydrates, g	59.0 ± 28.5	229 ± 78.9	25.8	59.2 ± 28.1	229 ± 78.7	25.9
Total sugars, g	24.6 ± 16.1	90.5 ± 43.1	27.2	24.9 ± 15.8	90.8 ± 42.9	27.4
Dietary fibre, g	4.68 ± 3.55	19.2 ± 7.87	24.4	4.77 ± 3.57	19.5 ± 7.88	24.5
Iron, mg	3.82 ± 3.29	12.1 ± 5.14	31.6	3.82 ± 3.22	12.2 ± 5.12	31.3
Calcium, mg	268 ± 151	900 ± 371	29.8	268 ± 149	905 ± 370	29.7
Sodium, mg	469 ± 366	2501 ± 903	18.8	457 ± 328	2494 ± 898	18.4
Folate, mcg	102 ± 81.0	319 ± 153	32.0	102 ± 79.8	322 ± 152	31.7
Vitamin B1, mg	0.48 ± 0.35	1.76 ± 2.21	27.3	0.48 ± 0.34	1.78 ± 2.26	27.0
Vitamin B2, mg	0.69 ± 0.50	1.89 ± 0.83	36.5	0.69 ± 0.49	1.91 ± 0.84	36.1
Vitamin B3, mg	5.02 ± 4.45	24.2 ± 10.7	20.7	5.02 ± 4.37	24.2 ± 10.6	20.7
Vitamin B12 mcg	0.97 ± 0.87	4.65 ± 3.5	20.9	0.97 ± 0.86	4.69 ± 3.53	20.7
Vitamin D, mcg	0.81 ± 1.15	3.26 ± 2.62	24.8	0.80 ± 1.15	3.30 ± 2.63	24.2

Regular breakfast consumers defined as consuming breakfast 3-4 out of 4 days and irregular breakfast consumers defined as consuming breakfast 1-2 out of 4 days. * - significantly different from regular breakfast consumers (p< 0.05). BF % daily – contribution of breakfast toward daily energy and nutrient intake

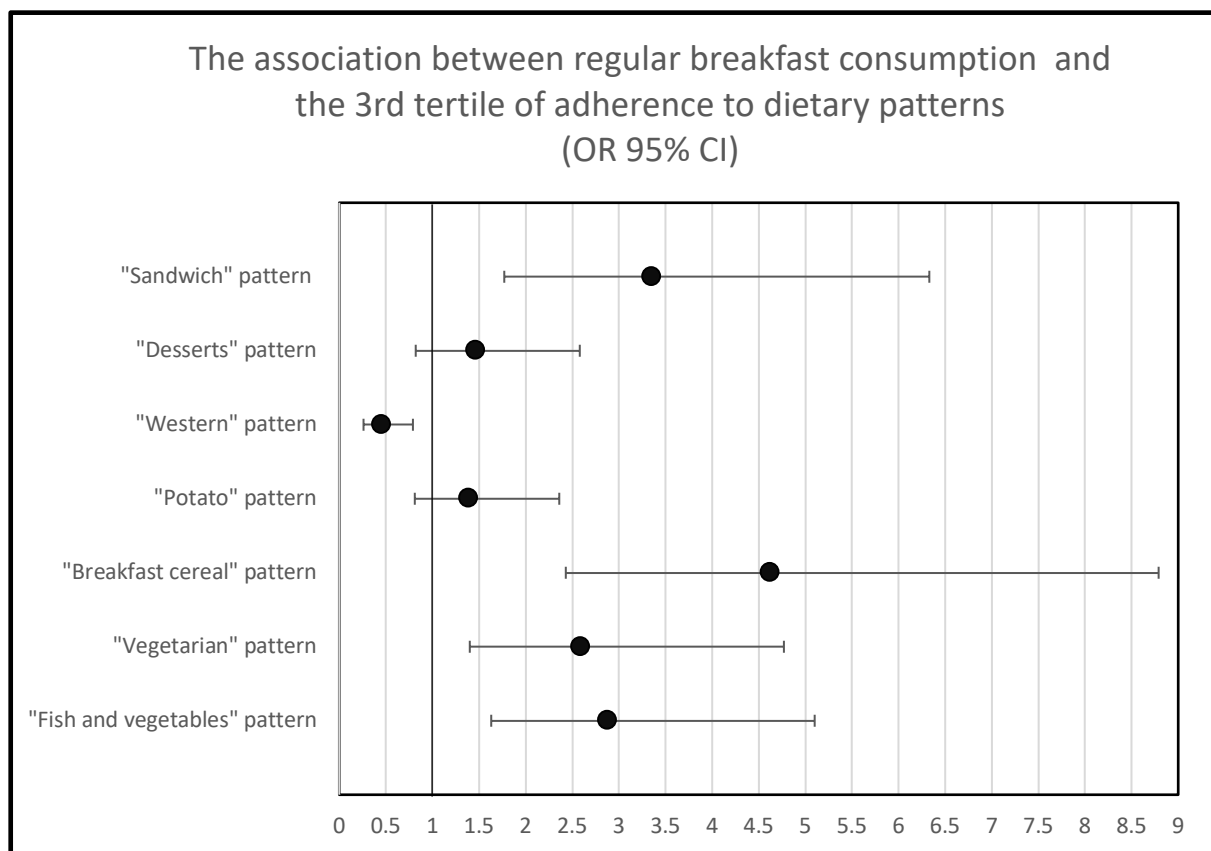
Table 3: A representation of the main food items characterizing each dietary pattern and its correlation with nutritional index NRF 9.3

