

A MULTI-DIMENSIONAL APPROACH OF FOOD CLASSIFICATIONS AND ITS ASSOCIATION WITH CARDIOMETABOLIC RISK FACTORS

Nadine Alkhoury

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UNIVERSITAT ROVIRA i VIRGILI

A multi-dimensional approach of food classifications and its association with cardiometabolic risk factors

Nadine Khoury



DOCTORAL THESIS
2024

Nadine Khoury

A multi-dimensional approach of food classifications and its association with cardiometabolic risk factors

DOCTORAL THESIS

Thesis supervised by

Prof. Jordi Salas-Salvadó, Prof. Nancy Babio and Dr. María Ángeles Martínez



UNIVERSITAT ROVIRA i VIRGILI

Human Nutrition Unit
Department of Biochemistry and Biotechnology
Rovira i Virgili University
Reus, Tarragona

2024

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FAIG CONSTAR que aquest treball, titulat "A multi-dimensional approach of food classifications and its association with cardiometabolic risk factors", que presenta Nadine Khoury per a l'obtenció del títol de Doctor, ha estat realitzat sota la meva direcció al Departament de Bioquímica i Biotecnologia d'aquesta universitat.

HAGO CONSTAR que el presente trabajo, titulado "A multi-dimensional approach of food classifications and its association with cardiometabolic risk factors", que presenta Nadine Khoury para la obtención del título de Doctor, ha sido realizado bajo mi dirección en el Departamento de Bioquímica y Biotecnologia de esta universidad.

I STATE that the present study, entitled "A multi-dimensional approach of food classifications and its association with cardiometabolic risk factors", presented by Nadine Khoury for the award of the degree of Doctor, has been carried out under my supervision at the Department Biochesmitry and Biotechnolog of this university.

El/s director/s de la tesi doctoral
El/los director/es de la tesis doctoral
Doctoral Thesis Supervisor/s

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Nadine Alkhoury

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ما في شكر كافي لحتى عبّر لوالديّ ساميرا وغسان عن كل شي عملتوه كرمالي

بعرف إنو الوداع بالمطار ما كان سهل، بس بتمَنّى دايماً إني خلّيكن فخورين فيي .شكراً وشكراً مرة تانية

بحبكن كتير

And finally, Freddy. I want to thank you because life hasn´t been easy for you, but you never *ever* gave up on us even at your lowest point. You have always had a vision for us despite the distance; through thick and thin, thank you again.

From the ashes, a phoenix shall rise.

To more success, dreams and good health. I love you beyond words.

Summary

UNIVERSITAT ROVIRA I VIRGILI
A MULTI-DIMENSIONAL APPROACH OF FOOD CLASSIFICATIONS AND ITS ASSOCIATION WITH CARDIOMETABOLIC RISK FACTORS
Nadine Alkhoury

SUMMARY

ENGLISH

Cardiovascular diseases remain the leading cause of mortality worldwide out of all non-communicable diseases. Current evidence suggests that diet is one of the most promising modifiable interventions for improving and reducing the risk of cardiometabolic risk factors. However, the role of the diet in human health is complex due to its multi-dimensional nature. Diet includes not only nutrients but also various other factors such as chemical contaminants, endocrine disruptors, degree of processing, formulation, additives, the food matrix itself and its sustainable profile.

The general objectives of this doctoral thesis were: 1) to prospectively assess the association between the Nutri-Score Nutrient Profiling system (NS-NPM) dietary index and cardiometabolic risk factors in an elderly population with overweight/obesity and metabolic syndrome, 2) to investigate the ability of the 2015 and 2023 NS-NPM to discriminate food consumption and nutrient intake in a population of French children and adolescents 3) to cross-sectionally assess the associations between ultra-processed foods (UPFs) consumption and cardiometabolic risk factors in a population of Spanish preschoolers, and, 4) to prospectively assess the association between the dietary intake of some important widespread endocrine disruptors (polychlorinated dibenzo-p-dioxins and furans (PCDD/F) and perfluorooctanesulfonic acid (PFOS)) present in food with cardiometabolic risk factors (adiposity, obesity risk and glucose homeostasis parameters) in an elderly Mediterranean population.

The results of this doctoral thesis show that the consumption of food with higher NS-NPM dietary index (lower nutritional quality) is associated with a 1-year increase in some cardiometabolic risk factors (adiposity parameters, fasting plasma glucose, triglycerides, and diastolic blood pressure). However, no significant associations were identified for critical cardiometabolic risk factors such as high-density lipoprotein cholesterol (HDL-cholesterol), low-density lipoprotein cholesterol (LDL-cholesterol), and systolic blood pressure. In addition, the results for the objective 3 suggest that higher consumption of UPFs was positively cross-sectionally associated with z score of body mass index, waist circumference, fat mass index, and fasting plasma glucose and negatively associated with high-density lipoprotein cholesterol concentrations. Higher dietary intake of endocrine disruptors (PCDD/Fs and PFOS) was cross-sectionally and longitudinally associated with cardiometabolic risk factors (adiposity, obesity status and glucose homeostasis parameters). Furthermore, the results for objective 4 suggest that the dietary intake of PCDD/Fs was associated with a higher prevalence of obesity and higher adiposity parameters at baseline and higher baseline dietary intake of PCDD/Fs was positively associated with changes in waist circumference after 1 year of follow-up. In addition, dietary intake of PFOS was associated with glucose homeostasis parameters. We found that high baseline dietary intake of PFOS was associated with a higher levels of fasting plasma glucose and HbA1c and with an increase in HOMA-IR and BMI after 1-year of follow-up.

Overall, the main conclusion of the present doctoral thesis is that the associations between each of the three food classification dimensions and different cardiometabolic

SUMMARY

risk factors are significant even after adjusting for diet quality, indicating that the three approaches should be considered independently when assessing the impact of food on cardiometabolic risk factors.

SUMMARY

CASTELLANO

Las enfermedades cardiovasculares siguen siendo la principal causa de mortalidad por enfermedades no transmisibles en todo el mundo. Las evidencias actuales sugieren que la dieta es un factor determinante modificable que permite mejorar y reducir el riesgo de factores de riesgo cardiometabólicos. Sin embargo, el papel de la dieta en la salud humana es complejo debido a su naturaleza multi-dimensional. La dieta incluye no sólo los nutrientes, sino también otros factores como los contaminantes químicos, los disruptores endocrinos, el grado de procesamiento, la formulación, los aditivos, la propia matriz alimentaria y su perfil más o menos sostenible.

Los objetivos generales de esta tesis doctoral fueron: 1) evaluar prospectivamente la asociación entre el índice dietético Nutri-Score Nutrient Profiling system (NS-NPM) y los factores de riesgo cardiometabólico en una población anciana con sobrepeso/obesidad y síndrome metabólico, 2) investigar la capacidad de los índices NS-NPM de 2015 y 2023 para determinar si ambos índices permiten caracterizar adecuadamente la dimensión nutricional de los alimentos en una población pediátrica y adolescente, 3) evaluar transversalmente las asociaciones entre el consumo de ultraprocesados y los factores de riesgo cardiometabólico en una población de preescolares españoles, 4) evaluar prospectivamente la asociación entre la ingesta dietética de algunos importantes disruptores endocrinos ampliamente utilizados (PCDD/F y PFOS) presentes en los alimentos con factores de riesgo cardiometabólico (adiposidad, riesgo de obesidad y parámetros de homeostasis de la glucosa) en una población mediterránea de edad avanzada.

Los resultados de esta tesis doctoral muestran que el consumo de alimentos con mayor índice dietético NS-NPM (menor calidad nutricional) se asocia con un aumento al año de algunos factores de riesgo cardiometabólico (parámetros de adiposidad, glucosa plasmática en ayunas, triglicéridos y presión arterial diastólica). Sin embargo, no se identificaron asociaciones significativas para factores de riesgo cardiometabólico críticos como el colesterol HDL, el colesterol LDL y la presión arterial sistólica. Además, los resultados del objetivo 3 sugieren que un mayor consumo de ultraprocesados se asoció positivamente con la puntuación z del índice de masa corporal, el perímetro de cintura, el índice de masa grasa y la glucosa plasmática en ayunas, y negativamente con las concentraciones de colesterol de lipoproteínas de alta densidad. Una mayor ingesta dietética de disruptores endocrinos (PCDD/Fs y PFOS) se asoció de forma transversal y longitudinal con factores de riesgo cardiometabólico (adiposidad, estado de obesidad y parámetros de homeostasis de la glucosa). Los resultados del objetivo 4 sugieren que la ingesta dietética de PCDD/Fs se asocia con una mayor prevalencia de obesidad y adiposidad. Además, una mayor ingesta dietética basal de PCDD/Fs se asoció positivamente con aumentos en la circunferencia de la cintura después de 1 año de seguimiento. La ingesta alimentaria de PFOS también se asoció con valores más altos de parámetros de homeostasis de la glucosa. Por último, una ingesta dietética basal elevada de PFOS se asoció con un aumento en los niveles de glucosa plasmática en

ayunas y HbA1c, así como a un aumento del HOMA-IR y el IMC tras 1 año de seguimiento.

La principal conclusión de la presente tesis doctoral es que las asociaciones entre cada una de las tres dimensiones de clasificación de los alimentos y diferentes factores de riesgo cardiometabólico son significativas incluso después de ajustar por la calidad de la dieta, lo que indica que los tres enfoques deben considerarse de forma independiente al evaluar el impacto que pueden tener los alimentos sobre los factores de riesgo cardiometabólico.

SUMMARY

CATALÀ

Les malalties cardiovasculars continuen sent la principal causa de mortalitat per malalties no transmissibles a tot el món. Les evidències actuals suggereixen que la dieta és un factor determinant modificable que permet millorar i reduir el risc de factors de risc cardiometabòlic. Malgrat tot, el paper de la dieta en la salut humana és complex degut a la seva naturalesa multi-dimensional. La dieta inclou no sols els nutrients, sinó també altres factors com els contaminants químics, els disruptors endocrins, el grau de processament, la formulació, els additius, la pròpia matriu alimentària i el seu perfil més o menys sostenible.

Els objectius generals d'aquesta tesi doctoral van ser: 1) avaluar de forma prospectiva l'associació entre l'índex dietètic NS-NPM i els factors de risc cardiometabòlic en una població d'edat avançada amb sobrepès/obesitat i síndrome metabòlica, 2) descriure l'índex dietètic NS-NPM utilitzant la versió actualitzada de l'algorisme Nutri-Score amb la versió anterior i determinar si tots dos índexs permeten caracteritzar adequadament la dimensió nutricional dels aliments en una població pediàtrica i adolescent, 3) avaluar transversalment les associacions entre el consum de UPFs i els factors de risc cardiometabòlic en una població de nens d'Espanya, 4) avaluar de forma prospectiva l'associació entre la ingesta dietètica d'alguns importants disruptors endocrins àmpliament utilitzats (PCDD/F i PFOS) presents en els aliments amb factors de risc cardiometabòlic (adipositat, risc d'obesitat i paràmetres d'homeòstasis de la glucosa) en una població mediterrània d'edat avançada.

Els resultats d'aquesta tesi doctoral mostren que el consum d'aliments amb alt índex dietètic NS-NPM (menor qualitat nutricional) s'associa amb un augment a l'any en alguns factors de risc cardiometabòlic (paràmetres d'adipositat, glucosa plasmàtica en dejú, triglicèrids i pressió arterial diastòlica). Malgrat tot, no es van identificar associacions significatives per a factors de risc cardiometabòlic crítics com el colesterol HDL, el colesterol LDL i la pressió arterial sistòlica. A més, els resultats de l'objectiu 3 suggereixen que un major consum de ultraprocessats s'associa positivament amb la puntuació z de l'índex de massa corporal, el perímetre de cintura, l'índex de massa grassa i la glucosa plasmàtica en dejú, així com negativament amb les concentracions de colesterol de lipoproteïnes d'alta densitat. Una superior ingesta dietètica de disruptors endocrins (PCDD/Fs i PFOS) es va associar de manera transversal i longitudinal amb factors de risc cardiometabòlic (adipositat, estat d'obesitat i paràmetres d'homeòstasis de la glucosa). A més, els resultats de l'objectiu 4 suggereixen que la ingesta dietètica de PCDD/Fs s'associa amb una més alta prevalença d'obesitat i adipositat, i una major ingesta dietètica basal de PCDD/Fs s'associa positivament amb augments en la circumferència de la cintura després d'1 any de seguiment. La ingesta alimentària de PFOS també es va associar amb valors més alts de paràmetres d'homeòstasis de la glucosa. Finalment, una ingesta dietètica basal elevada de PFOS es va associar a un augment en els nivells en dejú de glucosa plasmàtica i de HbA1c, així com a un augment del HOMA-IR i l'IMC després d'1 any de seguiment.

SUMMARY

La principal conclusió de la present tesi doctoral és que les associacions entre cadascuna de les tres dimensions de classificació dels aliments i els diferents factors de risc cardiometabòlic són significatives fins i tot després d'ajustar per la qualitat de la dieta, la qual cosa indica que les tres dimensions han de considerar-se de manera independent al avaluar l'impacte que poden tenir els aliments sobre els factors de risc cardiometabòlic.

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Abbreviations

A

ALADINO: Vigilancia de la ALimentación, Actividad física, Desarrollo INfantil y Obesidad

American College of Cardiology/American Heart Association: ACC/AHA

American Diabetes Association: ADA

B

Body mass index: BMI

C

Cardiovascular disease: CVD

Centers for Disease Control: CDC

Childhood Obesity Risk Assessments Longitudinal Study: CORALS

D

Dietary Approach to Stop Hypertension: DASH

Dioxin-like polychlorinated biphenyls: DL-PCBs

E

Endocrine disrupting chemicals: EDCs

Energy-reduced Mediterranean diet: er-MedDiet

Energy-reduced Mediterranean diet questionnaire: erMEDAS

ENRICA: Estudio Nacional de Nutrición y Riesgo Cardiovascular en España

European Food Safety Authority: EFSA

European Prospective Investigation into Cancer and Nutrition: EPIC

F

Fat mass index: FMI

Food and beverage frequency questionnaire: F&B-FQ

Food frequency questionnaire: FFQ

ABBREVIATIONS

Food-based dietary guidelines: FBDGs

G

Global Burden of Disease: GBD

Global greenhouse gas: GHG

H

Hemoglobin A1c: HbA1c

High density lipoprotein Cholesterol: HDL-cholesterol

Homeostatic Model Assessment of Insulin Resistance: HOMA-IR

Haut Conseil de la Santé Publique: HCSP

I

International Agency for Research on Cancer: IARC

International Food Information Council Foundation: IFIC

L

Limit of detection: LOD

Low density lipoprotein Cholesterol: LDL-cholesterol

Lower bound: LB

M

Metabolic equivalent task: MET

Mono-unsaturated fatty acids: MUFA

N

Non-communicable diseases: NCDs

Nutrient profiling system: NPS

Nutri-Score nutrient profiling model dietary index: NS-NPM DI

P

Per- and polyfluoroalkyl substances: PFAS

Perfluorohexane sulfonic acid: PFHxS

ABBREVIATIONS

Perfluorononanoic acid: PFNA

Perfluorooctanesulfonic acid: PFOS

Perfluorooctanoic acid: PFOA

Persistent organic pollutant: POP

Polychlorinated dibenzo-p-dioxins and furans: PCDD/F

S

Saturated fatty acids: SFA

T

The modified Food Standard Agency Nutrient Profiling System Dietary Index: FSAm-NPS DI

The PREvención con DIeta MEDiterránea Plus: PREDIMED-Plus

Tolerable weekly intake: TWI

Toxic Equivalents: TEQ

Type 2 diabetes mellitus: T2D

U

Ultra-processed food: UPF

Ultra-processed foods: UPF

University of North Carolina: UNC

Upper bound: UB

W

Waist circumference: WC

World Health Organization: WHO

"You have your Lebanon and I have mine.
You have your Lebanon with her problems, and I have my Lebanon with her beauty.
Your Lebanon is empty and fleeting, whereas My Lebanon will endure forever."

Gibran Khalil Gibran
1883-1931

To my parents, I give everything, including this.

I. INTRODUCTION

UNIVERSITAT ROVIRA I VIRGILI
A MULTI-DIMENSIONAL APPROACH OF FOOD CLASSIFICATIONS AND ITS ASSOCIATION WITH CARDIOMETABOLIC RISK FACTORS
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A. Diet and risk of cardiometabolic disease

1. Introduction

The first half of the 20th century witnessed a notable change in morbidity and mortality trends in the world's industrialized countries, transitioning from infectious diseases being predominant to the growing importance of chronic, non-communicable diseases (NCDs)[1]. NCDs are diseases that are not directly transmissible from one person to another. They have usually resulted from a combination of physiological, genetic, environmental and behavioral factors[2]. NCDs are chronic, and develop over time and contribute significantly to global morbidity and mortality[2]. According to the World Health Organization (WHO), NCDs are estimated to cause 74% of all deaths worldwide, with cancer accounting for 9.3 million deaths; chronic respiratory diseases for 4.1 million deaths and diabetes for 2.0 million deaths[2]. Cardiovascular disease (CVD) remains the leading cause of mortality worldwide out of all NCDs. The estimated death attributed to CVDs increased from approximately 12.1 million in 1990 (equally distributed between the sexes) to 18.6 million in 2019, comprising 9.6 million men and 8.9 million women, with ischemic heart disease the leading contributor to premature mortality in 146 countries for men and 98 countries for women[3].

Cardiovascular diseases in children

Although CVD is more prevalent in adults and in the elderly population, CVD has a long asymptomatic development phase and may begin during childhood, and its progression, even in its earliest stages, is related to the presence and severity of established cardiometabolic risk factors[4]. In Spain, according to the Global Burden of Disease (GBD), there are 136.42 prevalent cases of CVD per-100,000 in children aged 5 to 14 years. Longitudinal studies, such as the Cardiovascular Risk in Young Finns Study, have demonstrated the importance of early intervention and prevention efforts by following cardiovascular risk factors from childhood to adulthood[5].

Therefore, addressing the challenge of CVDs requires a comprehensive approach that compromises primary prevention, early detection and intervention and long-term management for all age groups.

2. Cardiometabolic risk factors and risk determinants

As defined by the Centers for Disease Control (CDC) a risk factor is *"an aspect of personal behavior or lifestyle, an environmental exposure, or an inborn or inherited characteristic that is associated with an increased occurrence of disease or other health-related event or condition*[6]". Therefore, risk determinants include physical inactivity, smoking, exposure to environmental toxic contaminants, smoking and unhealthy diets. In addition, cardiometabolic risk factors include a range of abnormalities that increase the likelihood of developing CVD and metabolic disorders and usually are related to the exposure to risk determinants. These risk factors include:

Dyslipidemia:

It is well established that dyslipidemia is associated with the onset of multiple CVDs such as myocardial infarction and stroke[7,8]. The Spanish National Institute of Statistics reported that circulatory system disorders accounted for 26.0% of deaths in 2022 of all deaths in Spain[9]. Dyslipidemia is one of the primary modifiable cardiometabolic risk factors[10]. In Spain, total cholesterol level is greater than 200 mg/dL in 50% of the adult's population[11]. Of them, 69% correspond to pure hypercholesterolemia, 26% to mixed dyslipidemia and 5% to hypertriglyceridemia[7]. Dietary lifestyle remains one of the most crucial determinants for management and prevention of dyslipidemia. Dietary guidelines targeting dyslipidemia such as the Mediterranean diet has been shown to reduce blood lipid levels[12,13].

Type 2 diabetes and glucose homeostasis parameters:

Type 2 diabetes mellitus (T2D) is a chronic metabolic disorder representing a major global health challenge. In 2021, the International Diabetes Federation estimated that 1 in 10 adults were living with T2D and this is predicted to rise to 1 in 9 adults by 2030[14]. The consequences of uncontrolled T2D can lead to serious complications. Chronic hyperglycemia may contribute to the development of microvascular complications, including diabetic retinopathy or nephropathy, and macrovascular complications such as stroke, peripheral artery disease and myocardial infarction, among others[15]. Fasting plasma glucose is a key measure used to assess blood glucose levels after fasting. It

serves as an important diagnostic criterion for glycemic control. According to the American Diabetes Association (ADA), T2D is diagnosed when on two separate tests, fasting plasma glucose levels range between 100 to 125 mg/dL[16]. In addition, another measure recommended by the ADA, is the hemoglobin A1c (HbA1c). HbA1c provides an indication of average blood glucose levels over the preceding 2 to 3 months, reflecting the extent of hyperglycemia during this period. In people diagnosed with diabetes, HbA1c levels range 6.5% or higher[16]. The ADA recommends targeting HbA1c levels of less than 7% for most adults with diabetes[16] to reduce the risk of diabetes complications. Furthermore, fasting plasma insulin is also an important parameter, elevated fasting insulin levels are often indicative of insulin resistance, which can lead to impaired glucose uptake and increased blood glucose levels[17]. Moreover, to estimate insulin resistance, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) has been proposed as a validated method for assessing insulin resistance in research and clinical settings. It is a mathematical model used to estimate insulin resistance based on fasting plasma glucose and fasting plasma insulin levels and the usual cut-off for HOMA-IR to discriminate insulin resistance is usually set at >2.0[18].

Hypertension:

Hypertension is a condition characterized by a persistently elevated blood pressure level. Normally, blood pressure is regulated by various physiological mechanisms to maintain adequate perfusion of organs and tissues. However, disturbance in these regulatory mechanisms, often due to lifestyle factors, genetic predisposition, or underlying health conditions can lead to hypertension. In 2017, the American College of Cardiology/American Heart Association (ACC/AHA) lowered the guidelines for blood pressure cutoffs to diagnose hypertension from 140/90 to 130/80 mmHg[19]. Hypertension is a significant risk factor for CVDs. In Spain, WHO reported a 27% age-standardized prevalence of hypertension among adults aged 30–79 years and the main cause were tobacco use, physical inactivity and obesity[20]. In children, a cross-sectional study including 1,604 Spanish children aged 4-6 years, found that the prevalence of hypertension was 18.2%, indicating a relatively high prevalence estimate[21].

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Adiposity parameters and obesity/abdominal obesity status:

Adiposity parameters are measures used to assess the excess accumulation of body fat. These parameters include indicators such as body mass index (BMI), waist circumference (WC) along with waist-to-height ratio and fat mass index (FMI) in children. Overweight or obesity are usually diagnosed when BMI is ≥25 and <30 kg/m^2 for overweight and ≥30 kg/m^2 for obesity[22]. On the other hand, abdominal obesity is defined when waist circumference is ≥88 cm for women and ≥102 cm for men[23]. Abdominal obesity, distinguished by the accumulation of fat around the abdomen, has been suggested as a more reliable indicator of adiposity, as overweight/obesity assessed by BMI does not differentiate between weight associated with muscle and weight associated with fat[24]. In addition, according to WHO, overweight is defined for children aged 5–19 years as a BMI-for-age greater than 1 standard deviation above the WHO Growth Reference median and for children <5 years old, overweight is defined as weight-for-height >2 standard deviations above the WHO Child Growth Standards median[25]. Obesity is defined for children aged 5–19 years as a BMI-for-age >2 standard deviations above the WHO Growth Reference median and for children <5 years old, obesity is defined as weight-for-height greater than 3 standard deviations above the WHO Child Growth Standards median[25]. Epidemiological studies conducted in Spain have shed light on this issue, revealing alarming trends. The ENRICA (Estudio Nacional de Nutrición y Riesgo Cardiovascular en España) study reported a high prevalence of obesity in Spain, with approximately 22.9% of adults classified as obese based on BMI criteria[26]. Additionally, the prevalence of abdominal obesity, as measured by waist circumference, was found to be 36%, indicating a concerning burden[26]. In children, the Spanish ALADINO (Vigilancia de la ALimentación, Actividad física, Desarrollo INfantil y Obesidad) cohort assessed the anthropometric measurements of 10,899 children aged 6-9 years from 168 primary education centers and revealed that the prevalence of overweight was 23.2%, of obesity 17.3% and of abdominal obesity 22.6%[27]. These findings highlight the urgent need for comprehensive public health interventions to address obesity and its associated cardiometabolic risks in the Spanish population.

Risk determinants

There are several risk determinants that can increase the likelihood of cardiometabolic risk factors and CVDs. Risk determinants can be classified as either non-modifiable, such as age, sex, race, family history, and genetic predisposition, or modifiable.

Modifiable risk factors are aspects of an individual's lifestyle and behavior that can be modified or controlled to reduce the risk of CVD such as, smoking, alcohol consumption, physical inactivity, sedentary behavior, and an unhealthy diet high in salt, sugar, saturated fat and trans-fat and low in fibers, phytochemicals and vitamins[28]. In addition, in recent years, environmental, anthropogenic contaminants and the degree of food processing have emerged as potential contributors to the development and progression of these cardiometabolic risk factors, with particular attention given to the role of endocrine disrupting chemicals (EDCs) [29–31] and ultra-processed foods (UPF)[32–35].

3. The role of the diet

The role of the diet in human health is complex due to its multi-dimensional nature. Dietary intake is affected not only by nutrients but also by various other factors such as chemical contaminants, endocrine disruptors, degree of processing, formulation, additives, the food matrix itself and its sustainable profile. Despite decades of research focusing on the associations between nutrients, food groups, or dietary patterns, we are only beginning to scratch the surface in uncovering the multiple effects of the multi-dimensional nature of the dietary exposome. It is important to note that no single food can be considered harmful, but rather it is the combination of foods and the amounts consumed that define a healthy or unhealthy diet[36]. Within food groups, there is considerable variation in the types and forms of foods, this variability extends to ingredients, nutritional composition, degree of processing, and the presence of chemicals and contaminants such as EDCs or its environmental impact.

Our understanding of what constitutes a healthy diet is always progressing, as it is adapting to the evolving studies about the effects of various foods and dietary combinations on health. Current evidence suggests that diet is one of the most promising modifiable interventions for improving and reducing the risk of

cardiometabolic risk factors. However, more research is needed to assess its multi-dimensional nature.

B. Multi-dimensional approach of food classification

1. The nutritional dimension of foods

a. Definition of the nutritional dimension of foods

The nutritional dimension of food includes a wide range of macronutrients and micronutrients that are essential components of a healthy diet, each playing an important role in supporting human health.

Macronutrients consist of 3 main categories: carbohydrates (found in foods such as grains, fruits, vegetables, and legumes), proteins (found in food such as meat, poultry, fish, eggs, dairy products, legumes, nuts, and seeds) and fats (found in food such as oils, butter, avocados, nuts, seeds, and fatty fish) while micronutrients consist of vitamins, which are classified as water-soluble (e.g., vitamin C and B-complex vitamins) or fat-soluble (e.g., vitamins A, D, E, and K) and are found mainly in fruits, vegetables, whole grains, dairy products, and meat. In addition, micronutrients include minerals, such as calcium, iron, potassium, magnesium, zinc, selenium, among others and are in dairy products, leafy greens, nuts, seeds, whole grains, and fortified foods.

Food of public health concerns related to macronutrients and micronutrients include a combination of unhealthy dietary habits. Excessive intakes of salt, added sugars, saturated and trans fats and low intakes of fibers and vitamins are of major concern[37–39]. Over the years, the nutritional dimension of food has been shown to have a significant impact on health. From 1990 to 2017, 11 million deaths were attributed to dietary intake with high sodium intake and low intake of whole grains and fruits being the leading dietary risks for mortality[37]. Consequently, addressing these public health concerns and informing consumers about the nutritional composition of foods is critical to reduce the deleterious impact of the diet.

b. Nutritional profile of food using front-of-pack labeling systems

The "UNICEF Nutrition Strategy 2020-2030" among other national and international organizations has restated the importance of informing consumers of the nutritional dimension of foods not only for adults but also for children[40]. Furthermore, the WHO recommended that governments should adopt front-of-pack labeling systems as part of a comprehensive policy response to encourage healthier diets[41]. Although labels on the back of the products are already mandatory in all European countries according to RE 1169/2011[42], there is evidence that consumers find it difficult to interpret this information. On the other hand, nutrition labels found on the front-of-pack of products are considered more helpful and efficient for consumers since nutritional information is simple, graphical, provides quick and easy to understand information about the nutritional quality of foods and beverages and it is available at a glance[43–47]. Emerging research suggests that front-of-pack labeling can play a significant role in improving consumer understanding of nutritional quality, it has the potential to encourage the selection and purchase of healthier foods while urging food industries to reformulate their products[40]. This dual impact on both consumer behavior and industry practices can contribute to reducing cardiometabolic risk factors.

A five-step approach was developed by WHO to help European countries to develop and implement an evidence-based front-of-pack labelling system[48]:

- Select the specific strategy: what is expected from a front-of-pack labelling system
- Select the type of the front-of-pack labelling system graphical design
- Determine the underlying nutrient profiling system
- Define studies to be performed to select the final format and nutrient profile model
- Establish monitoring procedures

In the present thesis, we focused of the fourth step approach which emphasizes defining study methodologies to validate a front-of-pack labelling system. To achieve this validation, two key steps should be considered. The validation process is built upon examining both the underlying nutrient profiling systems—assessing application-content validity, association with healthier diets, prospective association with health, and alignment with dietary guidelines—and the graphical format. This includes studying consumer perception, attitudes, understanding, use in purchasing situations, and the potential impact on health[48].

c. The modified Food Standard Agency Nutrient Profiling System (FSAm-NPS) underpinning the Nutri-Score label

As mentioned above, a front-of-pack nutrition labelling system reflects the nutritional quality of foods using a Nutrient Profiling System (NPS)[49,50]. Nutrient profiling is widely used to support public health initiatives to promote healthy diets[51]. Among the available nutrient profiling systems, the NPS developed by the UK Food Standard Agency (FSA-NPS) has been validated consistently in Europe[52–54]. This system was originally developed to differentiate foods on the basis of their nutritional composition in the context of television advertising aimed at children[55]. In France, after some modifications of the FSA-NPS by the French High Council for Public Health (Haut Conseil de la Santé Publique, HCSP) and demonstrating its applicability within the French context, the modified FSA-NPS (FSAm-NPS) was established in 2015 and a dietary index based on the FSAm-NPS (FSAm-NPS DI) was developed to validate the algorithm underlying the Nutri-Score label which is now called Nutri-Score Nutrient Profiling Model Dietary Index (NS-NPM DI)[56,57]. The DI based on the Nutri-Score algorithm has been shown to effectively reflect the nutritional quality of diets on an individual level and was linked to healthy dietary patterns, such as the Mediterranean diet[58]. The score for a given food or beverage is calculated by assigning points for the content per 100 g or 100 ml. This Nutri-Score algorithm takes into account both positive values (protein (g), fiber (g), fruits/vegetables/pulses/nuts and olive, walnut and canola oils (%)) and negative components (energy (kJ), saturated fat (g), sugar (g) and salt (g) or sodium (mg)), resulting in a final score based on a discrete continuous scale ranging from -15 to +40

points, which is then translated into a grade from A to E, corresponding to a color from dark green to dark orange[59]. The NS-NPM, which underpins the Nutri-Score label has been shown to be effective in ranking products by nutritional quality and improving food purchasing in real-life grocery shopping environments[60].

d. Nutri-Score update

Following the adoption of Nutri-Score by several European countries (including Belgium (2018), Spain (announced in 2018), Switzerland (2019), Germany (2020), Luxembourg (2021), the Netherlands (2023) and recently, Portugal (2024)), two committees were established: a steering committee responsible for making decisions regarding the overall development and implementation of Nutri-Score, and a scientific committee comprising independent scientists from each participating country tasked with reviewing and updating the algorithm, which was initially established in 2015. The primary objective of the scientific committee was to improve the alignment of the algorithm with national food-based dietary guidelines (FBDGs)[61].

In 2023, the NS-NPM has been updated to incorporate the latest scientific evidence and align with the primary FBDGs of European countries. The 2023 NS-NPM works in a similar way to its predecessor (2015 NS-NPM), however some modifications have been considered. These include an increase in the maximum number of points for sugar and salt, resulting in less favorable ratings for foods high in these components[61]. In addition, stricter criteria were applied to the allocation of points for the fiber and protein content to obtain positive points. Specific rules were introduced for red meat, limiting the maximum points it can receive in the positive category. For beverages, the presence of non-nutritive sweeteners was added as an additional unfavorable factor. Finally, the thresholds for converting the final score into a rating have been adjusted to align with the new algorithm[61].

e. The nutritional dimension of food and cardiometabolic risk factors

The nutritional dimension of food plays a crucial role in the prevention of cardiometabolic risk factors[28,62]. As stated before, consumption of food rich in salt,

sugar and saturated fats has been directly associated to various cardiometabolic risk factors, such as obesity[63,64], dislipidemia[64,65] and diabetes[64,66].

By assessing the nutritional content of foods using front-of-pack labeling systems, individuals can make informed dietary choices that can help mitigate these risk factors and reduce the likelihood of developing chronic diseases.

Front-of-pack nutrient profiling systems are increasingly being used to characterize the nutritional dimension of foods and have long been validated as an effective tool to help consumers make healthier nutritional food choices[52–54,56,57,60,67]. The NS-NPM DI was developed to assess the overall dietary intake and the nutritional quality of the individual´s dietary habits and has been associated with different health outcomes[68]. In the NS-NPM, total energy, salt, saturated fat, and sugar are considered negative components whereas fiber, protein, fruits and vegetables, legumes, and some vegetable oils are considered positive[59]. All of these nutrients have been shown to be related to cardiometabolic risk factors in numerous studies[63–66,69,70]. In particular, added sugar and saturated fat have been previously associated with higher levels of fasting blood glucose and triglycerides[64,71]. Therefore, these associations are consistent with the nature of the NS-NPM and with previous findings on diet and cardiometabolic risk factors.

2. The (Ultra)-processing/formulation dimension of foods

a. Definition and classification of the (Ultra)-processing/formulation dimension of foods

While there is no legal standard definition of “ultra-processed” foods, the authors of the NOVA classification system proposed a term to describe it. These authors describe ultra-processed foods as food products that have undergone significant processing, often resulting in high concentrations of added salt, sugar, oils and fats or other ingredients derived from industrial processes, such as the hydrogenation of fats or the hydrolysis of proteins[72–74]. In addition, in general, this high degree of processing is aimed at obtaining ready-to-eat products that can replace other unprocessed or minimally processed foods and to create products that are convenient, have a longer shelf life, and are palatable. They are often inexpensive to produce and can be marketed for their taste and

convenience[75–77]. Different systems have been proposed for classifying foods around the world and they present some distinguishments between them. For example, the IARC system was established in Europe by the International Agency for Research on Cancer (IARC) using the methodology designed for the European Prospective Investigation into Cancer and Nutrition (EPIC) and classifies food into 3 categories[78]. The International Food Information Council Foundation (IFIC) system was developed by the IFIC foundation, and the classification criteria are based on the increasing complexity of food processing and chemical composition, it divides foods into 5 categories[79,80]. The University of North Carolina (UNC) system was also developed in the USA by the University of North Carolina and classifies foods according to the degree of physicochemical changes produced by food processing and it classifies food into 7 categories[81]. The NOVA system classifies food into 4 categories (more details about the NOVA classification are in the next section), SIGA into 8 categories[82] and FoodEx2 into 3 categories[83]. The NOVA food classification system is the most commonly used in epidemiological studies to estimate the intake of ultra-processed foods. While the categorization of food according the degree of processing provides a useful framework, it also has several limitations. One is that it may oversimplify the complexity of food processing, as not all processed foods are unhealthy and it may overlook some other dimension of foods; for instance, some processed foods still provide essential nutrients and do not have a completely deficient nutritional profile such as pasteurized milk and frozen fresh vegetables retain high nutritional value despite being processed[84,85].

b. The NOVA classification

To assess the (Ultra)-processing/formulation dimension of food, the NOVA classification was proposed in 2009[86,87], it was originally developed in Brazil, and it categorizes foods based on the extent and purpose of their processing[88]. It is the most widely used and validated system[89]; it consists of four distinct groups classified according to the extent and purpose of the industrial processing they undergo. It considers all physical, biological and chemical methods used during the food manufacturing process, including the use of additives[86,88,90].

The current version of NOVA classifies foods as follows:

- ***Unprocessed and minimally processed foods:*** Foods of plant or animal origin that undergone preservation techniques that maintain the food's integrity and do not introduce salt, sugar, oils, fats, or other culinary ingredients. Examples include fresh or frozen fruits and vegetables, cereals, beans, eggs, milk.
- ***Processed culinary ingredients:*** Processed culinary ingredients, including oils, butter, sugar, and salt, are derived from foods in Group 1 or directly from nature through processes such as pressing, refining, grinding, milling, and drying. These processes aim to create products that serve to enhance the preparation, seasoning, and cooking of Group 1 foods, such as stews, soups, salads, breads, preserves, drinks, and desserts. These ingredients are not intended for consumption on their own but are combined with Group 1 foods.
- ***Processed foods:*** Processed foods, such as vegetables in bottles, fish in cans, fruits preserved in syrup and salted nuts, are primarily created by incorporating substances like salt, oil, sugar, or other elements from Group 2 into Group 1 foods. The objective of processing in this context is to enhance flavor and durability.
- ***Ultra-processed foods:*** Ultra-processed foods such as sugary drinks, packaged snacks, fast food, frozen pizzas, reconstituted meat products, instant noodles, confectionery, margarines, and energy drinks are not simply modified foods rather they are primarily or entirely composed of substances derived from foods and additives, with minimal intact from Group 1 food content. These formulations incorporate ingredients also found in processed foods, such as sugars, oils, fats, or salt. Additives present in ultra-processed foods include some shared with processed foods, such as preservatives, antioxidants, and stabilizers. However, ultra-processed products go beyond by including additional sources of energy and nutrients not usually utilized in culinary preparations (such as hydrogenated oils, hydrolyzed proteins, soy protein isolate, maltodextrin, and high-fructose corn syrup). Additionally, unique classes of additives are found only in ultra-processed products, aiming to mimic or enhance sensory qualities or mask undesirable aspects of the final product. These additives include color stabilizers, flavors, non-sugar sweeteners, and various processing aids like carbonating agents, firming agents, bulking agents, anti-bulking agents, de-

foaming agents, anti-caking agents, glazing agents and emulsifiers. The aim of ultra-processing is to craft convenient (long-lasting, ready to eat), appealing (highly palatable) and economically advantageous (utilizing low-cost ingredients) food items.

c. The (Ultra)-processing/formulation dimension of food and cardiometabolic risk factors

Processed foods have been part of the human diet since ancient times. Recently, the consumption of UPF has increased significantly and the issue of UPF has evolved rapidly to become one of the most innovative nutrition research topics[91,92]. Overall, most research that looked into the relationship between diet and health has relied on traditional food group classifications that do not take into account the method of production or the extent or type of food processing[87]. UPFs are becoming more common in modern diets, especially among children and adolescents and in families with lower income and education levels, where obesity is more frequent[93–96]. Furthermore, adopted in early life tend to persist over into adulthood, increasing the risk of CVD and other noncommunicable diseases[97]. In addition, some have argued that the association between ultra-processing dimension of foods and cardiometabolic risk factors could be addressed in the nutritional dimension as UPFs tend to be higher in calories, salts, sugars and fat. However, beyond the nutritional composition, UPFs also contain other bioactive substances that could affect human health and cardiometabolic health[87,88,98–100]. These substances include food additives, endocrine disrupting chemicals, compounds formed during the transformation or processing, and even chemicals coming from packaging. Therefore, given the increasing trends in the consumption of UPFs in children and adults and their possible health interactions, it is necessary to investigate the association between the consumption of UPFs and the risk of chronic diseases independently of the nutritional dimension of the food.

In adults, studies have shown that UPF consumption is associated with the incident risk of developing general and abdominal obesity[101,102], increased risk of hypertension[103], diabetes[104,105], and dyslipidemia[105]. In children, most studies have reported unfavorable associations with BMI, however, others have failed to find this association, and few have

focused on cardiometabolic risk factors[106]. Consequently, the evidence on cardiometabolic risk factors remains limited in both adults and children and sometimes controversial.

3. The chemical contamination dimension of foods

a. Endocrine disrupting chemicals: definition and origin

The topic of endocrine disrupting chemicals emerged in the 1960s, after ecologists discovered that phenomena affecting the long-term survival of various animal species (such as reduced reproduction in birds, the appearance of genital disorders in alligators[107], or sexual disruption in fish could be attributed to environmental or anthropometrical pollutants)[108]. Thus, the impact of these chemicals on the environmental ecosystems has generated significant concern among researchers. These chemicals can bioaccumulate and can be biomagnified, entering the food chain and reaching humans. Many of these chemicals have the capacity to alter our hormones, causing abnormal responses[29,109]. This issue gained increased attention in the 1990s, where the term "endocrine disruptors" was proposed at the Wingspread conference[109]. In 2002, WHO defined an EDC as "*an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations*"[110]. An EDC can act in different ways: it can have an agonistic or antagonistic effect by binding in the same way as a natural hormone to its receptor and either amplify its basic response or block its action; it can also modify the degradation or production of a hormone or its receptor, as well as alter its transport[111–113]. Thus, by interfering with the physiological hormonal signaling of the endocrine system, they disrupt homeostasis and lead to various deleterious health effects[29,113,114]. Furthermore, some EDC are classified as "persistent organic pollutants" (POPs) due to their highly lipophilic nature and their strong ability to resist environmental degradation[115]. As a result, they are able to accumulate in fatty tissues and organs, particularly in adipose tissue[116]. EDC are a heterogeneous group of substances that may occur naturally (as phytoestrogens from plants, as heavy metals such as lead, mercury and cadmium from volcanoes, as organochlorines from forest fires or volcanoes, as polycyclic aromatic hydrocarbon from coal combustion or forest fires

and as arsenic that occurs naturally and groundwater contamination), or synthetic human-made compounds released into the environment through human activities, often without prior understanding of their impacts on ecosystems, animal welfare, or human health[29,116]. Different classes of EDC can be cited, i) such as atmospheric pollutants from combustion or anthropogenic origin such as Organochlorines including dibenzo-p-dioxins/furans ii) synthetic chemicals carried out or manufactured by humans, not found naturally in nature such as the Per- and polyfluoroalkyl substances considered as an emergent EDC.

b. Food as a route of exposure

The food chain is considered to be the primary route of exposure for EDCs[117–123], it accounts for more than 90% of the total exposure due to several factors[124,125]. First, EDCs can persist in the environment for long periods of time, accumulating in soil, water, and plants moving eventually to the food chain[126,127]. Many EDCs possess lipophilic properties, making them prone to accumulation in fatty tissues[29]. This leads to higher concentrations of these chemicals in animal-based foods, such as meat, dairy products, and oily fish. Furthermore, EDCs can directly enter the food supply through food packaging materials or processing equipment that contains them[92]. In addition, the widespread use of EDCs in agricultural practices, such as pesticides and fertilizers, can directly contaminate crops and soil, further increasing the likelihood of EDCs entering the food chain[92,119]. Consequently, the complex interplay between the lipophilic nature of some EDCs, their persistence in the environment, their use in agriculture, and their presence in food packaging highlights why the food chain is the primary route of exposure for these chemicals.

All routes of human exposure are uncertain[128] and while dietary intake is the primary route of chemical exposure, there are several other routes by which an individual may be exposed to EDCs. Inhalation exposure to EDCs occurs when these substances are present in the air in the form of particles or dust and are subsequently inhaled into the respiratory system[119]. This route of exposure is significant because it allows EDCs to directly through the bloodstream via the lungs, bypassing the body's normal protective barriers[30]. Sources of EDCs in the air can vary widely and may include emissions from industrial processes, fossil fuels combustion, indoor air pollution from household

products, and environmental contamination from sources such as waste incineration or agricultural activities[29]. In addition, dermal exposure to EDCs occurs when these contaminants come into direct contact with the skin and can pass through the skin and into the bloodstream. Dermal exposure to EDCs can occur from a variety of sources. For example, individuals may encounter EDCs in personal care products such as cosmetics, lotions, and soaps, which often contain ingredients that act as EDCs or can be contaminated with them during manufacturing processes[129]. In addition, contaminated water and soil can also lead to dermal exposure, especially in occupational settings where individuals may come into direct contact with chemicals during activities such as agricultural work or handling contaminated materials[119].

Polychlorinated dibenzo-p-dioxins and furans (PCDD/F) are considered as classical types of EDCs that are generated from natural origins/sources; formed during, volcano eruptions or forest fires or formed by anthropogenic sources from industrial processes, such as waste, plastic incineration or thermal power plant, chemical manufacturing pesticides, automotive emissions, industrial applications as well as atmospheric or waste and water deposition which leads to an accumulation in the food chain[130] **(Figure 1).** Additionally, Per- and polyfluoroalkyl substances (PFAS) are considered as emerging EDCs that are currently widespread distributed and used in numerous countries. They are originally human made chemicals used for the production of stain resistant products, takeout containers, Teflon products or non-stick cookware, waterproof clothing, furniture, carpets, paper, personal care products, firefighting foams and waste infrastructure by-products. As PCDD/F, PFAS can reach the environment and water sources leading to a bioaccumulation and biomagnification in our food chain[131–134] **(Figure 2)**.

c. Polychlorinated dibenzo-p-dioxins and furans (PCDD/F)

PCDD/Fs are a group of highly toxic and persistent organic pollutants. As mentioned before, the most relevant reported exposure to PCDD/Fs worldwide comes from the food consumption, being meat, dairy, and fish products the main contributors, accounting for almost 80% of human exposure[130]. These EDCs could reach the human body and become significant because of the chronic exposure and the tendency of these

contaminants to bio-accumulate due to their lipophilic properties (octanol/water partition coefficient as log Pow= 6.6 and 6.92 for PCDD and PCDF, respectively[135,136]) and the high persistency potential, and resistance to degradation with an estimated half-life in the body ranging from 7 to 11 years[137]. PCCD/Fs were produced in large quantities for several decades with an estimated total world production of 1.2-1.5 million tons. However, since the 1980s, the production was banned in most European countries[130].

In 2018, the European Food Safety Authority (EFSA) Panel has set a new tolerable weekly intake (TWI) for PCDD/Fs in food of 2 pg/$kg_{body\ weight}$/week. The new TWI is seven times lower than the previous tolerable intake set by the panel in 2001 due to the epidemiological and experimental evidence of their toxicity[130].

d. Per- and polyfluoroalkyl substances (PFAS) and Perfluorooctanesulfonic acid (PFOS)

PFAS are a diverse group of almost 15,000 human-made chemicals[138]. These compounds have been widely used since the 1940s as water, grease, and stain resistant, due to their hydrophobic and lipophobic characteristics as well as their molecular stability. They are ubiquitous pollutants in the domestic environment and the main human exposure to PFAS is through food consumption mainly from drinking water, fish, fruit, eggs, and egg products[139,140]

Perfluorooctanesulfonic acid (PFOS) is a member of the PFAS family, it is an emerging EDC that has attracted considerable attention due to its widespread presence, particularly in food (specifically, those from animal origin)[140]. In addition, its structural similarity to naturally occurring fatty acids contributes to its prolonged elimination half-life and its ability to bind to specific proteins and hormones, leading to its accumulation in the human body[141,142]. PFOS is a POP and is known for its remarkable persistence, attributed to its extended half-lives in the body estimated to range between 3.4 and 5.7 years[143]. In addition, PFOSs are known for their significant hepatotoxicity and have been categorized as possible human carcinogens (class 2B)[144], leading to regulatory control on their use within the European Union. While Perfluorooctanoic acid (PFOA), another member of PFAS family, has been banned since 2015, the usage of PFOS persists, with

continued manufacturing and usage in other counties outside the European Union. Despite the decrease of most production in the world, PFAS persists in the environment due to their bio-accumulative nature[141,142] (octanol/water partition coefficient as log Pow=6.3[145]). PFOA and PFOS have become a major public health concern. In 2018 EFSA opinion set separate TWIs of 13 $kg_{body\ weight}$/week for PFOS and 6 $kg_{body\ weight}$/week for PFOA. However, in 2020, The EFSA assessment panel concentrated on four PFAS compounds (being PFOA and PFOS the most widespread and ubiquitous) and has set a new TWI of 4.4 $ng/kg_{body\ weight}$/week for PFAS[140] (including PFOS, PFOA, Perfluorononanoic acid (PFNA) and perfluorohexane sulfonic acid (PFHxS)).