Food groups	"Sandwich" pattern	"Desserts" pattern	"Western" pattern	"Potato" pattern	"Breakfast cereal" pattern	"Vegetarian" pattern	"Fish and vegetables" patterns
Factor loading							
Bread & rolls	0.819						
Butter & oils	0.795						
Cheese	0.391			-0.512			
Potatoes				0.748			
Baked goods		0.708					
Sugar, confectionary, snacks		0.639					-0.305
Desserts		0.513					
Beverages			0.708				
Meat			0.526				-0.465
Grains, pasta & savouries			0.485	-0.440			
Eggs			0.315		-0.370		
Dairy					0.668		
Breakfast cereal					0.534		
Nuts, seeds & herbs					0.366	0.529	
Supplements						0.654	
Soups & sauces						0.532	
Fruits						0.522	
Vegetables						0.320	0.568
Fish							0.740
Correlation coefficient between food – based dietary patterns and NRF 9.3							
NRF 9.3	-0.332**	-0.282**	-0.165**	-0.036	0.072**	0.283**	0.357**

* Values correspond to factor loadings (the higher the value, the more a particular food item contributes to the dietary cluster). Factor loadings below or above ± 0.03 were omitted for better interpretability

* p < 0.05; ** p < 0.01; † p < 0.001

Figure 1. The association between regular breakfast consumption with the highest adherence to the food-based dietary patterns (3rd tertile).



Model presented is a non-adjusted logistic regression model with odds ratios (OR) and 95% confidence interval (CI).

ONLINE APPENDIX

Regular breakfast consumption and diet quality among Irish population: National Adult Nutrition Survey

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Online Table 1. Demographic and lifestyle characteristics of population across breakfast consumption categories from a national dietary survey of Irish adults (N=1486).

		Frequency of breakfast consumption			
		4/4 days n = 1241	3/4 days n = 162	2/4 days n = 55	1/4 days n = 28
		N (%)			
Sex					
	Male	595 (47.9)	85 (52.5)	30 (54.5)	21 (75.0)
	Female	646 (52.1)	77 (47.5)	25 (45.5)	7 (25.0)
Age Group (years)					
	18-35	366 (29.5)	102 (63.0)	39 (70.9)	19 (67.9)
	36-50	371 (29.9)	42 (25.9)	10 (18.2)	8 (28.6)
	50-64	283 (22.8)	14 (8.60)	6 (10.9)	1 (3.60)
	>64	221 (17.8)	4 (2.50)	0 (0.00)	0 (0.00)
Education					
	Secondary school	540 (44.9)	74 (46.5)	21 (39.6)	15 (57.7)
	Professional training	98 (8.10)	10 (6.30)	4 (7.50)	2 (7.70)
	College degree	565 (47.0)	75 (47.2)	28 (52.8)	9 (34.6)
Marital status					
	Single	340 (27.6)	89 (54.9)	32 (60.4)	17 (63.0)
	Married	763 (61.8)	68 (42.0)	20 (37.7)	10 (37.0)
	Widowed	70 (5.70)	1 (0.60)	0 (0.00)	0 (0.00)
	Separated	61 (4.90)	4 (2.50)	1 (1.90)	0 (0.00)
Smoking habits					
	Current smoker	211 (17.3)	57 (35.2)	20 (36.4)	11 (42.3)
	Former smoker	355 (29.0)	30 (18.5)	12 (21.8)	3 (11.5)
	Never smoker	657 (53.7)	75 (46.3)	23 (41.8)	12 (46.2)

4/4 – breakfast consumed 4 out of 4 days; 3/4 – breakfast consumed 3 out of 4 days; 2/4 – breakfast consumed 2 out of 4 days; 1/4 – breakfast consumed 1 out of 4 days.

Online Table 2. Intakes of energy and macronutrients (units/d) from breakfast and contribution (%) to total intakes at breakfast from major food groups for breakfast consumers only from a national dietary survey of Irish adults (N=1486)

Food Group	Energy	Protein	Fat	Carbohydrate	Total sugar	Fiber	kcal/d or g/d (% of contribution to total intake)	
							402	14.0
Actual mean daily population intake	608 (100)	21.5 (100)	14.0	21.6 (100)	83.1 (100)	33.9 (100)	6.69 (100)	
Sum of the food groups	138.6 (22.8)	4.24 (19.7)	2.09 (9.76)	2.09 (9.76)	27.2 (32.7)	5.70 (16.8)	2.41 (36.0)	
Breakfast Cereals	120.2 (19.8)	4.35 (20.3)	1.43 (6.61)	1.43 (6.61)	23.0 (27.7)	1.92 (5.66)	2.22 (33.2)	
Bread & rolls	68.3 (11.2)	4.11 (19.1)	2.84 (13.1)	2.84 (13.1)	6.51 (7.83)	6.39 (18.8)	0.03 (0.45)	
Milk & yoghurt	48.8 (8.02)	0.07 (0.33)	5.37 (24.8)	5.37 (24.8)	0.10 (0.12)	0.06 (0.18)	0.00 (0.00)	
Butter, spreading fats & oils	43.2 (7.10)	0.57 (2.65)	0.13 (0.60)	0.13 (0.60)	10.5 (12.6)	10.1 (29.8)	1.08 (16.1)	
Fruit & fruit dishes	30.4 (5.00)	0.11 (0.51)	0.33 (1.53)	0.33 (1.53)	7.20 (8.66)	6.64 (19.6)	0.08 (1.20)	
Sugars, confectionary, preserves & savoury snacks	60.6 (10.0)	3.30 (15.4)	3.60 (16.7)	3.60 (16.7)	1.91 (2.30)	0.18 (0.53)	0.13 (1.94)	
Meat & meat products	31.2 (5.13)	2.25 (10.5)	2.45 (11.3)	2.45 (11.3)	0.02 (0.02)	0.02 (0.06)	0.00 (0.00)	
Eggs & egg dishes	11.7 (1.92)	0.37 (1.72)	0.49 (2.27)	0.49 (2.27)	1.49 (1.79)	0.39 (1.15)	0.13 (1.94)	
Grains, rice, pasta & savouries	16.0 (2.63)	0.27 (1.26)	0.76 (3.52)	0.76 (3.52)	2.33 (2.80)	0.97 (2.86)	0.13 (1.94)	
Biscuits, cakes & pastries	8.69 (1.43)	0.53 (2.47)	0.69 (3.19)	0.69 (3.19)	0.03 (0.04)	0.03 (0.09)	0.00 (0.00)	
Cheeses	3.32 (0.55)	0.19 (0.88)	0.01 (0.05)	0.01 (0.05)	0.73 (0.88)	0.65 (1.92)	0.00 (0.00)	
Beverages	3.52 (0.58)	0.38 (1.77)	0.16 (0.74)	0.16 (0.74)	0.11 (0.13)	0.06 (0.18)	0.05 (0.75)	
Supplements	23.8 (3.91)	0.74 (3.45)	1.27 (5.87)	1.27 (5.87)	1.98 (2.38)	0.83 (2.45)	0.43 (6.43)	
Other food groups *								

* Other food groups (N=6) include 'creams, ice-creams and desserts', 'potato and potato dishes', 'veg and veg dishes', 'fish and fish dishes', 'soups, sauces and miscellaneous foods', 'nuts, seeds, herbs and spices'