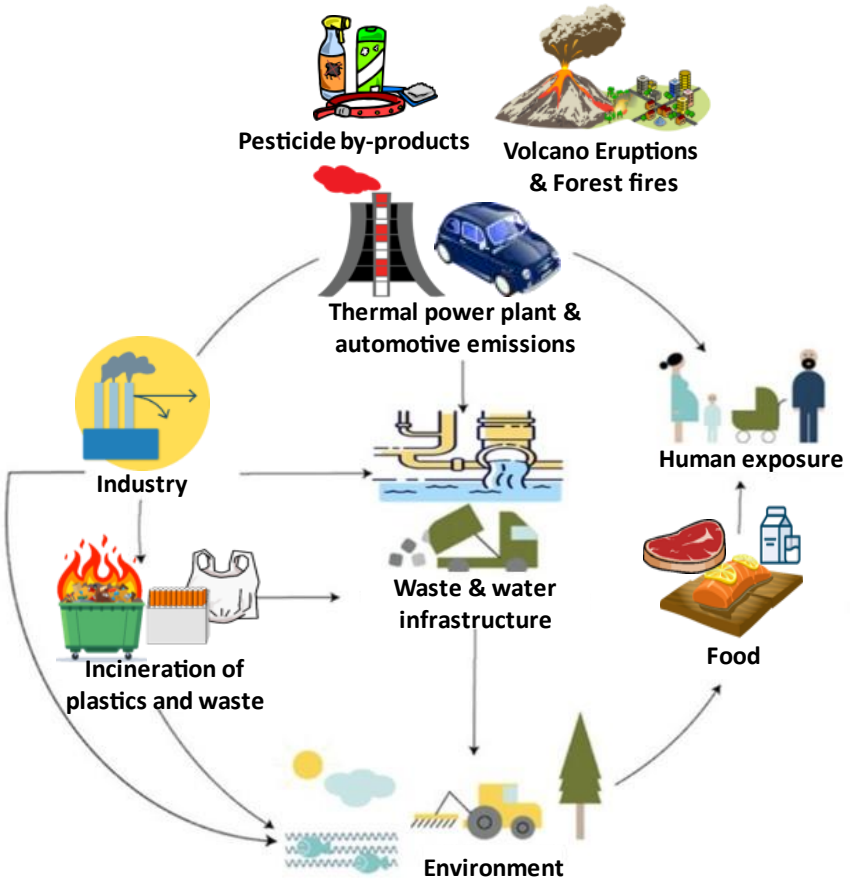


Figure 1. Human exposure to PCDD/Fs.

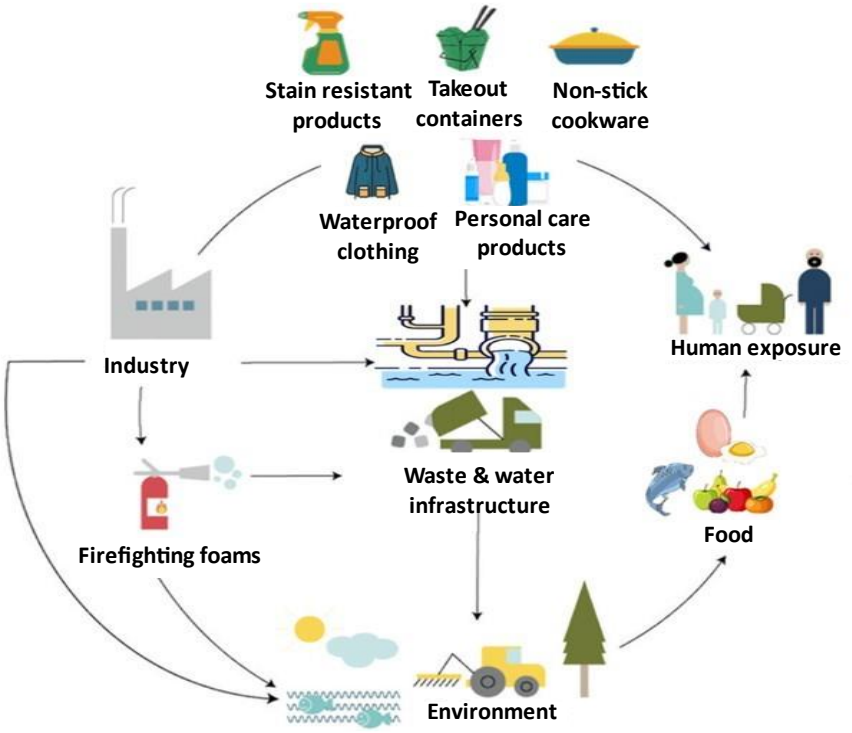


Figure 2. Human exposure to PFAS

e. The chemical contamination dimension of foods and cardiometabolic risk factors

Nowadays, it is known that there are multiple risk determinants that lead to the development of cardiometabolic risk factors (age, sex, smoking, sedentary lifestyle, and unhealthy dietary patterns etc..). However, in recent years, chemical contaminants present in food have started to receive some attention due to their potential contribution to the development and progression of cardiometabolic risk factors, with particular attention given to the role of EDCs, independently of the nutritional or processing dimension of the food.

As mentioned above, EDCs are capable of binding to cell receptors, they could mimic or block the functioning of natural hormones, leading to adverse health effects[29,113]. Among the most hazardous ones, PCDD/Fs and PFOSs are considered to pose serious risks for humans. The main human exposure to these human-made chemicals comes through food mostly from animal-origin products due to their lipophilic nature[130,140].

There is a growing body of evidence coming from the literature exploring the associations between PCDD/FS and increased risk of insulin resistance[146], diabetes[147], metabolic syndrome[148], or CVD[149]. Additionally regarding PFOS, the latter have been previously associated with cardiometabolic risk factors such as increased risk of obesity[150], dyslipidemia[151], and hypertension[152] and prior studies have shown the associations between PFAS exposure and elevated risk of diabetes[153,154]. However, the results are still inconsistent across all the literature and there is limited epidemiological evidence concerning the senior population's dietary intake of PCDD/Fs, PFOS and cardiometabolic risk factors. Additionally, the associations are predominantly analyzed cross-sectionally, and the majority of studies conducted have not specifically examined dietary exposure to PCDD/Fs and PFOS.

Despite the acknowledged vulnerability of specific groups, such as infants and pregnant women, to the adverse health effects of EDCs exposure, the elderly population is a vulnerable group often overlooked[155]. Therefore, it is essential to assess the association between EDCs and cardiometabolic risk factors in an elderly population since senior individuals frequently undergo prolonged lifetime exposure to different contaminants, including PFOS and PCDD/Fs, potentially resulting in increased cumulative effects over time[156]. Furthermore, age-related metabolic changes, diminished organ function, and potential alterations in immune response may affect how those EDCs are absorbed, distributed, and eliminated in this population[156].

4. The sustainability and environmental dimension of foods and cardiometabolic risk factors

According to WHO, sustainable healthy diets are “dietary patterns that promote all dimensions of individuals’ health and wellbeing; have low environmental pressure and impact; are accessible, affordable, safe and equitable; and are culturally acceptable[157]”.

Global dietary trends have shifted from predominantly plant-based diets to ones featuring an increased consumption of energy-dense and animal-based food, as well as increased consumption of added sugars, fats and UPFs with low nutritional value[158]. In parallel, global food systems contribute to 20-35% of global greenhouse gas (GHG) emissions, use approximately 40% of the Earth’s ice-free land area, contribute to

terrestrial and aquatic pollution due to excessive fertilizer and chemical usage being the primary cause of biodiversity loss[159–161]. The impact of the global dietary trends on the environment and human health is projected to worsen in the future if these trends continue[162]. Populations that are more urbanized have higher food demands including higher consumption of animal-based food, which have greater environmental footprint per calorie or gram of food produced[163]. These changes in dietary habits have been long associated with higher cardiometabolic risk factors. Results from the Mediterranean PREDIMED-Plus study showed that individuals with elevated CO_2 emissions were observed to have higher levels of glycaemia, and the risk of having severe metabolic syndrome was related to high CO_2 emissions[164]. In addition, another study conducted within the framework of the PREDIMED-Plus study showed that lower consumption of UPFs could contribute to environmental sustainability highlighting the importance of considering the processing level of food consumed for both health and as an environmental protection[165].

Therefore, adopting a low carbon diet was associated with a reduction in the severity of cardiometabolic risk factors. Additionally, a more sustainable diet has been shown to have dual benefits on health and the environment.

II. JUSTIFICATION

UNIVERSITAT ROVIRA I VIRGILI
A MULTI-DIMENSIONAL APPROACH OF FOOD CLASSIFICATIONS AND ITS ASSOCIATION WITH CARDIOMETABOLIC RISK FACTORS
Nadine Alkhoury

JUSTIFICATION

It is well established that diet is an important modifiable risk determinant that might contribute to the development of cardiometabolic risk factors. The definition of a "healthy" or "unhealthy" diet depends on a combination of several dimensions.

In fact, studies have shown that food may affect human health through several dimensions: the first dimension incorporates the nutritional composition and profile of foods, including the presence of favorable nutrients (fiber, protein, minerals, vitamins, etc.) or nutrients that should be limited (added sugars, saturated fats, salt, etc.). Secondly, the level of transformation and processing specifically UPFs that contain cosmetic food additives or other preservatives and colorants such as hydrogenated oils. Thirdly, the contamination dimension of food and the possible presence of EDCs such as PCDD/Fs and PFOS.

Although the three aspects diet quality—nutritional composition, ultra-processing, and EDCs exposures are essential, none of them alone can fully reflect the overall health impact of food on its own. Independent of each other, epidemiological research has demonstrated the significant associations of each of these dimensions on the onset of cardiometabolic risk. Indeed, several studies have shown that the detrimental impact of UPFs consumption or EDCs exposure persist, even after adjusting for diet quality. On the other hand, the association of the nutritional dimension and cardiometabolic risk factors remains significant even after adjusting for the other food dimensions.

Given that the associations between each of the three food dimension classifications are significant, the three approaches should be considered independently and equally important. However, the level of evidence of these dimensions varies, consequently further research is needed on the multi-dimensional approach of food classifications and their impact on cardiometabolic risk factors.

JUSTIFICATION

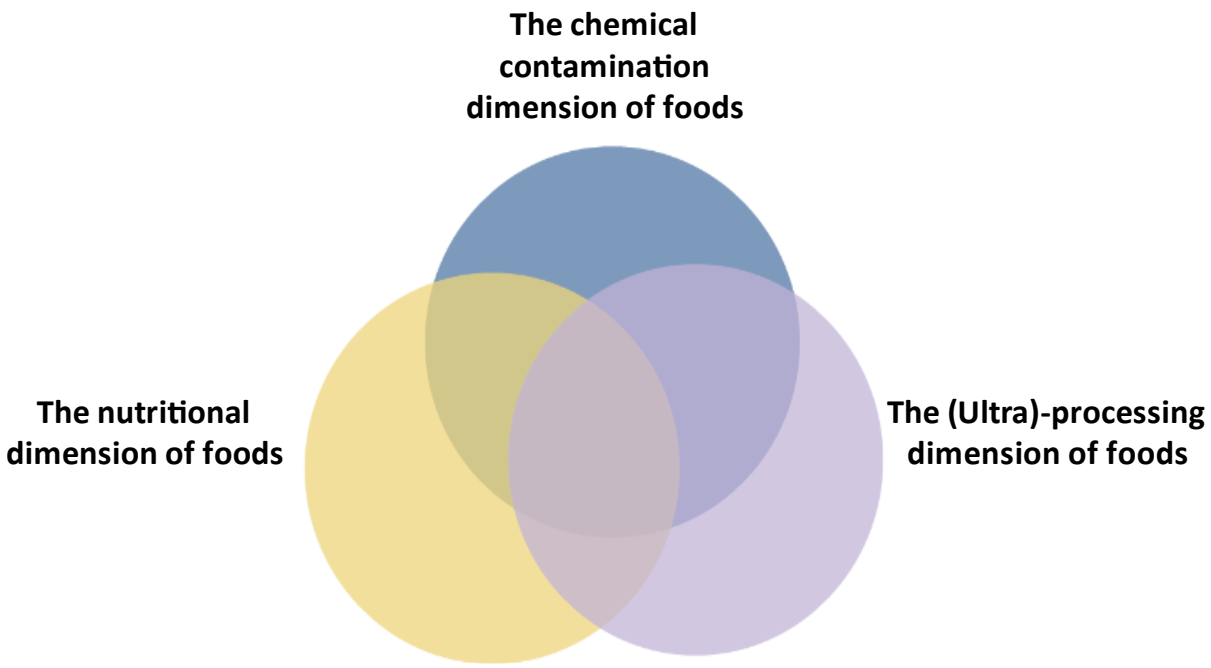


Figure 3. The multi-dimensional approach of food.

III. HYPOTHESIS AND OBJECTIVES

UNIVERSITAT ROVIRA I VIRGILI
A MULTI-DIMENSIONAL APPROACH OF FOOD CLASSIFICATIONS AND ITS ASSOCIATION WITH CARDIOMETABOLIC RISK FACTORS
Nadine Alkhoury

HYPOTHESIS AND OBJECTIVES

Hypothesis:

(1) The consumption of food with higher NS-NPM dietary index (lower nutritional quality) is associated with cardiometabolic risk factors.
(2) The updated 2023 NS-NPM discriminate food consumption and nutrient intake better than the 2015 NS-NPM in a population of French children and adolescents.
(3) Higher consumption of UPFs is associated with worse cardiometabolic risk indicators in children.
(4) Higher dietary intake of endocrine disrupting chemicals (PCDD/Fs and PFOS) is associated with cardiometabolic risk factors (adiposity, obesity status and glucose homeostasis parameters).

Objectives:

(1) To prospectively assess the association between the NS-NPM dietary index and cardiometabolic risk factors in an elderly population with overweight/obesity and metabolic syndrome.
(2) To investigate the ability of the 2015 and 2023 NS-NPM to discriminate food consumption and nutrient intake in a population of French children and adolescents.
(3) To cross-sectionally assess the associations between UPFs consumption and cardiometabolic risk factors in a population of Spanish preschoolers.
(4) To prospectively assess the association between the dietary intake of some important widespread endocrine disruptors (PCDD/F and PFOS) present in food and cardiometabolic risk factors (adiposity, obesity risk and glucose homeostasis parameters) in an elderly Mediterranean population.

IV. METHODOLOGY

UNIVERSITAT ROVIRA I VIRGILI
A MULTI-DIMENSIONAL APPROACH OF FOOD CLASSIFICATIONS AND ITS ASSOCIATION WITH CARDIOMETABOLIC RISK FACTORS
Nadine Alkhoury

METHODOLOGY

The present doctoral thesis includes 5 scientific articles. 3 articles have been conducted within the framework of the The PREvención con DIeta MEDiterránea Plus (PREDIMED-Plus) (objective 1 and 4), one article within the framework of the Childhood Obesity Risk Assessments Longitudinal Study (CORALS) cohort (objective 3), and another one within the framework of the French Esteban study (objective 2).

In the following sections the methodology of each of these articles will be described

1. The PREDIMED-Plus Study

a. Study design and objectives

The PREDIMED-Plus study, is a 6-year parallel-group, multi-center, randomized simple blind controlled clinical trial plus a 2-year of follow-up period without intervention conducted for the primary prevention of CVD.

Main objectives:

The primary objective of PREDIMED-Plus is to compare the outcomes of an intensive intervention with those of a control group. The study aims to assess the impact on:

- The incidence of cardiovascular events (non-fatal myocardial infarction, non-fatal stroke, or cardiovascular mortality).
- Weight loss and the long-term maintenance of weight loss.

Allocation

Population was randomly allocated to two lifestyle intervention groups: ***a)*** Intensive lifestyle intervention based on an energy-reduced Mediterranean diet (er-MedDiet) (energy reduction of ~600 kcal/day according to each participant's basal metabolic rate and physical activity level), physical activity promotion, and behavioral therapy, or **b)** a control group receiving recommendations of an *ad libitum* Mediterranean diet and standard care advice.

Recruitment

The recruitment process was carried out by family doctors at primary health care centers, who reviewed clinical records to identify potential participants meeting the

eligibility criteria. Subsequently, these potential participants were contacted by telephone to inform them about the study and to schedule an initial screening visit with trained study staff.

In the PREDIMED-Plus study, three screening visits were conducted as detailed below:

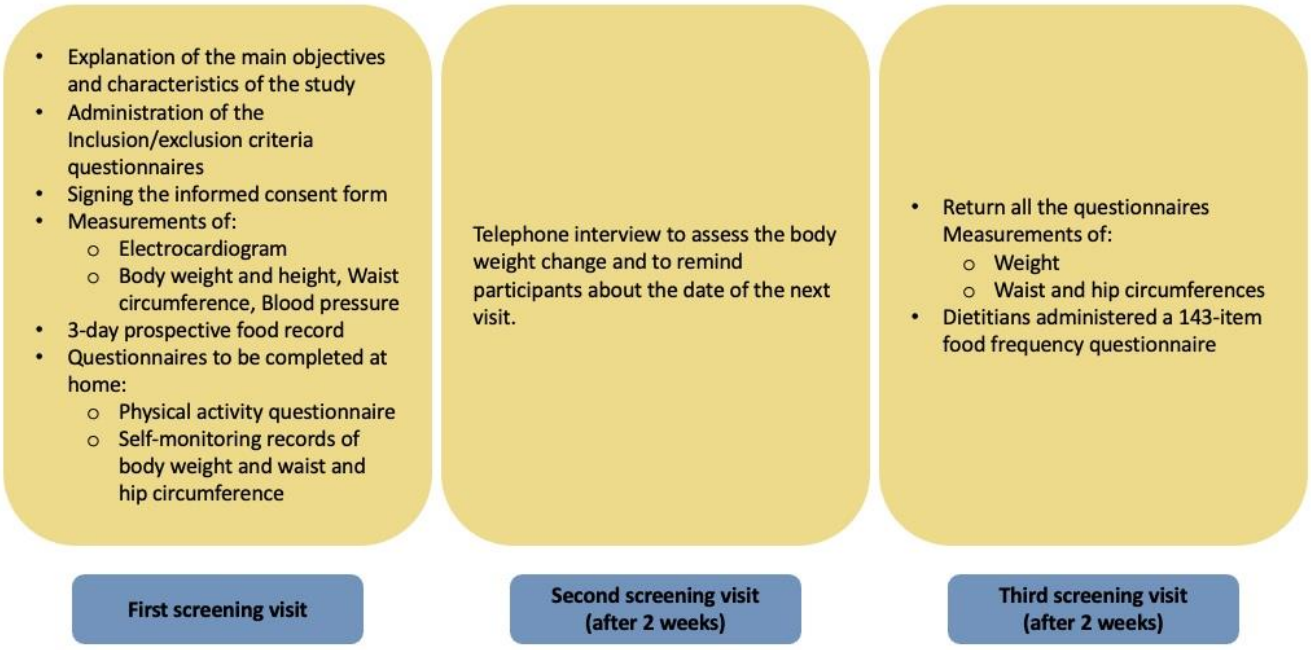


Figure 4. *PREDIMED-Plus screening visits.*

The PREDIMED-Plus trial was registered at the International Standard Randomized Controlled Trial (ISRCTN89898870).

As part of the trial intervention, participants in both groups attended periodic group sessions conducted by trained dietitians. These sessions included informative talks on lifestyle-related topics and motivational aspects. Additionally, to reinforce adherence to their assigned study group, participants were provided with free extra virgin olive oil (1 liter/month) and raw nuts (125g/month during the first year of the intervention).

Study design of the analysis

For the present doctoral thesis, data from all randomized PREDIMED-Plus participants was used to conduct both a cross-sectional and 1-year prospective analysis.

b. Study population

Participants in the PREDIMED-Plus study were randomly recruited from September 2013 to December 2016 across 23 centers in Spain, including research institutes, hospitals, and primary health care centers located in Málaga, Sevilla, Navarra, Palma de Mallorca, Barcelona, Reus, Granada, Madrid, Vitoria, Valencia, Las Palmas de Gran Canaria, Córdoba, Alicante, Jaén, and León.

The study included men (aged 55-75) and women (aged 60-75) with a BMI between 27 and 40 kg/m², who had no prior history of cardiovascular disease (CVD) or cancer. Additionally, participants had to meet at least three of the following metabolic syndrome criteria, as defined by the AHA[23]:

- Abdominal obesity (waist circumference ≥88 cm in women and ≥102 cm in men).
- High triglyceride levels (≥150 mg/dL or drug treatment for elevated triglycerides).
- Low HDL-cholesterol levels (<50 mg/dL in women and <40 mg/dL in men, or drug treatment for reduced HDL-cholesterol).
- High blood pressure (systolic BP >130 mm Hg or diastolic BP >85 mm Hg, or antihypertensive drug treatment).
- High fasting blood glucose (≥100 mg/dL or drug treatment for elevated glucose).

Exclusion criteria for the study included:

- Previous history of CVD (e.g., angina pectoris, myocardial infarction, stroke, peripheral arterial disease, ventricular arrhythmias, atrial fibrillation, heart failure, hypertrophic cardiomyopathy, aortic aneurism).
- Active cancer or a history of malignant tumors within the last five years, except non-melanoma skin cancer.
- Endocrine-related obesity (except for treated hypothyroidism).

- History of inflammatory bowel disease or bowel resection.
- History of weight-loss surgery or planned bariatric surgery within the next 12 months.
- Inability to adhere to the Mediterranean diet due to religious reasons, swallowing problems, or food allergies.
- Inability to perform physical activity.
- Alcohol dependence or daily alcohol intake >50 g.
- Drug abuse within the past six months.
- Cirrhosis or liver failure.
- Life expectancy of less than one year.
- Severe psychiatric disorders (e.g., schizophrenia, eating disorders, severe depression).
- Immunodeficiency or HIV-positive status.
- History of organ transplantation.
- Concurrent treatment with immunosuppressive drugs or cytotoxic agents.
- Current use of weight loss medication.

After completing the three screening visits, a total of 6,874 participants met the eligibility criteria and were randomly assigned to either the intervention or control group using a centrally assigned, computer-generated random number sequence with a 1:1 ratio. Participants in the intervention group were stratified by center, sex, and age group. Couples were randomized in the same group as clusters.

Study population for the analysis conducted in this doctoral thesis

For objective 1:

A total of 953 participants who did not complete a food frequency questionnaire (FFQ) at baseline and after 1 year of follow-up or with total calorie intake outside the pre-specified energy limits (women < 500 and >3,500 kcal/day, and men < 800 and >4,000 kcal/day)[166] were excluded from the analyses. Therefore, 5,921 participants aged 55-75 years with overweight/obesity and metabolic syndrome from the PREDIMED-Plus cohort were included in this study.

For objective 4:

Paper #4:

Participants without complete FFQs at baseline and after 1-year of follow-up or those with an energy intake outside the pre-specified energy limits (less than 500 and more than 3500 kcal/day, and less than 800 and higher than 4000 kcal/day in women and men respectively)[166] and participants without data for BMI and waist circumference after 1 year of follow-up n=22 were excluded. Therefore, 5,899 participants aged 55–75 years (48% women) living with overweight/obesity from the PREDIMED-Plus cohort were included in this study.

Paper #5:

A total of 4,600 participants without diabetes were included in the cross-sectional analysis. Participants had complete baseline data from FFQs. In addition, participants were excluded if their energy intake appeared implausible (women < 500 and >3,500 kcal/day, and men < 800 and >4,000 kcal/day)[166]. Additionally, individuals with T2D were excluded from the analysis. For the prospective analysis, out of the initial 4,600 participants, 504 were further excluded due to insufficient FFQ information, implausible energy intake, or because they had started insulin treatment during the one-year follow-up period. Therefore, 4,600 participants were included in the prospective analysis and 4,096 participants were included in the prospective analysis.

c. Exposure assessment

Dietary Index Based on the Modified FSA-NPS Computation (2015 NS-NPM) (objective 1):

The FSAm-NPS (or the 2015 NS-NPM) is a modified version of the original FSA-NPS. In 2015, modifications made by the French National Nutrition and Health Program and the French High Council for Public Health modified the scoring standards for cheese, added fats, and beverages. For all foods and beverages in the PREDIMED-Plus FFQ, the FSAm-NPS was computed per 100 grams of the product. Points were allocated as follows: 0–40 points for the content of critical nutrients that should be limited (0–10 points each for sugars, saturated fats, sodium, and energy), and 0–15 points for the content of beneficial nutrients that should be encouraged (0–5 points each for fibers, proteins, and

the percentage of fruits, vegetables, legumes, nuts, rapeseeds, walnuts, and olive oil in the total product)[59]. The total score was determined by subtracting the "negative" scores (nutrients to avoid) from the "positive" scores (nutrients to promote). Consequently, the final FSAm-NPS for each food/beverage was calculated on a scale ranging from -15 (healthiest) to 40 (least healthy).

To generate a dietary index at the individual level, the FSAm-NPS DI (or the 2015 NS-NPM DI) was calculated for each participant, taking into account their entire diet. This was done using energy-weighted means of the FSAm-NPS for all foods and beverages consumed using the following equation:

$$\text{Dietary Index} = \frac{\sum_{i=1}^{n} FSiEi}{\sum_{i=1}^{n} Ei} \quad \text{(Equation 1)}$$

Where "i" signifies a food or beverage consumed by the participant, "FSi" the food (or beverage) score, "Ei", the mean daily energy intake from this food (or beverage) and "n" the number of different foods.

Therefore, higher FSAm-NPS-DI reflect lower nutritional quality of the individual's overall diet.

Dietary intake of endocrine disrupting chemicals (objective 4):

A detailed explanation of the estimation of the dietary intake of PCDD/Fs and PFOS conducted for the present doctoral thesis is reflected in each of the publications included in the results section.

Briefly, the dietary intake was estimated using the FFQ accounting for the frequency of dietary consumption of each food item (in g/day) and the levels of PCDD/F or PFOS.

Estimation of the dietary intake of PCDD/Fs

For the estimation of the dietary intake of PCDD/Fs, the levels were expressed as Toxic Equivalents (TEQ). The TEQ is established by the World Health Organization (WHO)[167] to assess total PCDD/F exposure by the sum of an individual's congener concentration multiplied by their relative toxicity factor.

TEQ for each food item was obtained from the most updated studies, prioritizing those from the Mediterranean region since the participants are from Spain and typically follow a Mediterranean diet. The hierarchy for gathering TEQ data was: data from Spain > other Mediterranean countries > Europe > other available data.

The estimated dietary intake of PCDD/F (expressed in TEQ pg/week) was the sum of each TEQ (in TEQ pg/day) from a specific food item multiplied by the amount of each specific food consumed for each participant (in g/day) multiplied by seven considering the following equation:

Dietary intake of PCDD/F $_{(pg\ /week)}$ = $\sum TEQ_i * A_i) * 7$ (Equation 2)

Where TEQ_i represents the toxic equivalency of a specific food item (in pg PCDD/F/g), A_i represents the amount of a specific food consumed (in g/day) and it was multiplied by 7 to obtain the weekly dietary intake of PCDD/F in pg TEQ/week.

Estimation of the dietary intake of PFOS

For the estimation of the dietary intake of PFOS, we considered the most recent data on PFOS levels in food (measured in ng/g) from the EFSA. Food items were grouped into appropriate categories based on EFSA guidelines[140].

PFOS levels for each food group were provided from earlier EFSA reports, which analyzed PFOS concentrations in different food samples from European regions between 2012 and 2020. We primarily used data from 2020 as it provided the most recent. For the dietary intake estimation, we used the mean of the lower bound (LB) and upper bound (UB) levels within food categories defined by EFSA[140].

The terms “lower bound” and “upper bound” refer to the detection range of PFOS. The LB represents the lowest value, while the UB represents the highest estimated value for PFOS levels. The mean LB is considered the least conservative or "best-case scenario" because it assumes a concentration of zero for non-detects. The mean UB is the most conservative or "worst-case scenario" because it assumes the concentration for non-detects is at the limit of detection (LOD)[140]. Therefore, in this study, both LB and UB were considered to estimate the lower and upper limits of dietary intake and exposure to PFOS in our study population.

The estimated DI of PFOS UB and LB (expressed in ng/day) was obtained from the following equation:

$DI\ PFOS_{LB/UB}\ (ng/day) = \sum C_i * A_i$ (Equation 3)

Where DI represents the downward (LB) / upward (UB) Dietary intake of PFOS in ng/day; C_i represents the PFOS concentration of a specific food group (in ng/g); Ai signifies the quantity of a particular food item consumed, measured in grams per day (g/day).

d. Cardiometabolic risk factors outcomes (for objective 1 and 4)

For this doctoral thesis, all cardiometabolic risk factors were assessed at baseline and after 1-year of follow-up.

Waist Circumference

Waist circumference was measured midway between the lowest rib and the iliac crest using an anthropometric tape.

Body Mass Index

Body weight was measured twice with light clothes using high-quality electronic calibrated scales and the mean of both measurements was used. Height was measured twice using a wall-mounted stadiometer. BMI was then calculated by dividing weight in kilograms by height in meters squared.

Prevalence/incidence of obesity and abdominal obesity

Prevalence of obesity and abdominal obesity were defined as participants having higher levels of BMI and waist circumference at baseline Obesity was defined when BMI was higher than or equal to 30 kg/m^2. On the other hand, abdominal obesity was defined when waist circumference was higher than or equal to 88 cm for women and higher than or equals to 102 cm for men[168]. In addition, the incidence of obesity and abdominal obesity was defined when participants did not present these conditions (obesity and abdominal obesity) at baseline but presented higher levels of BMI and waist circumference than those indicated above after 1-year of follow-up.

Systolic and Diastolic Blood Pressure

Blood pressure was measured three times using a validated semiautomatic oscillometer (Omron HEM-705CP, Kyoto, Japan), with the mean of the repeated measures used.

Blood lipids

HDL-cholesterol and triglyceride were measured using standard enzymatic methods. LDL-cholesterol was calculated using the Friedewald formula when triglycerides were less than 300 mg/dL; for triglycerides over 300 mg/dL, LDL-cholesterol was measured using the standard method.

Glucose homeostasis parameters

Samples for fasting plasma glucose and HbA1c were obtained from our study cohort population and analyzed by a routine laboratory method. The fasting plasma insulin levels were measured centrally using an Elecsys immunoanalyzer (Roche Diagnostics, Meylan, France) with the assistance of an electrochemiluminescence immunoassay. To estimate insulin resistance, the HOMA-IR index was used following this formula[18]: HOMA-IR= ($Insulin_{(mU/mL)}$ * $glucose_{(mg/dL)}$) / 405 (Equation 4).

e. Covariates

At baseline, at 6 months, and annually, participants had face-to-face visits with trained PREDIMED-Plus staff (dietitians and nurses) at the primary care centers. During these visits, various questionnaires were administered, and several anthropometric and biochemical measurements were collected.

Sociodemographic and lifestyle data and family history of type 2 diabetes and cardiovascular diseases

General and follow-up questionnaires were performed to collect data about the study participants. These questionnaires included items related to personal and medical data, such as socioeconomic status, education level, marital status, family history of disease, illness history, smoking status, alcohol consumption, previous and current CVD risk factors, medication use, and the occurrence of new cardiovascular events or other medical conditions.

Adherence to the Mediterranean diet measurement

A validated short questionnaire was used to assess the adherence to the Mediterranean diet. The control group completed the 14-item validated Spanish version questionnaire, while the intervention group answered the 17-item energy-reduced Mediterranean diet questionnaire (erMEDAS)[169].

Physical activity assessment

A validated Spanish version of the Minnesota Leisure-time Physical Activity Questionnaire[169] was administered to each participant. This questionnaire included information about the monthly frequency (number of days) and duration (min/day) of several physical activities during the previous month. Total physical activity was estimated as the summed product of frequency and duration divided by 30 to obtain the amount of min/day. The intensity of each activity (expressed in metabolic equivalent task, MET) was assigned based on the Compendium of Physical Activity[170].

Dietary intake assessment

Dietary intake was assessed at baseline and after 1 year of follow-up using a validated FFQ administered face-to-face by trained dietitians[171]. The participants reported their average consumption in terms of frequency and quantity for 143 food and beverage items during the previous year. The frequency of consumption was shown through nine categories ranging from never or almost never to more than six times a day, and a commonly used portion size was specified (e.g., slices, glass, and teaspoons) to indicate serving sizes for each of the items. The nutrient intake was calculated as the sum of the frequency of daily consumption of each item multiplied by the nutrient composition of the specified serving size according to the Spanish food composition database[172]. The total energy intake was also estimated from the quantity and frequency of food and beverage consumption.

For the water intake assessment, a fluid-specific questionnaire that has been already validated as a tool for assessing intake of water and other types of beverages, was used in our population[173].

Table 1. Data collection used for the present doctoral thesis at baseline and after 1-year of follow-up in the PREDIMED-Plus trial

	Baseline	1-year
Anthropometric measurements	✓	✓
General questionnaire	✓	✓
143-item food frequency questionnaire	✓	✓
32-item Spanish fluid-intake questionnaire	✓	✗
Mediterranean diet questionnaire (17/14- item)	✓	✓
Physical activity questionnaire	✓	✓
Blood pressure measurement	✓	✓
Blood sample collection	✓	✓

2. Childhood Obesity Risk Assessments Longitudinal Study (CORALS)

a. Study design and objectives

The CORALS is an ongoing prospective, multicenter study with a 10-year follow-up period.

The objectives of this study are as follows:

Main objective:

- To longitudinally identify the risk factors for childhood obesity on long term in children initially aged 3 to 6 years old.

Secondary objectives:

- To assess the associations between perinatal risk factors, socioeconomic, cultural, and environmental factors, and the risk of developing obesity and its comorbidities.

- To assess the associations between lifestyle factors (diet, physical activity, sedentary behavior, sleep) and the risk of developing obesity and its comorbidities.
- To evaluate the association between genetic polymorphisms, metabolomic profiles, and metagenomic profiles (determined through omics techniques) and the development of obesity and its comorbidities.

The Ethics Committee of each recruitment center approved the study protocol (references: 051/2019, 4155/2019, 2019/18, 9/19, 19/162, 19/27, and 2019/131), which was conducted following the standards of the Declaration of Helsinki[174].

Study design of the analysis conducted in this doctoral thesis

For the present doctoral thesis, data from all CORALS participants was used to conduct the cross-sectional analysis.

b. Study population

Between March 22, 2019, and June 30, 2022, eligible participants aged 3 to 6 years at enrollment were recruited from schools across 7 cities in Spain.

To be enrolled in the study, participants had to meet the inclusion criteria:

- Be aged 3 to 6 years old at the time of inclusion.
- Compliance and completion of several questionnaires (the leisure-time physical activity questionnaire, sociodemographic questionnaire).
- Attendance to the scheduled inclusion visit.
- Submission of signed informed consent by parents or caregivers.

Exclusion criteria include:

- Children from families facing difficulties in participating or complying with the study protocol.
- Children experiencing comprehension and language difficulties.
- Unstable living conditions.

Since this study is school-based and aims to avoid stigmatizing children with any health issues (such as chronic conditions like diabetes, hypertension, hypercholesterolemia,

asthma, or cancer that could potentially interfere with the results), the exclusion of such children from the analysis has been considered once the results have been obtained.

Study population for the analysis *conducted in this doctoral thesis*

A total of 1,426 participants were included in the cross-sectional analysis after excluding 54 participants lacking food and beverage frequency questionnaire (F&B-FQ) and 29 participants with missing data or implausible reported energy intake.

c. Exposure assessment

UPF consumption:

The NOVA classification system was used to determine the consumption of food according on their degree of processing[175]. The categorization of food items was carried out by two different dietitians. In addition, a further dietitian carried out a thorough revision to ensure accuracy. Any discrepancies in the classification of particular food items were resolved through extensive discussions among the researchers, ultimately resulting in decisions based on consensus. The final categorization of food items according to the NOVA classification is shown below:

Table 2. Distribution of F&B-FQ items into four groups according to the degree of their processing established by NOVA classification system.

Group 1: Unprocessed or minimally processed foods	Milk (whole, semi-skimmed, skimmed), yogurt (without added sugar, skimmed), eggs, chicken (with and without skin), fresh meat (cow, turkey, pork, lamb, rabbit), liver meat, offal meat, fresh fish, oysters, calamari, fresh shrimps, fresh vegetables, gazpacho, fruits, dates, nuts, legumes, rice (white and whole), pasta (white and whole), water, fresh juices, coffee, tea and infusions.
Group 2: Processed culinary ingredients	Olive oil (extra virgin and refined), corn oil, sunflower oil, soybean oil, butter, salt, cacao and sugar.
Group 3: Processed foods	Condensed milk, lactose free yogurt, cooking cream, curd, cheeses (white cheese), ham serrano, bacon, salted fish, canned fish, homemade fries, fruits in syrup, olives, bread (white and whole), cereals (with no added sugars and whole) and non-dairy milk.
Group 4: Ultra-processed food and drink products	Milkshakes, yogurt (whole and with added sugars), fermented milk, cheese (Petite Suisse, soft cheese), custard, ice cream, ham, processed meat, pate, hamburger, surimi, potato chips, loaf of bread, bread sticks, cereals (with added sugar, flavored-stuffed cereals), pizza, margarine, biscuits (plain, whole, with chocolate), pastries, donuts, muffins, cakes, churros, chocolate, butter cookies, croquettes, ready-to-eat soups, mustard, mayonnaise, ketchup, jams, chocolate cream, snacks, candies, sparkling water, flavored water, bottled juices, soft drinks

Group 1 includes unprocessed or minimally processed foods consisting of natural and whole foods that have undergone little to no processing. Group 2 includes processed culinary ingredients, this group comprises substances extracted from Group 1 foods or nature, used to season and cook foods. Group 3 includes processed food that is manufactured by combining Group 1 or 2 ingredients and may involve some processing methods. Group 4 includes ultra-processed foods (UPF), this group consists of industrial formulations that are characterized by a high energy content, rich in saturated fats, added sugars, salt, with a low amount of fiber and vitamins, which may also contain additives like artificial colors and flavors or stabilizers. Ultra-processed foods are typically ready-to-eat or require minimal preparation (sugary drinks, packaged snacks, fast food, frozen pizzas, and many convenience foods).

d. Cardiometabolic risk factors outcomes

Assessment of adiposity measurements:

Weight and body fat mass content were assessed using a precision scale and an octopolar multifrequency bioelectrical impedance device (MC780MAS; Tanita). Height was measured with a portable SECA 213 stadiometer following standardized procedures. BMI was calculated and classified as underweight/normal weight or overweight/obesity based on pediatric cutoff points[176]. Waist circumference was measured using a flexible non-extensible measuring tape. Waist-to-height ratio was calculated by dividing the waist circumference (in centimeters) by the height (in centimeters)[177]. Fat Mass Index was estimated by dividing the body fat mass (in kilograms) by the square of the height (in meters)[178].

Assessment of cardiometabolic risk factors:

Blood pressure was measured three times in the non-dominant arm, with a 5-minute interval between each measurement, with an automatic oscillometer (M3 Intelligence HEM-75051-EV; Omron Healthcare Europe) and using child-specific cuff. The average of the three measurements was then estimated.

Blood samples were collected from participants after an 8-hour fast. Serum concentrations of total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides were measured using standard procedures. Plasma samples were used to assess fasting plasma glucose and fasting plasma insulin levels. Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was calculated as fasting insulin ($\mu IU\ ml^{-1}$) × fasting glucose ($mmol\ l^{-1}$)/22.5 (Equation 5).

Measurements of adiposity and assessments of cardiometabolic risk factors were conducted at Healthcare Centers.

e. Covariates

Early life and maternal factors

Questionnaires were used to evaluate early life factors and family factors, including birth weight (in kilograms), and specific maternal characteristics such as weight gain during pregnancy (in kilograms), maternal weight (in kilograms), height (in meters), educational level (primary or lower, secondary, academic-graduate, or not reported), socio-professional category (homemaker, student, retired, unemployed, or employee) and the duration of breastfeeding (in months). Maternal weight status was assessed using BMI and classified as underweight/normal weight (<25 kg/m^2) or overweight/obesity (≥25 kg/m^2).

Physical activity assessment

To assess physical activity, the total time (hours) of sports and physical activities per week was estimated using a previously validated questionnaire[179]. The total weekly duration of physical activity (in hours) was calculated by adding the time spent in physical education classes and extracurricular sports and physical activities.

Dietary intake assessment

To estimate the dietary intake, trained dietitians used a validated semi-quantitative 125-item COME-Kids F&B-FQ[180].

Parents or caregivers were asked to indicate, on average, how frequently the participant had consumed the specified serving of each item over the past year, rating their consumption frequency on a scale of 9 options ranging from "never" to "more than 6 times per day ". This questionnaire was designed in machine-readable format to be later scanned using the Evaldara® software. This software efficiently extracts the reported consumption, which was then integrated into the program e-Diet Base[181] to obtain the nutrient and energy values.

Diet quality assessment

An 18-item questionnaire for children was used to assess adherence to the Mediterranean diet, an indicator of diet quality[175].

3. Esteban Study

a. Study design and objectives

Esteban is a cross-sectional study conducted between 2014 to 2016 on a nationally representative sample of children aged 6 to 17 years old and adults aged 18 to 74 years old.

The objectives of this study are:

Main objectives:

- Describe the dietary intake, physical activity, sedentary behavior, and nutritional status of the population living in France.
- Estimate the levels of exposure of the population living in metropolitan France (6-74 years old) to environmental substances with presumed and/or observed impact on health and establish reference values.
- Estimate the prevalence of chronic diseases (diabetes, chronic kidney disease, asthma) and cardiometabolic risk factors (hypertension, dyslipidemia), as well as their undiagnosed proportion among adults.
- Estimate the prevalence of asthma, atopy, and allergic diseases among children.

Secondary objectives:

- Analyze the determinants of exposure, chronic diseases, and dietary behaviors.
- Compare the results with data from surveys conducted internationally.
- To have the necessary indicators to define and understand the monitoring of public health policies (Public Health Law, National Nutrition and Health Program, Obesity Plan, chemical management plans, etc.).
- Alert health authorities in case of emerging phenomena.

The three-stage sampling was implemented as follows: At the first stage, 190 geographic zones (municipalities or groups of municipalities) were randomly selected, stratified by eight large regions and by degree of urbanization (from "rural" to "cities of more than 100,000 inhabitants," with Paris as an additional category). The number of individuals included in each stratum was determined proportionally to the population size, with a

minimum of twenty-four dwellings to be surveyed. In the second stage, a random sample of households was selected by telephone. In each stratified sample, two household samples were independently drawn. These households included at least one adult aged 18-74 years. Household selection was achieved through the random generation of telephone numbers. The primary sample consisted of households reachable by landline telephone. To ensure the inclusion of households without a geographic landline, a supplementary sample was created. This "exclusive mobile" sample comprised households only reachable via mobile phone. At the third stage, a single individual (an adult or child, depending on the household sample) was selected randomly from among the eligible household members using Kish's method[182].

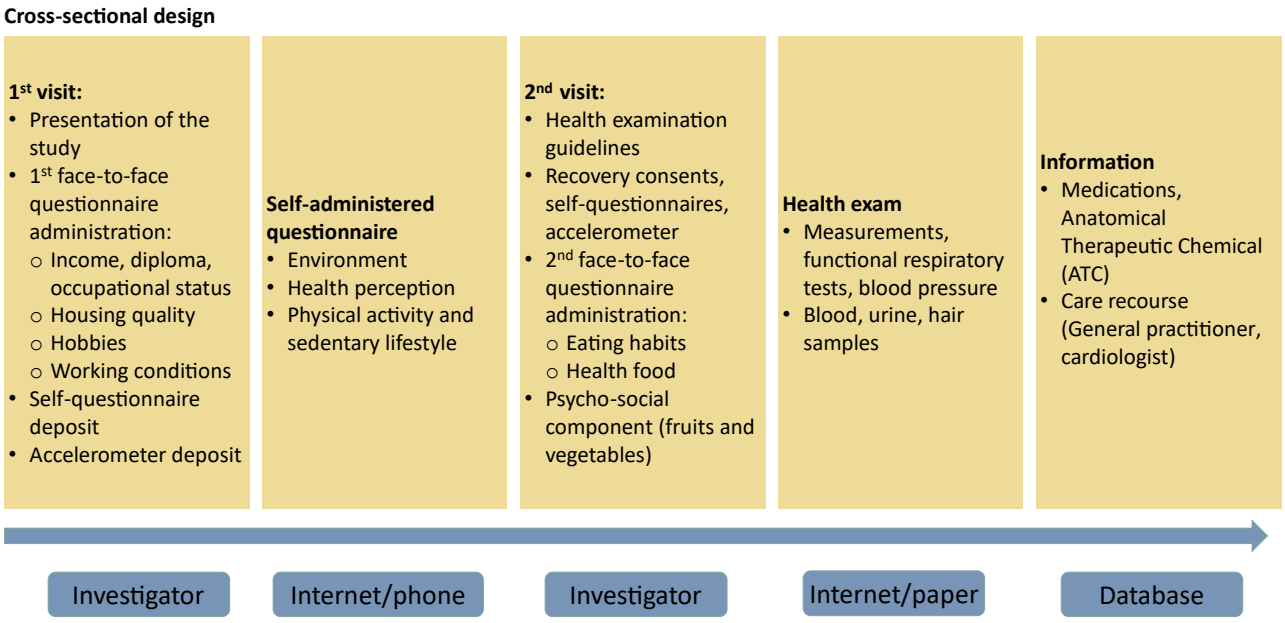


Figure 5. Protocol of data collection in the Esteban study (2014-2016)

This study received approval from the Advisory Committee on Information Treatment in Health Research (CCTIRS n° 13,002,294), the French Data Protection Authority (Cnil n° 2013–040), and the Personal Protection Committee (CPP n°2012-A00456-34). All participants provided informed consent.

Inclusion criteria:

- Reside in metropolitan France during the study period.

- Be aged 6 to 74 years old at the time of inclusion.
- Reside in a regular household reachable by a landline geographic phone or a mobile phone.
- Reside at least 4 days per week in their usual residence.
- Be able to understand, over the phone, the implications of participating in the study (hearing, understanding the French language) and be able to give consent.
- Have provided written consent to participate in the entire study, agreeing to complete the questionnaire survey component, including a home presentation visit, a dietary survey, administration of self-questionnaires, a second home visit, and a health examination including biological samples.
- For children aged 6 to 17 years old: written consent for participation is given by both parents of the child (or by their legal representatives).

Exclusion criteria:

- Have a condition that makes it impossible to complete the study (conditions involving enteral or parenteral nutrition, contraindication to blood sampling, etc.).
- Have plans to move within 2 months following inclusion outside the geographical areas of the study.
- Be unavailable (long travel abroad, etc.) during the 2 months following inclusion.

b. Study population

The target population of the Esteban study consists of all individuals residing in metropolitan France aged 6 to 74 years old and living in a regular household during the study period.

Study population for the analysis conducted in this doctoral thesis

For the study conducted within the framework of Esteban study (paper #2, objective 2), a total of 1,279 children and adolescents were included in the analysis. We included for this cross-sectional analysis data from only participants aged 17 and below and who provided at least two 24-h dietary records as a measurement of baseline dietary intake. Under-reporters for energy intake were identified using the Black method and excluded

from the analysis. In addition, data were weighted to account for potential selection bias and for the complex survey design and calibration using the “svyset” procedure in Stata.

c. Exposure assessment

- *NS-NPM underpinning the Nutri-Score*

The scoring for a given food or beverage is determined by assigning points based on its content per 100 g or 100 ml. This algorithm takes into account both positive scoring components and negative components[59], the points from the "favorable" component are subtracted from those of the "unfavorable" component, thus leading to a final score based on a discrete continuous scale going from -15 to +40 points[59]. This final score is then translated into a grade from A to E, corresponding to a color from dark green to dark orange.

- *2023* NS-NPM *underpinning the Nutri-Score update*

The 2023 NS-NPM aimed to integrate the latest scientific evidence and align with the primary food-based dietary guidelines of European countries.

The updated algorithm functions similarly to its predecessor, however some modifications have been considered. These include an increase in the maximal number of points for sugar and salt, resulting in less favorable ratings for foods high in these components. Additionally, stricter criteria were applied to the allocation of points for the fiber and protein components, requiring a higher content of fiber or protein to earn positive points. Specific rules were introduced for red meat, limiting the maximum points it could receive in the positive category. For beverages, the presence of non-nutritive sweeteners was added as an extra unfavorable factor. Finally, the thresholds for converting the final score into a rating were adjusted to align with the new algorithm[61].