Online Table 3. Intakes of micronutrients (units/d) from breakfast and contribution (%) to total intakes at breakfast from major food groups among breakfast consumers only (N=1486) from a national dietary survey of Irish adults

Food Group	Iron	Calcium	Folate	Thiamine	Riboflavin	Niacin	Vitamin B12	Vitamin D	Kcal/d or mcg/d (% of contribution to total intake)	
									Actual mean daily population intake	6.04
Sum of the food groups	8.11 (100)	397 (100)	202.1 (100)	2.05 (100)	2.21 (100)	11.2 (100)	3.58 (100)	2.52 (100)		
Breakfast Cereals	3.14 (38.7)	60.4 (15.1)	54.2 (26.8)	0.35 (17.0)	0.41 (18.3)	4.06 (36.3)	0.33 (9.20)	0.32 (12.6)		
Bread & rolls	0.99 (12.2)	76.9 (19.3)	21.1 (10.5)	0.13 (6.10)	0.04 (1.83)	1.21 (10.8)	0.00 (0.13)	0.02 (0.75)		
Milk & yoghurt	0.05 (0.58)	149 (37.6)	19.7 (9.73)	0.04 (2.02)	0.27 (12.3)	0.18 (1.61)	0.45 (12.7)	0.22 (8.77)		
Butter, spreading fats & oils	0.00 (0.00)	0.44 (0.11)	13.7 (6.75)	0.00 (0.07)	0.00 (0.15)	0.02 (0.18)	0.10 (2.70)	0.21 (8.44)		
Fruit & fruit dishes	0.21 (2.64)	10.7 (2.70)	12.3 (6.07)	0.05 (2.61)	0.03 (1.29)	0.35 (3.10)	0.00 (0.12)	0.00 (0.04)		
Sugars, confectionary, preserves & savoury snacks	0.07 (0.81)	3.92 (0.98)	0.92 (0.45)	0.01 (0.28)	0.01 (0.33)	0.07 (0.65)	0.00 (0.12)	0.00 (0.01)		
Meat & meat products	0.39 (4.79)	13.1 (3.28)	1.03 (0.51)	0.06 (3.09)	0.03 (1.19)	0.82 (7.34)	0.19 (5.22)	0.14 (5.63)		
Eggs & egg dishes	0.34 (4.22)	10.8 (2.72)	6.53 (3.23)	0.01 (0.63)	0.07 (2.95)	0.02 (0.15)	0.27 (7.46)	0.31 (12.3)		
Grains, rice, pasta & savouries	0.12 (1.45)	2.80 (0.70)	0.78 (0.38)	0.01 (0.60)	0.00 (0.19)	0.06 (0.52)	0.01 (0.17)	0.00 (0.18)		
Biscuits, cakes & pastries	0.07 (0.85)	3.59 (0.90)	0.76 (0.38)	0.01 (0.30)	0.00 (0.19)	0.04 (0.36)	0.01 (0.18)	0.01 (0.59)		
Cheeses	0.01 (0.12)	16.3 (4.10)	0.61 (0.30)	0.00 (0.05)	0.01 (0.45)	0.00 (0.02)	0.03 (0.73)	0.01 (0.22)		
Beverages	0.02 (0.23)	1.37 (0.34)	4.68 (2.32)	0.00 (0.02)	0.03 (1.46)	0.22 (1.96)	0.02 (0.56)	0.00 (0.00)		
Supplements	2.54 (31.4)	40.4 (10.1)	62.8 (31.1)	1.36 (66.2)	1.30 (58.7)	3.95 (35.3)	2.10 (58.8)	1.25 (49.1)		
Other food groups *	0.16 (1.98)	7.27 (1.83)	3.00 (1.49)	0.02 (0.98)	0.01 (0.66)	0.20 (1.78)	0.07 (1.97)	0.03 (1.33)		

* Other food groups (N=6) include 'creams, ice-creams and desserts', 'potato and potato dishes', 'veg and veg dishes', 'fish and fish dishes', 'soups, sauces and miscellaneous foods', 'nuts, seeds, herbs and spices'

Online Table 4. Intakes of micronutrients (units/d) from breakfast and contribution (%) to total intakes at breakfast from major food groups excluding nutritional supplements among breakfast consumers only (N=1486) from a national dietary survey of Irish adults