METHODOLOGY

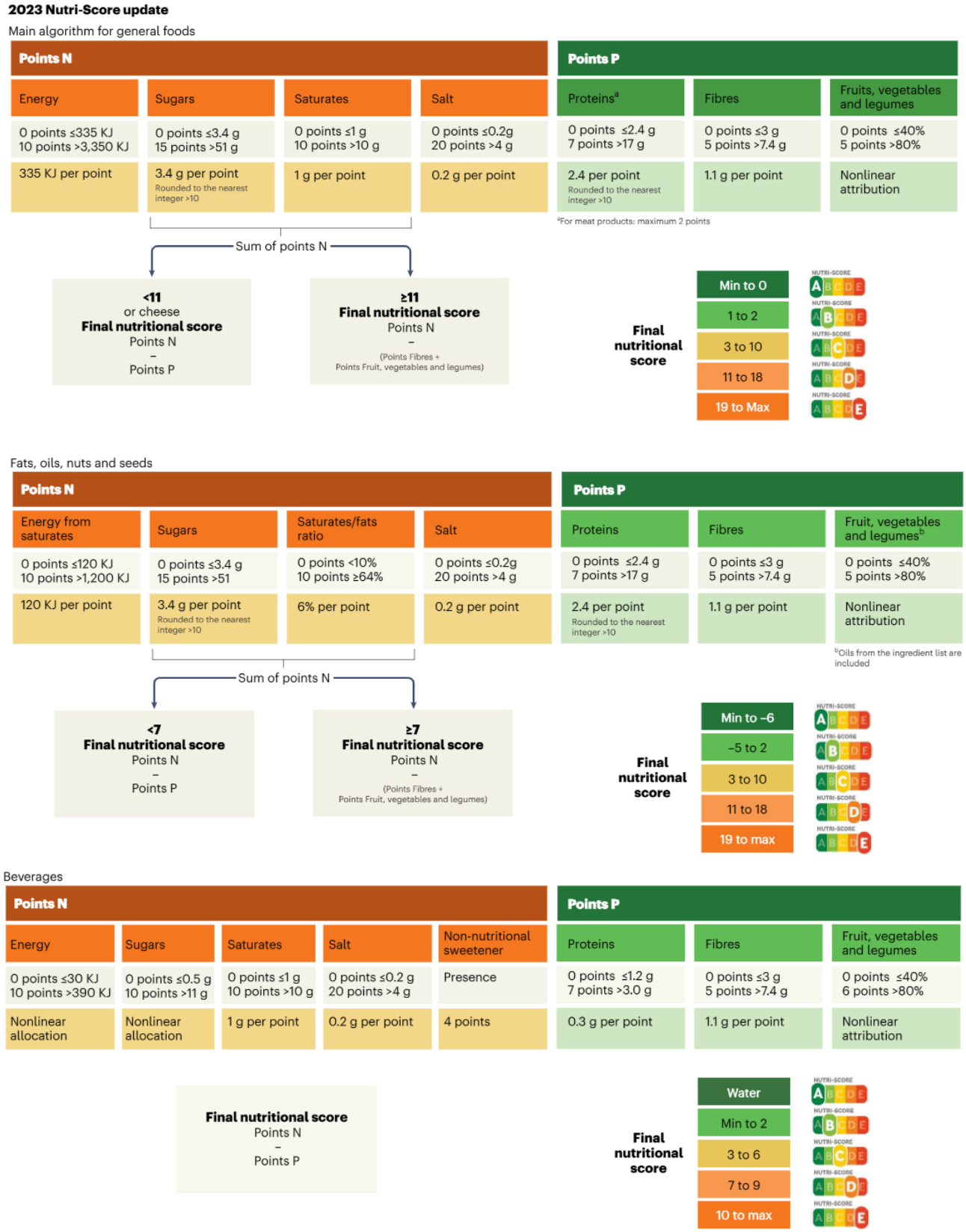


Figure 6. Nutri-Score updated algorithm for general foods, fats, oils, nuts and seeds, and beverages. N points refer to points attributed to unfavourable nutritional elements and P points refer to (negative) points attributed to favourable nutritional elements. Credit: Logo Nutri-Score, Santé publique France 2017. *Source: Merz, B., Temme, E., Alexiou, H. et al. Nutri-Score 2023 update. Nat Food 5, 102–110 (2024). https://doi.org/10.1038/s43016-024-00920-3*

In order to take into account, the differing range of distribution of values of the (2023 or 2015) NS-NPM in foods and beverages, we converted the (2023 or 2015) NS-NPM scores to a standardized scale of 0–100 for foods and beverages respectively. Therefore, a range from the healthiest (0 points), to least healthy (100 points) was used, following this formula[183]:

$$\text{Standardized score} = \frac{Score - Minimum\ possible\ score}{Maximum\ possible\ score - Minimum\ possible\ score} * 100 \quad \text{(Equation 6)}$$

- *Dietary index computation (at the individual level)*

To obtain a dietary score at the individual level, the 2015 and 2023 NS-NPM DI was computed as an energy-weighted mean of the NS-NPM scores of all foods and beverages consumed by each participant, using the following equation:

$$\text{Dietary Index} = \frac{\sum_{i=1}^{n} NS\text{-}NPMi * Ei}{\sum_{i=1}^{n} Ei} \quad \text{(Equation 7)}$$

Where "i" signifies a food or beverage consumed by the participant, "NS-NPMi" represents the score of the food (or beverage), "Ei" signifies the mean daily energy intake from this food (or beverage) and "n" the number of different foods consumed.

Therefore, higher NS-NPM DI scores reflected lower nutritional quality in the total foods consumed. The dietary index reflects the whole diet excluding only alcoholic beverages and herbs/spices. Consequently, both the 2015 and 2023 NS-NPM DI provide a comprehensive representation of the overall dietary patterns.

d. Covariates

Sociodemographic and lifestyle data

Social and demographic data were obtained through face-to-face questionnaires, which covered information about sex, age, educational level and occupational situation of the head of household.

Dietary data

The dietary survey consists of three 24-hour recordings for children aged 6-10 years and three 24-hour recalls for individuals aged 11-74 years, randomly distributed over three weeks. According to European recommendations[184], the days for the 24-hour recalls (two weekdays and one weekend day) are randomly selected, with the two weekday survey days being at least two weeks apart. Participants are not informed in advance of the survey days to minimize alterations to their diet. Participants are asked to describe what they consumed the day before the interview, from midnight to midnight, specifying, for each time of day (main meals and intervals between them, including at night), the nature and quantities of foods and beverages consumed (including water). Dietary consumption is entered into the NutriXpert© software (Medical Expert Systems, Paris). For participants aged 11 and older, the recalls are self-reported, potentially with parental assistance for younger participants, on a dedicated and secure website or collected by telephone by a trained dietitian. In this case, a validated photographic portion manual for the SU.VI.MAX study is provided to the participant by the dietitian investigator during the introductory visit and retrieved during the second home visit[185]. The comparability of dietary consumption data obtained through internet self-reporting and telephone interviews by a dietitian has been previously evaluated and deemed satisfactory[186]. For children aged 6 to 10 years, the recording is self-reported by the parents on a dedicated website or collected in a logbook retrieved at home by the dietitian investigator during a second visit. The quality of the logbooks is verified in the presence of the child's parents to help complete the collected data. Consequently, the average mean dietary intakes from the 24-hours dietary records of each participant were conducted and considered as a baseline usual dietary intake. Amounts consumed from composite dishes were estimated using pre-validated French recipes. Homemade dishes were deconstructed to list the specific ingredients used in their preparation[187]. Mean daily intakes were estimated using a detailed food composition table.

4. General statistical analysis

All the studies included in this doctoral thesis have a cross-sectional or prospective cohort design. Statistical analyses conducted are extensively described in each publication presented in the results section. All the analyses were performed using the statistical software Stata version 14.2 (StataCorp LP).

Briefly,

- To compare differences between exposure categories in the baseline characteristics of the study population, as well as in dietary and lifestyle factors, ANOVA or Pearson chi-square tests were used for continuous and categorical variables, respectively.
- In the cross-sectional analysis, associations between the exposures (in tertiles and continuous) and cardiometabolic outcomes were assessed at baseline using multivariable cox or linear regression models adjusted for potential confounder factors were used.
- In the prospective analysis, associations between the exposures (in tertiles and continuous) and changes or incidence of cardiometabolic outcomes were assessed using multivariable linear or logistic regression models.
- Dietary intake of endocrine disrupting chemicals (PCDD/Fs or PFOS) and the consumption of UPFs were adjusted for energy intake using the residuals method[188].
- Age- and sex-specific z scores of each cardiometabolic outcome were estimated from standardized residuals conducted using linear regression models in the CORALS cohort.
- Simulation model was fitted to substitute 100 g of consumed UPFs with 100 g of unprocessed or minimally processed food to examine the association of healthier food consumption with the outcomes. The theoretical impact of substituting 1 food group for another was assessed by introducing both variables simultaneously as continuous variables into the model.
- To assess the linear trend, it was assigned the median value to each exposure category and the new variables were modeled as continuous.
- Interaction between covariates and exposure variables was tested with likelihood ratio tests, which involved comparing models with and without cross-product terms.

V. RESULTS

UNIVERSITAT ROVIRA I VIRGILI
A MULTI-DIMENSIONAL APPROACH OF FOOD CLASSIFICATIONS AND ITS ASSOCIATION WITH CARDIOMETABOLIC RISK FACTORS
Nadine Alkhoury

RESULTS

Chapter 1: Association between the nutritional dimension of foods assessed with FSAm-NPS dietary index and cardiometabolic risk factors

a. Article 1

Title: Associations Between the Modified Food Standard Agency Nutrient Profiling System Dietary Index and Cardiovascular Risk Factors in an Elderly Population

Nadine Khoury, Clara Gómez-Donoso, María Ángeles Martínez, Miguel Ángel Martínez-González, Dolores Corella, Montserrat Fitó, J Alfredo Martínez, Ángel M Alonso-Gómez, Julia Wärnberg, Jesús Vioque, Dora Romaguera, Ana León-Acuña, Francisco J Tinahones, José M Santos-Lozano, Luís Serra-Majem, Paloma Massó Guijarro, Josep A Tur, Vicente Martín Sánchez, Xavier Pintó, Miguel Delgado-Rodríguez, Pilar Matía-Martín, Josep Vidal, Clotilde Vázquez, Lidia Daimiel, Emili Ros, Maira Bes-Rastrollo, Rocio Barragan, Olga Castañer, Jose D Torres-Peña, Leyre Notario-Barandiaran, Carlos Muñoz-Bravo, Itziar Abete, Lara Prohens, Naomi Cano-Ibáñez, Lucas Tojal Sierra, José Carlos Fernández-García, Carmen Sayon-Orea, Maria Pascual, Jose V Sorli, Dolores Zomeño, Patricia J Peña-Orihuela, Antonio J Signes-Pastor, F Javier Basterra-Gortari, Helmut Schröeder, Jordi Salas Salvadó, Nancy Babio

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Summary:

Compared to participants with a higher nutritional quality of diet (measured by a lower FSAm-NPS DI at baseline or a decrease in FSAm-NPS DI after 1 year), those participants with a lower nutritional quality of diet (higher FSAm-NPS DI or an increase in score) showed a significant increase in the levels of plasma glucose, triglycerides, diastolic blood pressure, BMI, and waist circumference (β coefficient [95% confidence interval]; P for trend) (1.67 [0.43, 2.90]; <0.001; 6.27 [2.46, 10.09]; <0.001; 0.56 [0.08, 1.05]; 0.001; 0.51 [0.41, 0.60]; <0.001; 1.19 [0.89, 1.50]; <0.001, respectively). No significant associations in relation to changes in HDL and LDL-cholesterol nor with systolic blood pressure were shown.

Conclusion: This prospective cohort study suggests that the consumption of food items with a higher FSAm-NPS DI is associated with increased levels of several major risk factors for CVD including adiposity, fasting plasma glucose, triglycerides, and diastolic blood pressure. However, results must be cautiously interpreted because no significant prospective associations were identified for critical CVD risk factors, such as HDL and LDL-cholesterol, and systolic blood pressure.

RESULTS

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ORIGINAL RESEARCH
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Associations Between the Modified Food Standard Agency Nutrient Profiling System Dietary Index and Cardiovascular Risk Factors in an Elderly Population

Nadine Khoury[1,2†], Clara Gómez-Donoso[1,2†], María Ángeles Martínez[1,2,3], Miguel Ángel Martínez-González[3,4,5], Dolores Corella[3,6], Montserrat Fitó[3,7], J. Alfredo Martínez[3,8,9], Ángel M. Alonso-Gómez[3,10], Julia Wärnberg[3,11], Jesús Vioque[12,13], Dora Romaguera[3,14], Ana León-Acuña[3,15], Francisco J. Tinahones[3,16], José M. Santos-Lozano[3,17], Luís Serra-Majem[3,18], Paloma Massó Guijarro[19,20,21], Josep A. Tur[3,22], Vicente Martín Sánchez[12,23], Xavier Pintó[3,24], Miguel Delgado-Rodríguez[9,25], Pilar Matía-Martín[26], Josep Vidal[27,28], Clotilde Vázquez[3,29], Lidia Daimiel[30], Emili Ros[3,31], Maira Bes-Rastrollo[3,4], Rocio Barragan[3,6], Olga Castañer[3,7], Jose D. Torres-Peña[3,15], Leyre Notario-Barandiaran[13], Carlos Muñoz-Bravo[11], Itziar Abete[3,8,9], Lara Prohens[3,14], Naomi Cano-Ibáñez[12,19,21], Lucas Tojal Sierra[3,10], José Carlos Fernández-García[3,16], Carmen Sayon-Orea[3,4], Maria Pascual[1,2], Jose V. Sorlí[3,6], Dolores Zomeño[3,7,32], Patricia J. Peña-Orihuela[3,15], Antonio J. Signes-Pastor[12,13], F. Javier Basterra-Gortari[4,33], Helmut Schröeder[3,7], Jordi Salas Salvadó[1,2,3‡] and Nancy Babio[1,2,3*‡]*

[1] Universitat Rovira i Virgili, Departament de Bioquímica i Biotecnologia, Unitat de Nutrició Humana, Reus, Spain, [2] Institut d'Investigació Sanitària Pere Virgili (IISPV), Reus, Spain, [3] Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y la Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain, [4] Department of Preventive Medicine and Public Health, IDISNA, University of Navarra, Pamplona, Spain, [5] Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA, United States, [6] Department of Preventive Medicine, University of Valencia, Valencia, Spain, [7] Unit of Cardiovascular Risk and Nutrition, Institut Hospital del Mar de Investigaciones Médicas Municipal d'Investigació Médica (IMIM), Barcelona, Spain, [8] Department of Nutrition, Food Sciences, and Physiology, Center for Nutrition Research, University of Navarra, Pamplona, Spain, [9] Precision Nutrition and Cardiometabolic Health Program, IMDEA Food, CEI UAM + CSIC, Madrid, Spain, [10] Osakidetza Basque Health Service, Araba University Hospital, Bioaraba Health Research Institute, University of the Basque Country UPV/EHU, Vitoria-Gasteiz, Spain, [11] Department of Public Health and Psychiatry, Biomedical Research Institute of Malaga (IBIMA), University of Malaga, Málaga, Spain, [12] CIBER de Epidemiología y Salud Pública (CIBERESP), Instituto de Salud Carlos III, Madrid, Spain, [13] Instituto de Investigación Sanitaria y Biomédica de Alicante, Universidad Miguel Hernández (ISABIAL-UMH), Alicante, Spain, [14] Health Research Institute of the Balearic Islands (IdISBa), Palma de Mallorca, Spain, [15] Department of Internal Medicine, Maimonides Biomedical Research Institute of Cordoba (IMIBIC), Reina Sofia University Hospital, University of Córdoba, Córdoba, Spain, [16] Department of Endocrinology, Virgen de la Victoria Hospital, Instituto de Investigación Biomédica de Málaga (IBIMA), University of Málaga, Málaga, Spain, [17] Research Unit, Department of Family Medicine, Distrito Sanitario Atención Primaria Sevilla, Seville, Spain, [18] Research Institute of Biomedical and Health Sciences (IUIBS), University of Las Palmas de Gran Canaria and Centro Hospitalario Universitario Insular Materno Infantil (CHUIMI), Canarian Health Service, Las Palmas de Gran Canaria, Spain, [19] Department of Preventive Medicine and Public Health, University of Granada, Granada, Spain, [20] Preventive Medicine Unit, Universitary Hospital Virgen de las Nieves, Granada, Spain, [21] Instituto de Investigación Biosanitaria de Granada (ibs.GRANADA), Granada, Spain, [22] Research Group on Community Nutrition and Oxidative Stress, University of Balearic Islands-IUNICS, Palma de Mallorca, Spain, [23] Institute of Biomedicine (IBIOMED), University of León, León, Spain, [24] Lipids and Vascular Risk Unit, Internal Medicine, Hospital Universitario de Bellvitge, Hospitalet de Llobregat, Barcelona, Spain, [25] Division of Preventive Medicine, Faculty of Medicine, University of Jaén, Jaén, Spain, [26] Department of Endocrinology and Nutrition, Instituto de Investigación Sanitaria Hospital Clínico San Carlos (IdISSC), Madrid, Spain, [27] CIBER Diabetes y Enfermedades Metabólicas (CIBERDEM), Instituto de Salud Carlos III (ISCIII), Madrid, Spain, [28] Department of Endocrinology, Institut d'Investigacions Biomédiques August Pi Sunyer (IDIBAPS), Hospital Clínic, University*

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Xiongfei Pan, Sichuan University, China

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***Correspondence:**
Nancy Babio
nancy.babio@urv.cat
Jordi Salas Salvadó
jordi.salas@urv.cat

†These authors have contributed equally to this work

‡These authors share senior authorship

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of Barcelona, Barcelona, Spain, [29] Department of Endocrinology and Nutrition, Hospital Fundación Jimenez Díaz, Instituto de Investigaciones Biomédicas IISFJD, University Autonoma, Madrid, Spain, [30] Nutritional Control of the Epigenome Group, Precision Nutrition and Obesity Program, IMDEA Food, CEI UAM + CSIC, Madrid, Spain, [31] Department of Endocrinology and Nutrition, Institut d'Investigacions Biomèdiques August Pi Sunyer (IDIBAPS), Hospital Clínic, Lipid Clinic, Barcelona, Spain, [32] School of Health Sciences, Blanquerna-Ramon Llull University, Barcelona, Spain, [33] Department of Endocrinology and Nutrition, Hospital Universitario de Navarra, Pamplona, Spain

Background: Helping consumers to improve the nutritional quality of their diet is a key public health action to prevent cardiovascular diseases (CVDs). The modified version of the Food Standard Agency Nutrient Profiling System Dietary Index (FSAm-NPS DI) underpinning the Nutri-Score front-of-pack label has been used in public health strategies to address the deleterious consequences of poor diets. This study aimed to assess the association between the FSAm-NPS DI and some CVD risk factors including body mass index (BMI), waist circumference, plasma glucose levels, triglyceride levels, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, and diastolic and systolic blood pressure.

Materials and Methods: Dietary intake was assessed at baseline and after 1 year of follow-up using a 143-item validated semi-quantitative food-frequency questionnaire. Dietary indices based on FSAm-NPS applied at an individual level were computed to characterize the diet quality of 5,921 participants aged 55–75 years with overweight/obesity and metabolic syndrome from the PREDIMED-plus cohort. Associations between the FSAm-NPS DI and CVD risk factors were assessed using linear regression models.

Results: Compared to participants with a higher nutritional quality of diet (measured by a lower FSAm-NPS DI at baseline or a decrease in FSAm-NPS DI after 1 year), those participants with a lower nutritional quality of diet (higher FSAm-NPS DI or an increase in score) showed a significant increase in the levels of plasma glucose, triglycerides, diastolic blood pressure, BMI, and waist circumference (β coefficient [95% confidence interval]; *P* for trend) (1.67 [0.43, 2.90]; <0.001; 6.27 [2.46, 10.09]; <0.001; 0.56 [0.08, 1.05]; 0.001; 0.51 [0.41, 0.60]; <0.001; 1.19 [0.89, 1.50]; <0.001, respectively). No significant associations in relation to changes in HDL and LDL-cholesterol nor with systolic blood pressure were shown.

Conclusion: This prospective cohort study suggests that the consumption of food items with a higher FSAm-NPS DI is associated with increased levels of several major risk factors for CVD including adiposity, fasting plasma glucose, triglycerides, and diastolic blood pressure. However, results must be cautiously interpreted because no significant prospective associations were identified for critical CVD risk factors, such as HDL and LDL-cholesterol, and systolic blood pressure.

Keywords: front of pack food labeling, cardiovascular risk factor, body weight, FSAm-NPS dietary index, PREDIMED-Plus study

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of mortality and is considered a major global public health problem (1, 2). According to the estimation in 2019, CVD burden was found to be responsible for 17.9 million deaths worldwide, accounting for approximately 32% of all global deaths, representing a huge economic and social cost (3).

A healthy diet is recognized as a lever for public health by using a modifiable determinant of CVD and other chronic diseases that can be addressed through primary prevention interventions (4). In contrast, an unhealthy diet characterized by an excess of energy, added sugar, salt and saturated fats, and a

lack of fruits, vegetables, and fibers has been recognized as an important causal CVD risk factor through the modulation of adiposity and other cardiometabolic risk factors (5).

Over the past decade, different strategies have been proposed to increase adherence to a healthy diet and reduce the risk of CVD. One of the recent initiatives in this regard is the adoption of front-of-pack (FOP) nutrition labels (6). Although labels on the back of the pack are already mandatory in all European countries according to RE 1169/2011 (7), there is evidence that this information is not easily understandable by consumers. In contrast, nutrition labels found on the front of pack of products are considered more helpful and efficient for consumers since nutritional information is summarized and available at a glance (8, 9). FOP labeling aims to help consumers make healthier choices at the point of purchase and to incentivize food manufacturers to reduce the content of nutrients that might compromise diet quality (e.g., salt, saturated fatty acids, and sugar) and/or increase the content of beneficial nutrients (e.g., fibers and vitamins) (10, 11).

Front-of-pack nutrition labels reflect the nutritional quality of food using a nutrient profiling system (NPS) (12). Nutrient profiling is widely used to support public health initiatives to promote healthy eating (12). Among the available nutrient profiling systems, the NPS developed by the UK Food Standard Agency (FSA-NPS) has been consistently validated in Europe (13). This system was originally developed to discriminate foods based on their nutritional composition in the context of television commercials targeting children (14). In France, after some modifications of the FSA-NPS by the French High Council for Public Health (Haut Conseil de la Santé Publique, HCSP) and demonstrating its applicability within the French context, the modified FSA-NPS (FSAm-NPS) was established and a dietary index (DI) based on the FSAm-NPS (FSAm-NPS DI) was developed to validate the algorithm underlying the Nutri-Score FOP label (15, 16). The score for a given food or beverage is calculated by allocating points for the content per 100 g or 100 ml. This algorithm considers both positive scoring components (protein, fiber, percentage of fruit, vegetables, legumes, nuts, rapeseed oil, walnut oil, and olive oil) and negative components (energy, sugars, saturated fatty acids, and salt) (17), and has been proved effective to rank products by nutritional quality and improve food purchases in real-life grocery shopping settings (18).

In addition, several large European prospective studies have shown associations between a higher FSAm-NPS (lower nutritional quality) and an increased risk of cancer (19), CVD (20), cancer mortality (21), CVD mortality (22), and all-cause mortality (23). In contrast, a lower FSAm-NPS (better nutritional quality) has been associated with a lower risk of long-term weight gain and metabolic syndrome incidence in the SU.VI.MAX French cohort study (24, 25). However, there is less information available about the association between the FSAm-NPS DI and CVD risk factors. The aim of this study was to evaluate the association between the FSAm-NPS DI and different CVD risk factors including body mass index (BMI), waist circumference, glucose, triglycerides, HDL- and LDL-cholesterol levels, as well as diastolic and systolic blood pressure in elderly adults with overweight/obesity and metabolic syndrome.

MATERIALS AND METHODS

Study Population and Design: The PREDIMED Plus Study

A prospective analysis was conducted using the data of the PREDIMED-Plus (PREvención con DIeta MEDiterránea) cohort. The PREDIMED-Plus trial is an ongoing randomized, controlled trial conducted in 23 Spanish centers that aims to compare the effect of an intensive weight loss intervention (based on an energy-reduced Mediterranean diet, physical activity promotion, and behavioral support) on the incidence of CVD events, to a control group that receives usual care advice and non-caloric reduced Mediterranean diet recommendations. A detailed description of the PREDIMED-Plus study is available at https://www.predimedplus.com. Between October 2013 and December 2016, 6,874 participants were recruited in Spain and were randomly assigned to either an intensive lifestyle intervention or standard medical care in a 1:1 ratio. Eligible participants were overweight or obese (BMI 27–40 kg/m^2) men and women aged 55–75 years who met at least three metabolic syndrome criteria as follows: waist circumference > 102 cm in men and >88 cm in women; serum triglycerides ≥ 150 mg/dl or drug treatment for elevated triglycerides; HDL-c < 40 mg/dl in men and <50 mg/dl in women or drug treatment for low HDL-cholesterol; blood pressure ≥ 130/85 mmHg or antihypertensive drug treatment; and fasting plasma glucose level ≥ 100 mg/dl or hypoglycemic treatment and without documented history of CVD. Extensive descriptions of inclusion and exclusion criteria can be found elsewhere (26). All subjects gave written informed consent, and the final protocol and methods were approved by the institutional review boards of each participating center. A total of 953 participants who did not complete a food frequency questionnaire (FFQ) at baseline and after 1 year of follow-up or with total calorie intake outside the pre-specified energy limits (women < 500 and >3,500 kcal/day, and men < 800 and >4,000 kcal/day) were excluded from the analyses (27) **Figure 1**.