Food Group	Iron	Calcium	Folate	Thiamine	Riboflavin	Niacin	Vitamin B12	Vitamin D	kcal/d or mcg/d (% of contribution to total intake)	
									Actual mean daily population intake	3.82
Sum of the food groups	5.57 (100)	356 (100)	139 (100)	0.69 (100)	0.91 (100)	7.25 (100)	1.48 (100)	1.27 (100)		
Breakfast Cereals	3.14 (56.3)	60.4 (16.9)	54.2 (39.0)	0.35 (50.4)	0.41(44.3)	4.06 (56.0)	0.33 (22.3)	0.32 (25.2)		
Bread & rolls	0.99 (17.7)	76.9 (21.5)	21.1 (15.2)	0.13 (18.0)	0.04(4.42)	1.21 (16.7)	0.00 (0.00)	0.02 (1.57)		
Milk & yoghurt	0.05 (0.89)	149 (41.7)	19.6 (14.1)	0.04 (5.97)	0.27(29.8)	0.18 (2.48)	0.45 (30.4)	0.22 (17.3)		
Butter, spreading fats & oils	0.00 (0.00)	0.44 (0.12)	13.6 (9.80)	0.00 (0.22)	0.00(0.36)	0.02 (0.28)	0.10 (6.76)	0.21(16.5)		
Fruit & fruit dishes	0.21 (3.77)	10.7 (3.00)	12.2 (8.81)	0.05 (7.72)	0.03(3.12)	0.35 (4.83)	0.00 (0.00)	0.00 (0.00)		
Sugars, confectionary, preserves & savoury snacks	0.07 (1.25)	3.92 (1.10)	0.92 (0.66)	0.01 (0.84)	0.01(0.80)	0.07 (0.97)	0.00 (0.00)	0.00 (0.00)		
Meat & meat products	0.39 (7.00)	13.1 (3.67)	1.03 (0.74)	0.06 (9.14)	0.03(2.88)	0.82 (11.3)	0.19 (12.8)	0.14 (11.0)		
Eggs & egg dishes	0.34 (6.10)	10.8 (3.03)	6.53 (4.69)	0.01 (1.87)	0.07(7.14)	0.02 (0.28)	0.27 (18.2)	0.31 (24.4)		
Grains, rice, pasta & savouries	0.12 (2.15)	2.80 (0.79)	0.78 (0.56)	0.01 (1.79)	0.00(0.46)	0.06 (0.83)	0.01 (0.68)	0.00 (0.00)		
Biscuits, cakes & pastries	0.07 (1.25)	3.59 (1.01)	0.76 (0.55)	0.01 (0.89)	0.00(0.47)	0.04 (0.55)	0.01 (0.68)	0.01 (0.79)		
Cheeses	0.01 (0.17)	16.3 (4.57)	0.61 (0.44)	0.00 (0.14)	0.01(1.08)	0.00 (0.00)	0.03 (2.03)	0.01 (0.79)		
Beverages	0.02 (0.35)	1.37 (0.38)	4.68 (3.36)	0.00 (0.07)	0.03(3.53)	0.22 (3.03)	0.02 (1.35)	0.00 (0.00)		
Other food groups *	0.16 (2.87)	7.27 (2.04)	3.00 (2.16)	0.02 (2.90)	0.01(1.60)	0.20 (2.76)	0.07 (4.73)	0.03 (2.36)		

* Other food groups (N=6) include 'creams, ice-creams and desserts', 'potato and potato dishes', 'veg and veg dishes', 'fish and fish dishes', 'soups, sauces and miscellaneous foods', 'nuts, seeds, herbs and spices'

Online Table 5. Recommended daily values (RDV) and maximum daily values (MDV) based on a 2000 kcal per day for selected nutrients by European Food and Authority (EFSA).