Dietary Intake Assessment

Dietary intake was assessed at baseline and after 1 year of follow-up using a validated FFQ administered face-to-face by trained dietitians (27). The participants reported their average consumption in terms of frequency and quantity for 143 food and beverage items during the previous year. The frequency of consumption was shown through nine categories ranging from never or almost never to more than six times a day, and a commonly used portion size was specified (e.g., slices, glass, and teaspoons) to indicate serving sizes for each of the items.

The nutrient intake was calculated as the sum of the frequency of daily consumption of each item multiplied by the nutrient composition of the specified serving size according to the Spanish food composition database (28). The total energy intake was

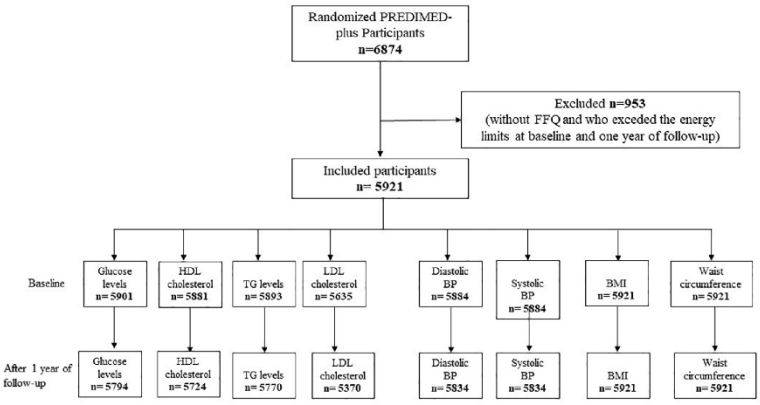


FIGURE 1 | Flow chart of participant's inclusion.

also estimated from the quantity and frequency of food and beverage consumption.

Dietary Index Based on the French Context, the Modified FSA-NPS Computation

The FSAm-NPS is a modified version of the original FSA-NPS (29). Modifications made by the French National Nutrition and Health Program and the French High Council for Public Health impacted the score standards for cheese, added fats, and beverages. For all foods and beverages in the PREDIMED-plus FFQ, the FSAm-NPS was computed per 100 g of the product as follows: 0–40 points was given for the content of critical nutrients that should be limited (0–10 points for each: sugars, saturated fats, sodium, and energy), and 0–15 points was given for the content of beneficial nutrients that should be encouraged (0–5 points for each: fibers, proteins, and the percentage of fruits, vegetables, legumes, nuts, rapeseeds, walnuts, and olive oil that make up the total product). The total score was calculated by subtracting the "negative" (nutrients to avoid) scores from the "positive" (nutrients to promote) scores. As a result, the final FSAm-NPS for each food/beverage was calculated using a discrete continuous scale with a theoretical range of −15 (healthiest) to 40 (least healthy). To generate a dietary index at the individual level, the FSAm-NPS DI was calculated for each participant accounting for their whole diet through energy-weighted means of the FSAm-NPS of all foods and beverages consumed with the following equation:

$$\text{Dietary Index} = \frac{\sum_{i=1}^{n} FSiEi}{\sum_{i=1}^{n} Ei} \quad (1)$$

where "i" signifies a food or beverage consumed by the participant, "FSi" the food (or beverage) score, "Ei", the mean daily energy intake from this food (or beverage), and "n" the number of different foods.

Therefore, a higher FSAm-NPS-DI reflects lower nutritional quality of the individual's overall diet.

Outcome (Cardiovascular Diseases Risk Factors) Assessment at Baseline and After 1 Year of Follow-Up

Qualified PREDIMED-Plus staff followed the study protocol to measure anthropometric variables and blood pressure. CVD risk factors such as glucose levels, triglyceride levels, HDL-cholesterol, LDL-cholesterol, diastolic and systolic blood pressure, BMI, and waist circumference were assessed at baseline and after 1 year of follow-up.

Waist Circumference

It was measured midway between the lowest rib and the iliac crest using an anthropometric tape, and body weight was measured twice using high-quality electronic calibrated scales and height was measured twice using a wall-mounted stadiometer.

Body Mass Index

It was computed by dividing the weight in kilograms by the square of height in meters.

Systolic and Diastolic Blood Pressure

They were measured three times using a validated semiautomatic oscillometer (Omron HEM-705CP, Kyoto, Japan) and the mean of repeated measures was used.

Fasting Plasma Levels of Glucose, Total Cholesterol, High-Density Lipoprotein-Cholesterol, and Triglycerides

They were measured using standard enzymatic methods. LDL-cholesterol was calculated using the Friedewald formula (whenever triglycerides were less than 300 mg/dl and whenever triglycerides were more than 300 mg/dl, LDL-cholesterol was calculated with the standard method).

Assessment of Covariates

Covariates were assessed by trained staff in a face-to face interview using self-reported general questionnaires that collect information on socio-demographics (sex, age, marital status, and level of education), lifestyle (smoking habits and physical activity), and medication use. Leisure time physical activity was estimated using the validated Minnesota-REGICOR Short Physical Activity questionnaire (30).

Statistical Analyses

For our analyses, we used the PREDIMED-Plus database updated in December 2020. Analysis of descriptive baseline characteristics of sociodemographic, dietary, and biomedical variables was carried out and reported as means ± SD or median [P25–P75] and percentages (number) for continuous and categorical variables, respectively.

Participants were categorized by tertiles of the FSAm-NPS DI, ranging from tertile 1 (T1) for the best nutritional quality to tertile 3 (T3) for the lowest nutritional quality. The Chi-square test for categorical variables and one-way ANOVA for continuous variables were used to compare the baseline characteristics between the tertiles.

Linear regression models were fitted to assess the associations [β-coefficient (95% confidence interval (CI)] between FSAm-NPS DI (in tertiles and as continuous variables) and CVD risk factors at baseline. We also used linear regression models to explore the prospective associations between changes in the FSAm-NPS DI (in tertiles and as continuous variable) and changes in CVD risk factors after 1 year of follow-up. Changes in the FSAm-NPS-DI were calculated by subtracting 1 year from the baseline values. Missing data after 1 year of follow-up for BMI (four participants) and diastolic BP (one participant) were imputed using the mean value method.

Three models were fitted as follows: crude model, model 1, and the fully adjusted model. Model 1 was adjusted for age (years), sex, BMI (kg/m^2), educational level (primary or lower, secondary or academic, or graduate), smoking habit (never, former, or current), total energy consumption (kcal/day), physical activity (METs. min/week), and marital status (married, widowed, single or divorced or separated, or religious). The fully adjusted model was further adjusted for medication for the treatment of hypercholesterolemia, hypertension and diabetes, size of the recruitment centers (<250, 250 to <300, 300 to <400, ≥400), and intervention group. In the prospective analysis, each CVD risk factor was adjusted for its baseline level (in model 1 and fully adjusted).

To indirectly assess the effect of olive oil on the associations with cardiovascular risk factors, we conducted a sensitivity analysis where olive oil was valued −8 (the healthiest value in the score, such as for fruits, vegetables, or legumes) in the computation of the FSAm-NPS-DI.

The statistical significance threshold for the results was set at $p < 0.05$. All analyses were conducted with robust estimates of the variance to correct for intra-cluster correlation and using the Stata 14 software program (StataCorp).

RESULTS

A total of 5,921 participants (52% men and 48% women, with the mean age of 65 years) were included in this study. Baseline characteristics of the participants overall and according to the tertiles of the FSAm-NPS are shown in **Table 1**. Participants with a higher FSAm-NPS DI were more likely to be men of younger age, to have a higher level of education, to smoke, to be less physically active, and to have a higher alcohol intake. As expected, participants with a lower FSAm-NPS DI, reflecting a higher nutritional quality (Tertile 1), consumed higher amounts of vegetables, fruits, legumes, unrefined cereals, dairy products, white meat, fish and shellfish, nuts, and fiber. In contrast, participants with a higher FSAm-NPS DI (Tertile 2 and 3), reflecting a lower nutritional quality, consumed higher amounts of refined cereals, biscuits, red meat and processed meat, olive oil, alcohol, and sugar, and showed higher intakes of total energy and saturated fat. In relation to biochemical parameters, the participants in the higher tertile of the FSAm-NPS DI showed higher serum concentrations of triglycerides and LDL-cholesterol and lower levels of HDL-cholesterol (**Table 1**).

The cross-sectional association between the FSAm-NPS DI and CVD risk factors at baseline is shown in **Table 2**. Compared to those participants with a lower FSAm-NPS DI (T1) reflecting a healthier diet, those with a higher FSAm-NPS DI (T3) showed lower HDL-cholesterol levels (β: −0.77 [95% CI = −1.47, −0.06]; *P* for trend = 0.027). This association was consistent for the HDL-cholesterol and FSAm-NPS DI as a continuous variable (β: −0.21 [95% CI = −0.37, −0.05]). A direct association was observed between the FSAm-NPS DI as continuous and baseline BMI and waist circumference (β: 0.08 [95% CI = 0.03, 0.13] and β: 0.22 [95% CI = 0.09, 0.35], respectively).

Associations between 1 year changes in the FSAm-NPS DI and changes in the CVD risk factors are shown in **Table 3**. Compared to participants with a score change resulting in a lower FSAm-NPS DI (T1), those participants with a higher FSAm-NPS DI (T3) after 1 year of follow-up showed a significant increase in the levels of plasma glucose, triglycerides, diastolic blood pressure, BMI, and waist circumference (β: 1.67 [95% CI = 0.43, 2.90]; *P* for trend < 0.001; β: 6.27 [95% CI = 2.46, 10.09]; *P* for trend < 0.001; β: 0.56 [95% CI = 0.08, 1.05]; *P* for trend = 0.001; β: 0.51 [95% CI = 0.41, 0.60]; *P* for trend < 0.001; β: 1.19 [CI = 0.89, 1.50]; *P* for trend < 0.001, respectively).

In **Figure 2**, we present the β coefficient (95% CI) for the prospective associations between changes in the FSAm/NPS DI (continuous) and changes in the CVD risk factors after 1 year of follow-up. Consistent with the results described

RESULTS

TABLE 1 | Baseline characteristics of participants overall and by tertiles of FSAm-NPS DI score, PREDIMED-plus study.

	All	Tertiles of the FSAm-NPS DI			
	n = 5,921	T1 Higher quality *n* = 1,974	T2 Moderate quality *n* = 1,974	T3 Lower quality *n* = 1,973	*P*-value
FSAm-NPS DI range	1.51–16.6	1.51–7.37	7.38–8.87	8.88–16.6	<0.001
Sex					
Men	51.7 (3,065)	44.0 (869)	51.3 (1,012)	60.0 (1,184)	<0.001
Women	48.3 [2,856)	56 (1,105)	48.7 (962)	40.0 (789)	
Age (years)	65.0 ± 4.89	65.6 ± 4.78	65 ± 4.87	64.5 ± 4.95	<0.001
Marital status					
Married	76.8 (4,545)	74.7 (1,475)	77 (1,518)	78.7 (1,552)	0.005
Widowed	10.4 (616)	12.5 (245)	10.4 (206)	8.36 (165)	
Single/divorced/separated/Religious	12.5 (741)	12.6 (249)	12.3 (242)	12.7 (250)	
Educational level					
Up to primary	49.7 (2,942)	54.1 (1,066)	50 (986)	45.1 (890)	<0.001
Secondary	28.7 (1,701)	27 (531)	28.2 (557)	31.1 (613)	
University	21.6 (1,278)	19.1 (377)	21.8 (431)	23.9 (470)	
Smoking status					
Never	44.7 (2,646)	50.7 (1,000)	44.4 (877)	40 (769)	<0.001
Current	12.4 (734)	9.02 (178)	13.2 (260)	15.0 (296)	
Former	42.9 (2,541)	40.3 (796)	42.4 (837)	46.0 (908)	
Physical activity (MET min/week)	2,508 ± 2,322	2,648 ± 2,408	2,469 ± 2,207	2,411 ± 2,342	0.045
Waist circumference (cm)					
Men	111 ± 8.72	110 ± 8.6	111 ± 8.4	111 ± 9.0	0.201
Women	104 ± 9.2	103 ± 9.2	104 ± 9.1	104 ± 9.1	0.12
BMI (kg/m^2)	32.4 ± 3.44	32.4 ± 3.43	32.5 ± 3.41	32.5 ± 3.46	0.376
Dietary assessment					
Vegetables (g/day)	329 ± 137	372 ± 147	331 ± 129	283 ± 120	<0.001
Fruits (g/day)	359 ± 205	419 ± 226	361 ± 194	298 ± 171	<0.001
Legumes (g/day)	20.6 ± 11.0	22.8 ± 12.3	20.5 ± 10.9	18.3 ± 9.21	<0.001
Total cereals (g/day)	151 ± 78	148 ± 79	155 ± 79	149 ± 76	0.006
Refined cereals (g/day)	110 ± 87.8	104 ± 91.0	112 ± 88.7	112 ± 83.3	0.001
Unrefined cereals (g/day)	41.1 ± 63.4	43.88 ± 60.97	42.7 ± 66.3	36.6 ± 62.6	0.001
Biscuits (g/day)	26.8 ± 29.7	13.3 ± 15.1	23.6 ± 21	43.5 ± 38.8	<0.001
Dairy products (g/day)	344 ± 200	385 ± 217	334 ± 189	314 ± 187	<0.001
Red meat and derivatives (g/day)	82.3 ± 44.8	76.93 ± 43.57	83.1 ± 43.46	86.9 ± 46.8	<0.001
White meat intake (g/day)	62 ± 34	67.7 ± 36.3	62.6 ± 33.5	55.4 ± 31.2	<0.001
Fish and shellfish intake (g/day)	102 ± 47.4	113 ± 50.6	102 ± 44.8	91.4 ± 43.9	<0.001
Nuts intake (g/day)	15 ± 17	21.2 ± 21.6	14 ± 14.4	9.99 ± 11.4	<0.001
Total olive oil (g/day)	40.1 ± 16.8	33.4 ± 15.2	42.3 ± 15.6	44.7 ± 17.2	<0.001
Refined olive oil (g/day)	8.15 ± 15.1	6.22 ± 12.1	7.96 ± 15.1	10.3 ± 17.4	<0.001
Virgin olive oil (g/day)	31.9 ± 20.7	27.2 ± 18.2	34.3 ± 20.6	34.4 ± 22.2	<0.001
Alcohol (g/day)	11.2 ± 15.1	8.92 ± 13.42	10.9 ± 14.3	13.7 ± 17	<0.001
Dietary fiber intake (g/day)	26.1 ± 8.72	28.7 ± 8.94	26.2 ± 8.53	23.5 ± 7.88	<0.001
Ultra-processed food (g/day)	158 ± 158	107 ± 117	148 ± 137	218 ± 186	<0.001
Total sugar intake (g/day)	6.73 ± 12	4.32 ± 8.76	6.67 ± 11.7	9.21 ± 14.3	<0.001
Total energy (kcal/day)	2,367 ± 550	2,207 ± 519	2,363 ± 521	2,531 ± 560	<0.001
Saturated fat intake (g/day)	26.2 ± 8.45	22.2 ± 6.83	26.0 ± 7.30	30.5 ± 8.93	<0.001
Sodium intake (mg/day)	2,426 ± 773	2,340 ± 770	2,427 ± 756	2,509 ± 784	<0.001
Biomedical parameters					
Glucose levels, mg/dL (*n* ± 5,901)	113 ± 29	114 ± 30.2	113 ± 28.7	113 ± 27.9	0.28
TG levels, mg/dL (*n* ± 5,893)	135 [102–178]	136 [102–177]	133 [100–179]	137 [106–178]	0.011
HDL-cholesterol, mg/dL (*n* ± 5,881)	47 [40–55]	48 [41–56]	47 [41–55]	45 [39–53]	<0.001
LDL-cholesterol, mg/dL (*n* ± 5,635)	117 [96–140]	115 [94–140]	118 [97–140]	118 [98–141]	0.028
Systolic BP, mmHg (*n* ± 5,884)	139 ± 16.9	139 ± 16.9	140 ± 17.24	140 ± 16.45	0.119
Diastolic BP, mmHg (*n* ± 5,884)	80.7 ± 9.88	80.4 ± 9.49	80.8 ± 10.14	82 ± 10	0.209

FSAm-NPS, Food Standard's Agency Nutrient Profiling System (modified version) Dietary Index; BMI, body mass index; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; BP, blood pressure. Data were expressed as means ± SD or median [P25–P75] and percentages (number) for continuous and categorical variables, respectively. P-values for comparisons were tested by the one-way ANOVA or chi-square test, as appropriate according to the FSAm-NPS tertiles.

RESULTS

TABLE 2 | Association between FSAm-NPS DI and CVD risk factors at baseline, and β coefficient (95% CI).

	Tertiles of the FSAm-NPS DI			R-squared	*P*-trend	FSAm-NPS DI at baseline (continuous)
	T1	T2	T3			
Glucose levels (mg/dL)						
n	1,967	1,967	1,967	0.04	0.177	5,901
Crude Model	*Ref*	−1.31 (−3.14, 0.53)	−1.25 (−3.07, 0.57)	1.84	0.059	−0.21 (−0.63, 0.20)
Model 1	*Ref*	−1.57 (−3.40, 0.27)	−1.80 (−3.68, 0.07)	30.5	0.832	−0.36 (−0.79, 0.07)
Fully adjusted	*Ref*	−0.24 (−1.80, 1.32)	0.16 (−1.41, 1.73)			0.11 (−0.25, 0.47)
HDL-cholesterol (mg/dL)						
n	1,961	1,960	1,960	0.5	<0.001	5,897
Crude Model	*Ref*	−0.52 (−1.25, 0.22)	−1.98 (−2.73, −1.24)	15.4	0.128	−0.49 (−0.66, −0.33)
Model 1	*Ref*	0.19 (−0.49, 0.88)	−0.52 (−1.24, 0.19)	0.17	0.027	−0.15 (−0.31, 0.01)
Fully adjusted	*Ref*	−0.01 (−0.69, 0.67)	−0.77 (−1.47, −0.06)			−0.21 (−0.37, −0.05)
TG levels (mg/dL)						
n	1,965	1,964	1,964	0.15	0.004	5,893
Crude Model	*Ref*	1.89 (−2.77, 6.55)	7.15 (2.35, 11.95)	2.88	0.387	1.46 (0.44, 2.49)
Model 1	*Ref*	−0.94 (−5.62, 3.74)	1.96 (−2.97, 6.89)	3.35	0.238	0.19 (−0.89, 1.26)
Fully adjusted	*Ref*	−0.36 (−5.04, 4.31)	2.75 (−2.18, 7.68)			0.36 (−0.72, 1.44)
LDL-cholesterol (mg/dL)						
n	1,879	1,878	1,878	0.12	0.027	5,635
Crude Model	*Ref*	2.53 (0.45, 4.61)	2.33 (0.26, 4.39)	4.04	<0.001	0.44 (−0.01, 0.89)
Model	*Ref*	3.12 (1.06, 5.17)	3.66 (1.57, 5.75)	26	0.052	0.75 (0.29, 1.22)
Fully adjusted	*Ref*	1.59 (−0.23, 3.41)	1.76 (−0.05, 3.57)			0.35 (−0.05, 0.75)
Diastolic BP (mmHg)						
n	1,962	1,961	1,961	0.05	0.076	5,884
Crude Model	*Ref*	0.39 (−0.23, 1.01)	0.55 (−0.06, 1.16)	6.52	0.18	0.16 (0.02, 0.30)
Model 1	*Ref*	−0.06 (−0.67, 0.55)	−0.41 (−1.02, 0.20)	7.58	0.063	−0.08 (−0.22, 0.06)
Fully adjusted	*Ref*	−0.14 (−0.75, 0.47)	−0.57 (−1.18, 0.04)			−0.11 (−0.25, 0.03)
Systolic BP (mmHg)						
n	1,962	1,961	1,961	0.07	0.059	5,884
Crude Model	*Ref*	0.87 (−0.20, 1.95)	1.01 (−0.04, 2.06)	3.36	0.159	0.18 (−0.05, 0.42)
Model 1	*Ref*	0.83 (−0.24, 1.90)	0.79 (−0.28, 1.87)	5.29	0.201	0.12 (−0.13, 0.36)
Fully adjusted	*Ref*	0.50 (−0.56, 1.56)	0.72 (−0.35, 1.78)			0.10 (−0.15, 0.34)
BMI (kg/m^2)						
n	1,974	1,974	1,973	0.03	0.17	5,921
Crude Model	*Ref*	0.10 (−0.12, 0.31)	0.15 (−0.07, 0.37)	3.18	0.063	0.06 (0.01, 0.11)
Model 1	*Ref*	0.12 (−0.09, 0.33)	0.22 (−0.01, 0.44)	4.27	0.054	0.08 (0.03, 0.13)
Fully adjusted	*Ref*	0.13 (−0.08, 0.34)	0.22 (0.00, 0.44)			0.08 (0.03, 0.13)
Waist circumference (cm)						
n	1,979	1,979	1,979	0.06	<0.001	5,921
Crude Model	*Ref*	0.70 (0.10, 1.29)	1.87 (1.27, 2.47)	65.4	0.803	0.51 (0.38, 0.65)
Model 1	*Ref*	−0.21 (−0.57, 0.15)	0.04 (−0.32, 0.41)	17	0.381	0.03 (−0.05, 0.11)
Fully adjusted	*Ref*	0.12 (−0.44, 0.67)	0.60 (0.03, 1.18)			0.22 (0.09, 0.35)

FSAm-NPS, Food Standards Agency Nutrient Profiling System (modified version) Dietary Index; CVD, cardiovascular disease; CI, confidence interval; HDL, high-density lipoprotein; TG, triglycerides; LDL, low-density lipoprotein; BP, blood pressure; BMI, body mass index. Linear regression models were fitted. Model 1: adjusted for age, sex, physical activity, smoking status, BMI, total energy intake at baseline, education level, and marital status. Fully adjusted: Model 1 additionally adjusted for medication for treatment of hypercholesterolemia, of hypertension and of diabetes and size of the recruitment centers (<250, 250 to <300, 300 to <400, ≥400). All analyses were conducted with robust estimates of the variance to correct for intra-cluster correlation. R-squared was multiplied by 100.

above, positive associations were found between changes in the FSAm-NPS DI and changes in the glucose and triglyceride levels, diastolic blood pressure, BMI, and waist circumference after 1 year of follow-up. Non-significant association was observed in the case of HDL-cholesterol, LDL-cholesterol, and systolic blood pressure changes. Nevertheless, after 1 year of follow-up, 79.36 and 19.4% of the population remained in the recommended levels of HDL and LDL-cholesterol, 12.82 and 8.9% of the participants showed increased levels, and only 7.82 and 10.9% of the participants showed a decrease in HDL-cholesterol or LDL-cholesterol, respectively (data not shown).

The cross-sectional and longitudinal associations remained significant when olive oil was considered the healthiest food option in the computation of the FSAm-NPS DI (data not shown).

RESULTS

TABLE 3 | Association between 1-year change of FSAm-NPS and changes of the CVD risk factors levels after 1 year of follow-up, and β coefficient (95% CI).