Nutrients	DV	MDV
Nutrients to encourage		
Protein (g)	0.83 per 1 kg of body mass *	
Fibre (g)	25 *	
Vitamin A (RE)	750 – men *	
	650 – women *	
Vitamin C (mg)	111 – men *	
	95 – women *	
Calcium (mg)	950 *	
	1000 – 18-24y *	
Iron (mg)	11 *	
	16 / 11 – pre-/postmenopausal *	
Vitamin E (mg)	13 – men *	
	11 – women *	
Potassium (mg)	3500 *	
Magnesium (mg)	350 – men *	
	300 – women *	
Nutrients to limit		
Saturated fat (g)		20 *
Total sugar (g)		125 *
Sodium (mg)		2400 *

RDV = recommended daily value; MDV = maximum daily value

* EFSA Panel on Dietetic Products, Nutrition and Allergies

Online Table 6. Overview of nutrient rich foods NRF 9.3 score algorithm

Model	Algorithm	Comment
NR _{100 g}	$\sum_{i=1}^9 (Nutrient_i / DV_i) \times 100$	<i>Nutrient_i</i> = content of nutrient in 100g of selected food group
NR _{100 kcal}	$(NR_{100 g} / ED) \times 100$	
LIM3 _{100 g}	$\sum_{i=1}^9 (L_i / MDV_i) \times 100$	<i>L_i</i> = content of limiting nutrient in 100g of selected food group
LIM3 _{100 kcal}	$(LIM3_{100 g} / ED) \times 100$	
NRF9.3 _{100 kcal}	NR _{100 kcal} - LIM3 _{100 kcal}	

DV = recommended daily value; MDV = maximum daily value; ED = energy density (kcal/100g)

CONCLUSIONS

1. Compared to MDP, individuals in the lowest quintile of adherence to the WDP, characterised by high intake of red meat, fast food, dairy, and cereals, presented on average 4.6 mg/dL lower HDL-c levels ($P < .001$), 8 mg/dL lower APOA1 levels ($P = .005$) and a greater risk of having decreased HDL-c (odds ratio = 3.19; 95% confidence interval, 1.36-7.5; P -trend = .03). Adherence to a Mediterranean dietary pattern is associated with 3.3mg/dL higher high-density lipoprotein cholesterol levels ($P < .001$), and a ratio of triglycerides to high-density lipoprotein cholesterol that was 0.43 times lower compared to WDP ($P = .043$).
2. An adherence to a newly identified dietary pattern, so-called Social-Business eating pattern, which is characterised by a high consumption of red meat, pre-made foods, snacks, alcohol, and sugar-sweetened beverages and frequent eating-out behaviour is associated with a significantly worse cardiovascular risk profile and, after adjustment for risk factors, increased odds of presenting subclinical atherosclerosis (OR: 1.31; 95% CI: 1.06 to 1.63) in a population of asymptomatic middle-aged adults.
3. Higher aMED score was associated with the presence of plaque in femoral arteries (odds ratio highest vs lowest aMED score quartile: 0.63; 95% confidence interval: 0.48-0.83; P trend = .045) independently of risk factors and mediators. The strongest association between aMED score and the presence of plaque was found among smokers, both in femoral (0.39 [0.22-0.69]; P trend = .001) and in any territory (0.33 [0.14-0.79], P trend = .008). aMED was inversely associated with the number of plaques in all territories except for carotids.
4. Moderate alcohol consumption, as well as higher adherence to aMED score is independently associated with a 6% lower risk of having subclinical atherosclerosis. Smoking abstinence is associated with a 11% lower risk of subclinical atherosclerosis. Those who follow 3 lifestyle behaviours (Mediterranean diet, non-smoking, and moderate alcohol intake) have 18% lower odds of presenting subclinical atherosclerosis compared with those who do not follow these protective lifestyle habits.
5. LCA identified 7 meal consumption patterns among the Irish population. The typical meal pattern followed by the majority was characterised by consumption of cereal or toast for breakfast, skipping or consuming a sandwich for light meal, and meat or fish with potatoes,

pasta or vegetables for the main meal. Eating patterns differed on weekends, and those participants who consumed meat and eggs for breakfast instead of breakfast cereal and skipped light meal were more likely to have an unhealthier dietary pattern, a higher diastolic blood pressure, and increased serum ferritin.

6. Independent of the presence of traditional and dietary CV risk factors, habitual skipping breakfast was associated with a higher prevalence of noncoronary (odds ratio: 1.55; 95% confidence interval: 0.97 to 2.46) and generalized (odds ratio: 2.57; 95% confidence interval: 1.54 to 4.31) atherosclerosis in a population of asymptomatic middle-aged adults.