	Tertiles of one-year FSAm-NPS DI change			R-squared	*P*-trend	FSAm-NPS DI one-year change (continuous)
	T1	T2	T3			
Glucose levels change (mg/dL)						
n	1,932	1,931	1,931	0.27	<0.001	5,794
Crude Model	Ref	2.51 (1.17, 3.86)	2.47 (1.06, 3.88)	18.4	<0.001	0.50 (0.20, 0.81)
Model 1	Ref	2.06 (0.82, 3.31)	2.56 (1.29, 3.83)	23.3	<0.001	0.42 (0.14, 0.70)
Fully adjusted	Ref	1.76 (0.54, 2.98)	1.67 (0.43, 2.90)			0.35 (0.09, 0.62)
HDL-cholesterol change (mg/dL)						
n	1,908	1,908	1,908	0.1	0.021	5,724
Crude Model	Ref	−0.40 (−0.87, 0.07)	−0.54 (−1.00, −0.08)	8.44	0.019	−0.09 (−0.19, 0.0.1)
Model 1	Ref	−0.28 (−0.73, 0.17)	−0.53 (−0.98, 0.09)	9.31	0.024	−0.03 (−0.12, 0.07)
Fully adjusted	Ref	−0.16 (−0.60, 0.29)	−0.27 (−0.72, 0.18)			−0.02 (−0.12, 0.07)
TG levels change (mg/dL)						
n	1,924	1,923	1,923	0.43	<0.001	5,770
Crude Model	Ref	4.43 (0.04, 8.81)	11.0 (6.70, 15.25)	25.3	<0.001	2.75 (1.87, 3.63)
Model 1	Ref	3.85 (0.04, 7.65)	8.84 (5.06, 12.63)	26.5	<0.001	1.81 (1.05, 2.56)
Fully adjusted	Ref	2.75 (−1.06, 6.56)	6.27 (2.46, 10.09)			1.75 (1.00, 2.51)
LDL-cholesterol change (mg/dL)						
n	1,790	1,790	1,790	0.03	0.673	5,370
Crude Model	Ref	−0.77 (−2.59, 1.05)	0.41 (−1.35, 2.17)	19.2	0.282	−0.04 (−0.42, 0.33)
Model 1	Ref	−0.92 (−2.56, 0.72)	−0.86 (−2.46, 0.74)	21.6	0.287	−0.34 (−0.69, 0.00)
Fully adjusted	Ref	−1.06 (−2.68, 0.56)	−0.88 (−2.48, 0.72)			−0.34 (−0.68, 0.00)
Diastolic BP change (mmHg)						
n	1,945	1,945	1,944	0.13	0.007	5,834
Crude Model	Ref	0.50 (−0.02, 1.03)	0.72 (0.19, 1.24)	20	0.002	0.17 (0.05, 0.28)
Model 1	Ref	0.58 (0.12, 1.05)	0.76 (0.28, 1.24)	20.8	0.001	0.14 (0.04, 0.24)
Fully adjusted	Ref	0.45 (−0.02, 0.92)	0.56 (0.08, 1.05)			0.15 (0.04, 0.25)
Systolic BP change (mmHg)						
n	1,945	1,945	1,944	0.09	0.03	5,834
Crude Model	Ref	0.45 (−0.49, 1.39)	1.08 (0.11, 2.05)	22.8	0.028	0.31 (0.10, 0.51)
Model 1	Ref	0.70 (−0.13, 1.53)	0.96 (0.10, 1.82)	23.6	0.041	0.18 (0.00, 0.37)
Fully adjusted	Ref	0.45 (−0.39, 1.28)	0.49 (−0.39, 1.36)			0.17 (−0.01, 0.35)
BMI change (kg/m^2)						
n	1,974	1,974	1,973	4.04	<0.001	5,921
Crude Model	Ref	0.41 (0.31, 0.51)	0.77 (0.67, 0.87)	5.08	<0.001	0.17 (0.14, 0.19)
Model 1	Ref	0.40 (0.30, 0.50)	0.75 (0.65, 0.85)	16.1	<0.001	0.11 (0.09, 0.13)
Fully adjusted	Ref	0.27 (0.18, 0.36)	0.51 (0.41, 0.60)			0.11 (0.09, 0.13)
Waist circumference change (cm)						
n	1,967	1,966	1,966	2.77	<0.001	5,899
Crude Model	Ref	1.22 (0.90, 1.55)	2.09 (1.76, 2.41)	9.74	<0.001	0.47 (0.40, 0.54)
Model 1	Ref	0.11 (0.79, 1.43)	1.99 (1.68, 2.31)	15.5	<0.001	0.29 (0.23, 0.36)
Fully adjusted	Ref	0.73 (0.42, 1.03)	1.19 (0.89, 1.50)			0.28 (0.22, 0.35)

FSAm-NPS, Food Standards Agency Nutrient Profiling System (modified version) Dietary Index; CVD, cardiovascular disease; CI, confidence interval; HDL, high-density lipoprotein; TG, triglycerides; LDL, low-density lipoprotein; BP, blood pressure; BMI, body mass index. Linear regression models were fitted. Model 1: adjusted for age, sex, physical activity, smoking status, BMI, total energy intake at baseline, education level and marital status and each CVD risk factor was adjusted to its level at baseline. Fully adjusted: Model 1 additionally adjusted for medication for treatment of hypercholesterolemia, of hypertension and of diabetes, size of the recruitment centers (<250, 250 to <300, 300 to <400, ≥400) and the intervention groups. All analyses were conducted with robust estimates of the variance to correct for intra-cluster correlation. R-squared was multiplied by 100.

DISCUSSION

To the best of our knowledge, this study is one of the first to evaluate the prospective associations between the nutritional quality of diet assessed using the FSAm-NPS DI (algorithm underpinning the Nutri-Score FOP label) and different CVD risk factors. Findings of this study showed that the consumption of foods with higher scores of FSAm NPS (foods with less favorable rating in the Nutri-Score scale) was associated with unfavorable changes in CVD risk factors after 1 year of follow-up, specifically with an increase in adiposity (BMI and waist

RESULTS

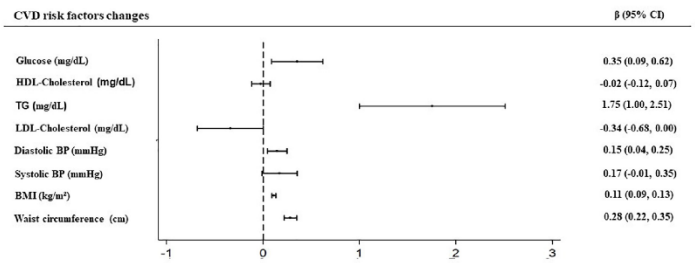


FIGURE 2 | Prospective associations between dietary index based on the French context, the modified FSA-NPS (FSAm-NPS DI) and cardiovascular diseases (CVD) risk factors changes after 1 year of follow-up.

circumference) and the levels of plasma glucose, triglycerides, and diastolic blood pressure.

Our results are partially in line with the SU.VI.MAX study where the authors assessed the prospective association between the FSAm-NPS DI and metabolic syndrome and its components (24). Even though, these authors found significant and positive association between the FSAm-NPS DI and the incidence of metabolic syndrome, when the components were assessed individually, only systolic and diastolic blood pressure showed a significant association. In our study, we also found significant and positive association with diastolic blood pressure, but not for systolic. These results may be explained because the FSAm-NPS DI considers the salt content of food that has been proved to be related to hypertension (31). However, we cannot discard that other factors of the score, as well as their synergistic effect, may also explain these results on blood pressure. In contrast to the SU.VI.MAX study, we have demonstrated significant prospective associations with triglycerides and fasting glucose. These results in relation to triglycerides and glucose may be explained because the participants in our study were elderly Mediterranean individuals (aged between 55 and 75 years) with overweight/obesity and metabolic syndrome. Therefore, as they were already at the risk of CVD, the results of our study could be influenced by their pre-existing risk and conditions. In contrast, in the SU.VI.MAX study, the participants were younger and healthier (women aged between 35 and 60 years and men aged between 45 and 60 years).

In our study, no prospective associations were observed between changes in the FSAm-NPS DI and changes in HDL-cholesterol or LDL-cholesterol concentrations. Even though, in the cross-sectional analysis, we found an association between HDL-cholesterol and FSAm-NPS DI at the baseline, the lack of association in the prospective analysis might be explained because most of the population did not show changes in the levels of HDL-cholesterol after 1 year of follow-up. In a previous study, analysis conducted in the SU.VI.MAX cohort reported a negative association between the FSAm-NPS DI and LDL-cholesterol concentration (16). According to the authors, the absence of association or the negative association observed in the case of the LDL-cholesterol could be partly explained by the fact that, even when saturated fat is considered in the score, the FSAm-NPS DI does not distinguish between fatty acid subtypes (monounsaturated or polyunsaturated fatty acids) (25).

In our study, a higher 1-year increase in the FSAm-NPS, reflecting a decrease in the nutritional quality of diet, was also positively associated with increases in adiposity measured by BMI and waist circumference. These findings are in line with other studies using other nutritional quality indexes (32, 33), and with other two studies analyzing the FSAm-NPS. In the SU.VI.MAX French cohort, a positive association between the FSAm-NPS DI and body weight and BMI gain (24) was found in both men and women. In addition, an increased risk of obesity was observed only in men after 13 years of follow-up. A study using data from the French NutriNet-Santé cohort has recently analyzed the prospective associations between different nutrient profiling systems (the original Food Standards Agency nutrient profiling system and three variants, Food Standards Australia New Zealand Nutrient Profiling Scoring Criterion (NPSC), Health Star Rating NPS and the French NPS (HCSP-NPS), and adiposity markers and overweight/obesity risk. The results showed that participants with a dietary index reflecting lower diet nutritional quality (irrespective of the nutrient profiling used) were more likely to increase the BMI over time and had an increased risk to develop overweight/obesity (34). It is important to remark that whilst differences were small, the French FSAm-NPS one appeared to show a significantly greater association with the risk of overweight compared to other nutrient profile scores. It is important to highlight that the reclassification of olive oil in the FSAm-NPS computation only induced small effects in our study, whereas previous evidence from the SUN cohort found that the reclassification of olive oil strengthened the associations between the FSAm-NPS and total mortality (23).

In the FSAm-NPS, total energy, salt, saturated fat, and sugar are considered negative components whereas fiber, protein, fruits and vegetables, legumes, and some vegetable oils are considered positive. The FSAm-NPS DI resumes the FSAm-NPS scores of all food items mostly consumed by an individual, by assigning points based on the consumption of foods, food groups, or nutrients

relevant to the risk of chronic diseases. All these nutrients or food groups have been demonstrated to be related to CVD risk factors in numerous studies (5, 35, 36). Particularly, added sugar and saturated fat have been previously associated with higher levels of fasting blood glucose and triglycerides (37, 38). Therefore, the observed associations are consistent with the nature of the FSAm-NPS and with previous findings regarding diet and CVD.

Furthermore, there is also increasing evidence that ultra-processed foods (UPF) have detrimental effects on CVD risk (39), In this sense, we should bear in mind that the FSAm-NPS DI and the NOVA classification (based on degree of food processing) are two different complementary approaches to assess nutritional quality and healthiness (40). Indeed, in our study, those participants allocated in the highest FSAm-NPS DI tertiles had higher UPF consumption. It is acknowledged that the FSAm-NPS DI does not cover all the health dimensions of food (e.g., food processing, additives, and presence of pesticides). It considers neither the added sugar included in food nor the monounsaturated or polyunsaturated fat because this score has been based on those nutrients that, in Europe, are mandatory to be disclosed in the list of nutrients in the food labeling. However, at the moment, there is no classification system including all these nutrients and dimensions of food in a single indicator. Therefore, FSAm-NPS focuses on the nutritional dimension and serves as the underlying system of some front-of-pack labels to allow consumers to easily compare foods belonging to the same category. Importantly, FSAm-NPS has the advantage of considering a large number of elements from a nutritional point of view, in particular the content, per 100 g of food of fruits and vegetables (proxy of the amount of antioxidants, vitamins, and minerals), legumes, nuts, proteins (e.g., proxy of the amount of calcium and iron), olive oil, rapeseed oil, and walnut oils.

Overall, our results support the suitability of the FSAm-NPS as a nutritional quality indicator aimed at improving diets and preventing the development of chronic diseases. Further prospective studies analyzing the effect of modifying the nutritional quality of diet through changes in this dietary index are warranted in the future to confirm our results.

Strengths and Limitations

Our study has several limitations that deserve to be discussed. First, the results cannot be generalized to other populations since participants included in the analysis were elderly Mediterranean individuals with overweight/obesity and metabolic syndrome. Therefore, as they were already at the risk of CVD, the results of this study could be influenced by this condition. Second, the assessment of food intake through a FFQ is prone to possible measurement errors. However, despite this limitation, food-based FFQs have been widely used as a tool in epidemiological studies since the 1990s (41). Third, we cannot rule out bias due to unmeasured potential confounders related to the risk of CVD.

This study also has some strengths such as its prospective design, which reduces the possibility of reverse causation bias, the control for several potential confounding factors, the large sample size, and the extensive data collected by trained staff.

In conclusion, the results of this prospective cohort study suggest that the consumption of foods with higher FSAm-NPS (reflecting a lower nutritional quality) is associated with an increase in some CVD risk factors (adiposity, fasting plasma glucose, triglycerides, and diastolic blood pressure). However, no significant associations were identified for critical CVD risk factors such as HDL-cholesterol, LDL-cholesterol, and systolic blood pressure.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because the datasets generated and analyzed during the current study are not publicly available due to data regulations and for ethical reasons, considering that this information might compromise research participants' consent because our participants only gave their consent for the use of their data by the original team of investigators. However, collaboration for data analyses can be requested by sending a letter to the PREDIMED-Plus steering Committee (predimed_plus_scommittee@googlegroups.com).The request will then be passed to all the members of the PREDIMED-Plus Steering Committee for deliberation. Requests to access the datasets should be directed to predimed_plus_scommittee@googlegroups.com.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by JW: CEI Provincial de Málaga-Servicio Andaluz de Salud (O01_feb_PR2), José Lapetra: CEI de los Hospitales Universitarios Virgen Macarena y Virgen del Rocío-Servicio Andaluz de Salud (PI13/00673), JAM: CEIC Universidad de Navarra (053/2013), DR: CEI de las Illes Balears – Conselleria de Salut Direcció General de Salut Publica i Consum (IB 2242/14 PI), MF: CEIC Parc de Salut Mar y IDIAP Jordi Gol (PI13/120), JSS: CEIC del Hospital Universitari Sant Joan de Reus y IDIAB Jordi Gol (13-07-25/7proj2), Aurora Bueno: CEI de la Provincia de Granada- Servicio Andaluz de Salud (MAB/BGP/pg), CV: CEIC de la Fundacion Jiménez Díaz (EC 26-14/IIS-FJD), MM-G: CEIC Universidad de Navarra (053/2013), Fernando Aros: CEIC Euskadi (PI2014044), DC: CEIC Corporativo de Atención Primaria de la Comunitat Valenciana (2011-005398-22), LS-M: CEI Humana de la Universidad de las Palmas de Gran Canaria (CEIH-2013-07), XP: CEIC del Hospital de Bellvitge (PR240/13), José López Miranda: CEI de Cordoba-Junta de Salud (3078), José María Ordovás: CEI de la Fundación IMDEA Alimentación (PI-012), PM-M: CEIC Hospital Clínico San Carlos de Madrid-Piloto-CEIC Servicio Madrileño de salud-General (30/15), FT: CEI Provincial de Málaga-Servicio Andaluz de Salud, JT: CEI de las Illes Balears – Conselleria de Salut Direcció General de Salut Publica i Consum (IB 2251/14 PI), JoV: CEIC del Hospital Clínic de Barcelona (HCB/2017/0351), JeV: CEIC del Hospital General Universitario de Alicante (CEIC PI2017/02),

M-DR: CEIC de la Investigación Biomédica de Andalucía (CCEIBA), VM: CEI de la Universidad de León (ÉTICA-ULE-014-2015), and Ramon Estruch: CEIC del Hospital Clínic de Barcelona (HCB/2016/0287). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

NK, CG-D, MM, JSS, and NB conceived and designed the study. MM, DC, MF, JAM, ÁA-G, JW, JV, DR, FT, JS-L, LS-M, JT, VM, XP, MD-R, PM-M, JVi, CV, LD, and ER conducted data acquisition. NK, CG-D, and NB performed statistical analyses. NK, CG-D, MM, JSS, and NB carried out interpretation of the data for the study. All authors were involved in draft redaction, revision for important intellectual content, read, and approved the final manuscript.

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

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b. Article 2

Title: Ability of a dietary index based on the updated algorithm underpinning the Nutri-Score to discriminate food consumption and nutrient intake in a French population of children and adolescents

Nadine Khoury, Barthélémy Sarda, Mathilde Touvier, Emmanuelle Kesse-Guyot, Jordi Salas-Salvadó, Nancy Babio, María Ángeles Martínez, Serge Hercberg, Pilar Galan, Pauline Ducrot, Valérie Deschamps, Chantal Julia

Journal: Under Review

Summary:

1,279 participants (50.2% boys and 49.8% girls, mean age 11 years) were included in this study. Using the 2023 Nutri-Score Nutrient Profiling Model Dietary Index (NS-NPM DI), participants with more favorable DI (lower NS-NPM DI) consumed higher amounts of fruits, vegetables, whole grain cereals, milk, poultry, fish and seafood and vegetable oils and lower amounts of cheese, processed meat and sweetened foods. In addition, using the NS-NPM DI 2023, participants with more favorable DI (thus more favorable Nutri-Score) had higher intakes of proteins, fiber, vitamins and minerals, and lower intakes of lipids, SFA, MUFA and simple sugars. Compared to the NS-NPM DI 2015, with the updated algorithm, higher differences between quartiles were observed for fish and seafood, poultry, processed meat and vitamin D, all in the expected direction (better discrimination).

Conclusion: The results of the present study suggest that the NS-NPM algorithm underlying Nutri-Score is a useful tool for characterizing the diet quality of children and adolescents. The updated version of this algorithm showed a more discriminatory ability when assessing some animal food groups and vitamin D, indicating that the revision of the Nutri-Score represents an improvement for public health purposes.

RESULTS

Discrimination ability of the updated NS-NPM dietary index underpinning the Nutri-Score front-of-pack nutrition label in a French children and adolescent population

Objective: to investigate the ability of the 2015 and 2023 NS-NPM to discriminate food consumption and nutrient intake in a population of French children and adolescents.

NUTRI-SCORE A B C D E

$$(2015\&\ 2023)\ \mathrm{NS-NPM\ Dietary\ Index} = \frac{\sum_{i=1}^{n} \mathrm{NS-NPMi} * \mathrm{Ei}}{\sum_{i=1}^{n} \mathrm{Ei}}$$

esteban

Children and adolescents
n=1,279
50.2% boys and 49.8% girls
with mean age of 11 years

Participants with lower NS- NPM DI Using the 2023 NS-NPM consumed:

↑
- Fruits & vegetables
- Whole grain cereals
- Milk
- Poultry
- Fish and seafood
- Vegetable oils.
- Proteins carbohydrates
- Fiber
- Vitamins & minerals

↓
- Cheese
- Processed meat
- Sweetened foods
- Lipids
- SFA & MUFA
- Simple & added sugars

Compared to 2015 NS-NPM DI, **2023 NS-NPM DI** showed higher discriminative differences for:

↑
- Fish and seafood
- Poultry
- Processed meat
- Vitamin D

Conclusion:
The NS-NPM algorithm is a useful tool for characterizing the diet quality of children and adolescents.
The updated version showed a more discriminatory ability compared to the previous one, indicating that this revision represents an improvement for public health purposes.

Ability of a dietary index based on the updated algorithm underpinning the Nutri-Score to discriminate food consumption and nutrient intake in a French population of children and adolescents

Nadine Khoury[1,2,3], Barthélémy Sarda[4], Mathilde Touvier[4], Emmanuelle Kesse-Guyot[4], Jordi Salas-Salvadó[1,2,3], Nancy Babio[1,2,3], María Ángeles Martínez[1,2,3], Serge Hercberg[4,5], Pilar Galan[4], Pauline Ducrot[6], Valérie Deschamps[7], Chantal Julia[4,5*]

1. Universitat Rovira i Virgili, Departament de Bioquímica i Biotecnologia, Unitat de Nutrició Humana, ANUT-DSM group. Spain.
2. Institut d'Investigació Sanitària Pere Virgili (IISPV), Reus, Spain.
3. Consorcio CIBER, M.P. Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III (ISCIII), Madrid, Spain.
4. Université Sorbonne Paris Nord and Université Paris Cité, INSERM, INRAE, CNAM, Center of Research in Epidemiology and StatisticS (CRESS), Nutritional Epidemiology Research Team (EREN), F-93017 Bobigny, France
5. Public health Department, Hôpital Avicenne, Assistance Publique-Hôpitaux de Paris (AP-HP), Bobigny, France.
6. Santé publique France, French national public health agency, F-94415, Saint-Maurice, France.
7. Nutritional Epidemiology Surveillance Team (ESEN), Santé Publique France, The French Public Health Agency, Bobigny, France.

***Corresponding author:** Chantal Julia, c.julia@eren.smbh.univ-paris13.fr, 74 rue Marcel Cachin, 93017 Bobigny

Highlights

- The nutrient profiling model dietary index underlying Nutri-Score (NS-NPM DI) updated in 2023 was able to adequately discriminate food consumption and nutrient intake
- Differences between quartiles of NS-NPM DI for fish, poultry, processed meat and dietary vitamin D intakes were higher with the new version of the NPM compared to the previous one (dated 2015)
- NS-NPM algorithm is a useful tool for characterizing the diet of children and adolescents

Abstract

Introduction: Preventing chronic diseases among children and adolescents requires a comprehensive approach. The Nutri-Score, a front-of-pack nutrition labeling system, may play an important role in public health by enhancing overall nutritional quality of dietary intake, a key modifiable risk factor. The aim of this study was to investigate the ability of the 2015 and 2023 NS-NPM to discriminate food consumption and nutrient intake in a population of French children and adolescents.

Methods: Children and adolescents (6 to 17 years old) participating in the cross-sectional representative ESTEBAN survey conducted in mainland France were included.

Food consumption was assessed with three repeated 24h dietary recalls. To obtain a dietary score at the individual level, the Nutri-Score nutrient profiling model dietary index (NS-NPM DI) was computed as an energy-weighted mean using the 2015 and the 2023 NS-NPMs and categorized into quartiles. Linear contrasts were used to assess the linearity of the association between the indexes and the intakes.

Results: 1,279 participants (50.2% boys and 49.8% girls, mean age 11 years) were included in this study. Using the 2023 NS-NPM DI, participants with more favorable DI (lower NS-NPM DI) consumed higher amounts of fruits, vegetables, whole grain cereals, milk, poultry, fish and seafood and vegetable oils and lower amounts of cheese, processed meat and sweetened foods. In addition, using the NS-NPM DI 2023, participants with more favorable DI (thus more favorable Nutri-Score) had higher intakes of proteins, fiber, vitamins and minerals, and lower intakes of lipids, SFA, MUFA and simple sugars. Compared to the NS-NPM DI 2015, with the updated algorithm, higher differences between quartiles were observed for fish and seafood, poultry, processed meat and vitamin D, all in the expected direction (better discrimination).

Conclusion: The results of the present study suggest that the NS-NPM algorithm underlying Nutri-Score is a useful tool for characterizing the diet quality of children and adolescents. The updated version of this algorithm showed a more discriminatory ability when assessing some animal food groups and vitamin D, indicating that the revision of the Nutri-Score represents an improvement for public health purposes.

Key words: NS-NPM DI; Nutri-Score; Children; Adolescents; ESTEBAN study

Abbreviations: NS-NPM DI: Nutri-Score nutrient profiling model dietary index; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty; FSA NPS: Food Standard Agency Nutrient Profiling System

Introduction

Addressing the growing burden of chronic diseases in children and adolescents and fostering prevention for long-term health requires a multi aspect approach. The World Obesity Federation estimates that 206 million children and adolescents aged 5 to 19 years will be obese by 2025, and 254 million by 2030 (Lobstein, 2019). Among the key modifiable risk factors, efforts should focus on promoting healthy lifestyles, mainly, improving access to nutritious and healthy foods, regulating advertising food high in fat, sugar and salt, increasing opportunities for physical activity, and creating supportive environments for children's well-being (De Rezende et al., 2014; WHO, 2017; Escalon et al., 2021; Kelishadi, 2019; Weihrauch-Blüher et al., 2018). One of the main public health strategies that aims to reduce the prevalence of chronic diseases in children and adolescents, is the implementation of preventive policies with a goal of promoting healthier eating such as the implementation of front-of-pack nutrition labels (UNICEF, 2021; Pettigrew et al., 2022). Nutri-Score is a front-of-pack nutrition labeling system that may contribute to public health efforts by promoting healthier food choices and to help consumers to improve the nutritional quality of their diets(Alrige et al., 2017). It is used to provide consumers with easily understandable information about the nutritional quality of a food product(Egnell et al., 2019). The Nutri-Score system is based on the UK

Food Standard Agency Nutrient Profiling System (FSA NPS) originally developed to discriminate foods based on their nutritional composition in the context of the regulation of television commercials targeting children. This system assigns a color-coded grade to each food product, ranging from dark green (A) to dark orange (E), wherein A signifies the healthier option and E indicates less healthy option(Hercberg et al., 2021). The purpose of Nutri-Score is to represent in a simplified way the global nutritional quality of a product, allowing consumers to compare easily different products - with the same utilization - and quickly at the point of sale. By helping consumers to identify the nutritional value of food products and the possibility at a glance to compare food products, Nutri-Score aims to improve public health and encourage healthier eating habits (Julia et al., 2014a, 2016a).