7. Regular breakfast consumption was associated with higher overall dietary quality. Participants who consumed breakfast regularly had the highest adherence to the healthiest dietary patterns, namely “Vegetarian”, “Fish and vegetables” and “Breakfast cereals” dietary patterns. Regular breakfast consumption contributed significantly to the total micronutrients profile, as such it provided on average as much as 24% of dietary fibre, 32% of iron, 30% of calcium, 32% of folate, and 37% of riboflavin to the daily micronutrient intakes.

CONCLUSIONES

1. El quintil más bajo de adherencia al patrón de dieta occidental, rico en carnes rojas, comida rápida, productos lácteos y cereales presenta cifras de colesterol unido a lipoproteínas de alta densidad 4,6 mg/dl menores ($p < 0,001$) y de apolipoproteína A1, 8 mg/dl menores ($p = 0,005$) y mayor riesgo de mostrar una disminución del colesterol unido a lipoproteínas de alta densidad (odds ratio = 3,19; intervalo de confianza del 95%, 1,36-7,5; p de tendencia = 0,03). La adherencia al patrón de dieta mediterránea (MDP), rica en verduras, frutas, pescado, carnes blancas, frutos secos y aceite de oliva se asocia con cifras de colesterol unido a lipoproteínas de alta densidad 3,3 mg/dl mayores ($p < 0,001$) y un cociente de triglicéridos/colesterol unido a lipoproteínas de alta densidad 0,43 veces inferior ($p = 0,043$).
2. Un patrón dietético Social-Negocios, caracterizado por un elevado consumo en de carnes rojas, comida rápida, bocadillos, alcohol y bebidas azucaradas, y comidas fuera de casa de forma habitual se relaciona con un riesgo cardiovascular mas elevado, incluida una prevalencia elevada de aterosclerosis subclínica (odds ratio = 1,31; intervalo de confianza del 95%: 1,06 - 1,63)..
3. MDP, rica en verduras, frutas, pescado, carnes blancas, frutos secos y aceite de oliva se asocia con una menor prevalencia de placas de aterosclerosis en las arterias femorales de forma independiente de la presencia de otros factores de riesgo convencionales. La asociación más fuerte se encuentra entre los fumadores, tanto en femoral (odds ratio = 0,39; intervalo de confianza del 95%: 0,22-0,69), tendencia $P = 0,001$) y en cualquier territorio (odds ratio = 0,33; intervalo de confianza del 95%: 0,14-0,79, tendencia $P = 0,008$). aMED se asocia con menos placas en todos los territorios, con excepción de las carótidas.
4. El consumo moderado de alcohol, así como la mayor adherencia a MDP se asocian de forma independiente con una prevalencia reducida de aterosclerosis subclínica (odds ratio = 0,94; intervalo de confianza del 95%: 0,91-0,99, tendencia $P < 0,001$; odds ratio = 0,94; intervalo de confianza del 95%: 0,89-0,98, tendencia $P < 0,001$). La adherencia a MDP combinada con consumo moderado de alcohol y el abandono del hábito de fumar resulta en una reducción adicional en el riesgo de presentar aterosclerosis subclínica (odds ratio = 0,82 intervalo de confianza del 95%: 0,75-0,88, tendencia $P < 0,01$).

5. LCA identificó 7 patrones alimentarios entre la población irlandesa. Los patrones alimentarios difieren significativamente en los fines de semana y los participantes que prefieren carne y huevos para el desayuno en lugar de cereal y omiten la comida ligera más tarde durante el día, presentan un patrón de dieta menos saludable, una presión arterial diastólica más alta y nivel de ferritina elevado.

6. La omisión del desayuno es un marcador de un patrón alimentario y estilo de vida poco saludable y que está asociado con una mayor prevalencia de aterosclerosis no-coronaria (odds ratio: 1,55; intervalo de confianza del 95%: 0,97 a 2,46) y generalizada (odds ratio: 2,57; 95% intervalo de confianza: 1.54 a 4.31) de forma independiente de la presencia de otros factores de riesgo convencionales.

7. El consumo regular de desayuno se asocia con el patrón dietético más favorable y la mayor adherencia a los patrones dietéticos, como "Vegetariano", "Pescado y verduras" y "Cereales para el desayuno". El consumo regular de desayuno aporta hasta 24% de las necesidades diarias de fibra dietética, 32% de las necesidades diarias de hierro, 30% de las necesidades diarias de calcio, 32% de las necesidades diarias de ácido fólico y 37% de las necesidades diarias de vitamina B2.

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