Nutri-Score has been first implemented in France in 2017 and adopted by 8 European countries to date. Recently, the international scientific committee of Nutri-Score has updated the algorithm underlying the label to correct some identified limitations, while maintaining the overall consistency of the system and the classification of the products and permitting a better alignment to current nutritional recommendations(Merz et al., 2024). This update includes a higher strictness for high-sugar and high-salt products, as well as improved classification for fibers and for specific food groups including fish, vegetable oils and nuts (Merz et al., 2024).

Although the Nutri-Score algorithm has been long validated and proved its adequacy to evaluate the nutritional quality of diets(Andreeva et al., 2021; Barrett et al., 2024; Julia et al., 2014a, 2016a; Montericcio et al., 2024), further investigations are needed to assess the validity and applicability of the updated algorithm, in particular among children. Additionally, the efforts to address chronic diseases through diet, have been focused mainly on the adult population, with limited investigation in children and adolescents. Therefore, the aim of this study is to investigate the ability of the 2015 and 2023 NS-NPM to discriminate food consumption and nutrient intake in a population of French children and adolescents.

Material and methods

Study design and participants

A total of 1,279 children and adolescents aged between 6 to 17 years old from the French national ESTEBAN study were included in the present analysis. ESTEBAN is a cross-sectional survey conducted from 2014 to 2016, aimed to assess dietary intake, physical activity, sedentary behaviors, and nutritional status of the French population. The survey ensured a representative sample of the general population through a multi-stage sampling approach, involving random selection of geographic zones (stratified by regions and degree of urbanization), random selection of households based on a randomly generated phone lists, and random selection of eligible subjects. Recruitment occurred across mainland France for 1 year to consider seasonal dietary variations. The survey's protocol included socio-demographic assessments and dietary surveys with three 24-hour recalls. Detailed information about the ESTEBAN study design can be found elsewhere(Balicco et al., 2017).

This study received approval from the Advisory Committee on Information Treatment in Health Research (CCTIRS n° 13,002,294), the French Data Protection Authority (Cnil n° 2013–040), and the Personal Protection Committee (CPP n°2012-A00456-34). All participants provided informed consent.

Sociodemographic data

Social and demographic data were obtained through face-to-face questionnaires, which covered information about sex, age, educational level and occupational situation of the head of household.

Dietary data

Dietary information was gathered through three non-consecutive 24-hour dietary records (for participants aged between 6 to 10 years) or three non-consecutive 24-hour dietary recalls (for participants aged 11 to 17 years), scheduled on varied days (two weekdays and one weekend day) and distributed randomly over a two-week duration. This process was conducted either via a specialized online platform or through telephone interviews conducted by trained dietitians (Lassale et al., 2015; Touvier et al., 2011). Participants were given the choice between an online or telephone interview based on their preference, with all interviews overseen and supervised by trained dietitians. Individuals were requested to provide comprehensive details about their food consumption, including details about homemade recipes and quantities consumed. To facilitate the description of portion sizes, a validated photographic guide displaying food portions or standard measurements such as grams or household containers was utilized (Moullec et al., 1996). Consequently, the average mean dietary intakes from these 24-hour dietary records and recalls of each participant were computed. Amounts consumed from composite dishes were estimated using pre-validated French recipes. Homemade dishes were deconstructed to list the specific ingredients used in their preparation and mean daily intakes were estimated using a detailed food composition table (Inserm, 2013). Under-reporters for energy intake were identified using the Black method and excluded from the analysis (Black, 2000).

NS-NPM underpinning the Nutri-Score

The scoring for a given food or beverage is determined by assigning points based on its content per 100 g or 100 ml. This algorithm considers both positive scoring components and negative components (Hercberg et al., 2021), overall, the points from the "favorable" component are subtracted from those of the "unfavorable" component, thus leading to a final score based on a discrete continuous scale going from -15 to +40 points for the 2015 NS-NPM (Hercberg et al., 2021). This final score is then translated into a grade from A to E, corresponding to a color from dark green to dark orange.

2023 NS-NPM *underpinning the Nutri-Score update*

The 2023 NS-NPM, determined and validated by an international scientific committee aimed to integrate the latest scientific evidence and align with the primary food-based dietary guidelines of European countries.

The updated algorithm functions similarly to its predecessor, however some modifications have been considered. These include an increase in the maximal number of points for sugar and salt, resulting in less favorable ratings for foods high in these components. Additionally, stricter criteria were applied to the allocation of points for the fiber and protein components, requiring a higher content of fiber or protein to earn positive points. Specific rules were introduced for red meat, limiting the maximum points it could receive in the positive category. For beverages, the presence of non-nutritive sweeteners was added as an extra unfavorable factor. Finally, the thresholds for converting the final score into a rating were adjusted to align with the new algorithm(Merz et al., 2024). More details for the comparison and calculation of the initial and updated versions of the Nutri-Score, can be found in **Supplementary Material File S1**.

NS-NPM standardization

In order to take into account the differing range of distribution of values of the (2023 or 2015) NS-NPM in foods and beverages, we converted the (2023 or 2015) NS-NPM scores to a standardized scale of 0–100 for foods and beverages respectively. Therefore, a range from the healthiest (0 points), to the least healthy (100 points) was used, following this formula(Pan et al., 2020):

$$\text{Standardized score} = \frac{Score - Minimum\ possible\ score}{Maximum\ possible\ score - Minimum\ possible\ score} * 100 \quad \text{(Equation 1)}$$

Dietary index computation (at the individual level)

To obtain a dietary score at the individual level, the 2015 and 2023 NS-NPM DI were computed as an energy-weighted mean of the NS-NPM scores of all foods and beverages consumed by each participant per day, using the following equation:

$$\text{Dietary Index} = \frac{\sum_{i=1}^{n} NS\text{-}NPMi * Ei}{\sum_{i=1}^{n} Ei} \quad \text{(Equation 2)}$$

Where "i" signifies a food or beverage consumed by the participant, "NS-NPMi" represents the score of the food (or beverage) obtained from equation 1, "Ei" signifies the mean daily energy intake from this food (or beverage) and "n" the number of different foods consumed.

Therefore, higher NS-NPM DI scores reflected lower nutritional quality in the total foods consumed. The dietary index reflects the whole diet excluding only alcoholic beverages and herbs/spices. Consequently, both the 2015 and 2023 NS-NPM DI provide a comprehensive representation of the overall dietary patterns.

Statistical analysis

Analysis of descriptive characteristics of sociodemographic variables was carried out and reported as mean ± SD and percentages (number) for continuous and categorical variables, respectively. Participants were categorized into quartiles of the NS-NPM DI (for the 2 versions), ranging from quartile 1 (Q1) -representing the highest nutritional quality- to quartile 4 (Q4) -indicating the lowest nutritional quality-. The Chi-square test for categorical variables and Kruskal-Wallis for continuous variables were used to compare the characteristics across the quartiles.

The two computed dietary indexes were categorized into quartiles, where lower quartile represented the individuals with healthier diet, and highest quartile represented the individuals with the least healthy diet. All nutrient intakes were adjusted for energy intake using the residual method (Willett & Stampfer, 1986).

Spearman correlation coefficients were computed to measure the relationships between the two versions of Nutri-Score algorithm (continuous coding). The correlation coefficients were interpreted in terms of their strength, considering a correlation above 0.50 in absolute value as indicative of a high correlation (Hemphill, 2003).

Discriminations across quartiles of NS-NPM DI and dietary intakes were examined using ANOVA with linear contrast across quartiles of the 2015 and 2023 NS-NPM DI expressed as mean (SE). This analysis allowed the assessment of how food consumption varied across quartile 4 and quartile 1 of the dietary indexes. Absolute and relative differences between Q4 and Q1 were computed as descriptive indicators of the discrimination power of each NS-NPM DI. When absolute and relative differences varied by more than 5 percentage points between 2015 NS-NPM DI and 2023 NS-NPM DI the differences in discriminating power were considered substantial.

Data were weighted to account for potential selection bias and for the complex survey design and calibration using the "svyset" procedure. The statistical significance threshold for the results was set at $p < 0.01$. All analyses were conducted using the Stata 14 software program (StataCorp).

Results

A total of 1,279 participants (50.2% boys and 49.8% girls, with mean age of 11 years) were included in this study. Socio-demographic characteristics of the participants across quartiles of the NS-NPM DI (2023 and 2015 version) are shown in **Table 1**. For both Nutri-Score algorithms, no differences across quartiles of NS-NPM DI were observed for age, sex and occupational level of the head of household. Participants with a lower NS-NPM DI, meaning better nutritional quality, were more likely to have parents with high education level.

Spearman correlation coefficient between the dietary indexes using the new and old version of Nutri-Score algorithm was of 0.953 (p-value <0.001) **(Table 2)**.

Food group assessment across quartiles of NS-NPM DI (2023 and 2015 version) in children and adolescents of the ESTEBAN study are shown in **Table 3**. For both Nutri-score versions (2015 and 2023), participants with a lower NS-NPM DI (meaning a higher nutritional quality of the diet) showed a higher consumption of fruits, vegetables, whole grain cereals, milk, poultry, fish and seafood and vegetable oil compared to those in

quartile 4. Additionally, they showed a lower consumption of cheese, processed meat, sweetened products (food and beverages).

The relative differences between participants in the extreme quartiles of NS-NPM DI for both Nutri-Score versions (2015 and 2023) were similar regarding the intake of fruits, vegetables, tubers, cereals, milk, yogurt and Greek yogurt, red meat, eggs, sweetened food, and sweetened beverages.

The relative differences between participants in the extreme quartiles of NS-NPM DI were higher with the 2023 NS-NPM DI compared to the 2015 NS-NPM DI for the consumption of poultry, fish and seafood, processed meat, nuts, and vegetable oil, demonstrating an enhanced discriminatory power for these food groups.

The relative differences between participants in the extreme quartiles of NS-NPM DI were slightly lower for the 2023 NS-NPM DI compared to the 2015 NS-NPM DI in case of the intake of bread, whole grain cereals, cheese, and fruits and vegetable juices, showing a slightly lower discriminatory power.

Nutrient intake assessment across quartiles of NS-NPM DI (utilizing the 2023 and 2015 version of Nutri-Score) in children and adolescents of the ESTEBAN study is shown in **Table 4**. Children with a lower 2023 NS-NPM DI (meaning a higher nutritional quality of the diet), showed a higher intake of proteins, carbohydrates, fibers, calcium, potassium, magnesium, phosphorus, iron, zinc, vitamin B1, B2, B9 and B12, β-carotene and vitamin C, D and E compared to those in quartile 4. Additionally, they showed a lower intake of energy, lipids, MUFA and SFA and simple sugars.

The relative differences between extreme quartiles of NS-NPM DI for both Nutri-Score versions (2023 and 2015) were consistent in case of energy intake and age-adjusted energy intake, proteins, carbohydrates, lipids, SFA, MUFA, PUFA, simple sugars, added sugars, fibers, calcium, potassium, magnesium, phosphorus, sodium, iron, zinc, retinol, vitamin B1, B2, B9, B12, β-Carotene, vitamin C and vitamin E. Relative differences for vitamin D between participants in the extreme quartiles were higher when using the 2023 NS-NPM DI, meaning a higher discriminating capacity for this vitamin in the case of the updated algorithm.

Discussion

To the best of our knowledge, the present study is the first that assessed the relevance of the 2023 updated Nutri-Score algorithm on evaluating its discriminatory power and comparing to the 2015 version of the algorithm in French children and adolescents. Our results showed that nutritional quality of the diet is in general higher for participants in the lowest quartile of NS-NPM DI compared to those in the highest quartile, meaning that both Nutri-Score algorithms are able to characterize the quality of children and adolescent's diet. In addition, our results suggest that compared to the previous algorithm, with the updated algorithm higher discriminative differences between quartiles were observed for fish and seafood, poultry, processed meat, nuts, vegetable oils and vitamin D, all in the expected direction, indicating that this updated version of the algorithm of Nutri-Score represents an improvement to characterize diet quality.

In our study, participants with more favorable DI (lower NS-NPM DI) corresponding to participants consuming foods with more favorable rating in the Nutri-Score scale, consumed higher amounts of fruits, vegetables, whole grain cereals, milk, poultry, fish

and seafood, vegetable oils, proteins, carbohydrates, added sugars, fibers and vitamins and minerals. Additionally, they consumed lower amounts of, processed meat, sweetened foods, lipids, SFA, MUFA and simple sugars.

Past studies assessed the validity and the discriminatory power of the initial Nutri-Score and found that overall, the updated FSA NPS demonstrates strong consistency in characterizing individual diets within the French context and different other countries(Dréano-Trécant et al., 2020; Julia et al., 2014b, 2016b; Lee et al., 2023; Szabo De Edelenyi et al., 2019). In a recent Canadian study conducted by Lee et al., with 13,495 adult participants, it was reported that the NS-NPM DI based on 2015 Nutri-Score algorithm, showed better capacity to discriminate diet quality than the Dietary Approach to Stop Hypertension (DASH) dietary index, showing a moderate alignment with the dietary index scoring system based on Canada's Food Guide(Lee et al., 2023). In addition, Paper et al., assessed the cross-sectional association between dietary indexes that underpin 3 front-of-pack labels: the Nutri-Score, the proposed Canadian 'High In' Symbol and the Diabetes Canada Clinical Practice Guidelines in 1,836 adults from the ESTEBAN study and found that the dietary index underpinning the Nutri-Score algorithm discriminated adequately individuals according to the nutritional quality of their diets through food consumption and nutrient intakes (Paper et al., 2023). These results reinforce our findings and show the consistency of algorithm to be applied in populations of different life stages.

Some of the observed results in our study can be linked to the elements taken into account in the Nutri-Score algorithm and its ability to assess the overall nutritional quality of foods and beverages(Julia & Hercberg, 2017). The NS-NPM, takes into account various nutrients and food components, assigning scores that reflect the overall nutritional of a product. Since energy, saturated fat, simple sugars, salt, fibers, and proteins are factored into the computation of Nutri-Score(Hercberg et al., 2021), it is anticipated that a discrimination would be observed at the individual level. In addition, while vitamins and minerals are linked to the presence of proteins, fruits, and vegetables (elements considered in the score calculation), they are not explicitly included in the algorithm, which strengthens the discriminatory ability of the NS-NPM to characterize the overall nutritional quality beyond the elements taken directly into consideration in the NS-NPM algorithm.
Furthermore, both DIs showed an adequate ability to discriminate the intake of added sugars. While the two algorithms (2015 and 2023 NS-NMP) only take into account total sugars, the results showed that the classification is consistent and that the main type of sugar discriminated is indeed added sugars not the sugars naturally present in fruit or dairy products, which is of importance for public health purposes for addressing obesity, particularly in children who have a high intake of added/free sugars.
Overall, the distribution of the various food groups within the updated Nutri-Score was aligned with the previous one. The recent update has resulted in improved ratings for certain food groups, specifically fish and seafood, vegetable oils, nuts, poultry and processed meat. These enhancements can be attributed to the adjustment in the maximum points allocated for protein and fiber content and for nuts and vegetable oils. This adjustment helps offset the higher caloric density of these foods, which are rich in essential micronutrients, such as vitamin D in fish. Additionally, the modifications in the

attribution of protein points in the updated algorithm improved the initial classification and ensured a good discrimination between red meat (including processed meat) and poultry. Consequently, while poultry received relatively favorable ratings in both versions of the Nutri-Score algorithm, unprocessed meat classification – except for very lean cuts – was shifted towards less favorable ratings in the updated version. These modifications may explain the higher discrimination in the consumption of poultry and red meat in the 2023 NS-NPM DI compared to the 2015 NS-NPM DI.

The 2023 NS-NPM modified the overall classification for breakfast cereals, with a shift towards less favorable rating. This shift tended to group these products within similar ratings and somewhat reduced the algorithm´s discriminant capacity within this specific food group, which may explain the relatively lower discriminatory capacity of the 2023 NS-NPM DI in this group compared to the 2015 version. Furthermore, in our analysis, when using the 2015 NS-NPM DI, we observed an unexpected direction in bread and breakfast cereals consumption among participants. We found that participants in quartile 4 consumed less bread/breakfast cereals compared to those in quartile 1 (better nutritional quality). Typically, we might expect higher consumption in quartile 4, as those products tend to have lower nutritional quality overall (high sugar content and/or low fiber content) However, it is important to note that while the relative difference in bread consumption between the quartiles was statistically significant for the 2015 NS-NPM, it was no longer the case for the 2023 NS-NPM. Although bread and breakfast cereals consumption trends between the highest and lowest quartiles did not align with initial expectations for the 2015 NS-NPM, the lack of statistical significance in the 2023 NS-NPM DI suggests an enhancement in the reliability and validity of the updated dietary index.

Our study has some limitations that need to be discussed. Firstly, it is important to approach the generalization of the present findings with caution, as the participants in this study were primarily healthy children and adolescents from mainland France. Another limitation is inherent to the use of 24-hour records, which may bias the estimation of the intake of nutrient intakes, which in turn is crucial for the correct estimation of the NS-NPM DI. However, we employed high-quality dietary data, incorporating repeated 24-hour records obtained through a validated web-based tool (Ma et al., 2009). This approach ensures precise estimates of individual intakes.

The main strengths of our study notably include the utilization of a large representative sample size of the French population. In addition, to the best of our knowledge, this is the first study that assessed the discriminatory power of the 2023 NS-NPM DI at the individual´s level in children and adolescents. Moreover, the translation of nutrient profiles from the food level to individual dietary indices enabled us to consider the complexity of the entire diet. This approach facilitates a more comprehensive evaluation of dietary quality, considering the interactive or synergistic effects that individual dietary nutrients may exert within the context of the food matrix(Jacobs et al., 2009).

Conclusion

In conclusion, the results of the present study suggest that the Nutri-Score algorithm accurately discriminates diet quality, reinforcing the utility of this nutritional front-of-pack nutrition label as a useful tool for promoting healthier dietary choices in children and adolescents. Furthermore, the 2023 NS-NPM DI exhibited greater discriminatory

power in assessing fish and seafood, poultry, processed meat, nuts, vegetable oils and vitamin D compared to the previous version. This enhancement indicates that this revision represents a valuable improvement for public health purposes.

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Conflict of interest

Prof Salas-Salvadó reported being a nonpaid member of the scientific boards of the International Nut and Dried Fruit Foundation, Danone Institute International, and Fundación Eroski; receiving institutional grants from the International Nut and Dried Fruit Foundation; and receiving personal fees from Danone Institute Spain.

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RESULTS

Table 1. Socio-demographic characteristics across quartiles of NS-NPM DI (2023 and 2015 versions) in children and adolescents of the ESTEBAN study (n=1,279)

	Quartiles of 2023 NS-NPM DI *(New version)*					Quartiles of 2015 NS-NPM DI *(Old version)*				
	Q1 (%) *(Highest nutritional quality)*	**Q2** (%)	**Q3** (%)	**Q4** (%) *(Lowest nutritional quality)*	**p-value**	**Q1** (%) *(Highest nutritional quality)*	**Q2** (%)	**Q3** (%)	**Q4** (%) *(Lowest nutritional quality)*	**p-value**
Age					0.464					0.257
6 to 10 years	23.7	21.4	27	27.9		21.7	25	25.5	27.9	
11 to 14 years	24.6	27.8	22.8	24.8		24.3	26.6	23.1	26	
15 to 17 years	28.9	26.2	20.2	24.7		32.5	19.4	25.2	22.9	
Sex					0.507					0.234
Boys	23.3	27.1	22.8	26.8		22.2	26.7	25.3	25.8	
Girls	27.2	23.5	24.4	24.8		28.4	22.1	23.4	26.1	
Occupational situation (head of household)					0.758					0.747
Employed	23.9	25.4	24.4	26.3		23.8	25.2	25.1	25.9	
Unemployed	34.1	24.6	23.6	17.8		37.2	15.4	26.4	21	
Student, student in training, unpaid internship	38.3	22.8	20.7	18.2		32.2	28.9	22.6	16.3	
Retired, pre-retired	37.1	18.0	14.5	30.4		25	30.2	33.1	11.8	
Housewife or house-husband	27.0	28.9	12	32.1		29.5	22.8	13.1	34.6	
Other inactive (pension, disabled, etc.)	11.1	22.9	45.3	20.7		11.7	22.2	23.3	42.7	
Parents education level					0.221					0.169
Primary or lower education	27.9	32.8	14.7	24.6		25.8	32.3	13.9	27.9	
Highschool education	25.0	22	24.9	28.1		24.9	21.2	26.1	27.8	
University education	24.8	29.9	22.8	22.5		25.4	28.9	24	21.7	

Abbreviations: NS-NPM DI: Nutri-Score nutrient profiling model dietary index.
Data were expressed as percentages for categorical variables.
P-values for comparisons were tested by chi-square test according to the NS-NPM DI quartiles.

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RESULTS

Table 2. Spearman correlation coefficients between the 2015 NS-NPM DI version and the 2023 NS-NPM DI

	2023 NS-NPM DI (new version)	2015 NS-NPM DI (old version)
2023 NS-NPM DI (**new version**)	1	-
2015 NS-NPM DI (**old version**)	0.953*	1

*p-value <0.001

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RESULTS

Table 3. Food groups assessment across quartiles of NS-NPM DI (2023 and 2015 versions) in children and adolescents of the ESTEBAN study (n=1,279)

	Quartiles of 2023 NS-NPM DI *New version*							Quartiles of 2015 NS-NPM DI *Old version*						
	Q1 *(Highest nutritional quality)*	Q2	Q3	Q4 *(Lowest nutritional quality)*	p-value (Linear contrast)	Δ_{Q4-Q1}	Relative difference (%)	Q1 *(Highest nutritional quality)*	Q2	Q3	Q4 *(Lowest nutritional quality)*	p-value (Linear contrast)	Δ_{Q4-Q1}	Relative difference (%)
Food groups														
Fruits (g/day)	153 (11.6)	129 (7.77)	114 (7.77)	72.4 (6.24)	<0.001	-80.2 (13.2)	-53%	153 (11.7)	122 (7.56)	122 (7.56)	72.1 (6.28)	<0.001	-81.2 (13.3)	-53%
Vegetables (g/day)	158 (6.53)	146 (6.97)	119 (5.14)	93.2 (4.93)	<0.001	-64.8 (8.23)	-41%	160 (6.26)	137 (5.88)	127 (6.90)	92.5 (4.84)	<0.001	-67.9 (7.96)	-42%
Tubers (g/day)	65.3 (3.99)	52.0 (4.47)	55.4 (3.16)	49.8 (3.84)	0.005	-15.5 (5.54)	-24%	61.9 (3.89)	54.1 (4.59)	55.1 (3.32)	51.3 (3.80)	0.053	-10.5 (5.44)	-17%
Bread (g/day)	65.2 (3.08)	59.4 (3.65)	61.1 (3.11)	61.9 (3.38)	0.477	-3.25 (4.56)	-5%	70.3 (3.18)	61.9 (3.90)	59.3 (3.13)	56.2 (2.99)	0.001	-14.1 (4.34)	-25%
Cereals (g/day)	130 (6.46)	114 (5.39)	92.1 (4.15)	87.2 (4.88)	<0.001	-42.6 (8.05)	-33%	130 (6.82)	110 (5.04)	96.1 (4.18)	87.0 (4.81)	<0.001	-43.3 (8.29)	-20%
Breakfast cereals (g/day)	12.3 (1.25)	18.9 (1.70)	12.1 (1.41)	11.2 (1.29)	0.556	-1.06 (1.79)	-9%	14.8 (1.46)	14.9 (1.65)	13.7 (1.37)	11.3 (1.29)	0.072	-3.52 (1.95)	-24%
Whole grain cereals (g/day)	14.4 (2.16)	10.8 (1.78)	6.92 (1.12)	4.89 (1.17)	<0.001	-9.54 (2.44)	-66%	14.7 (2.19)	11.3 (1.77)	7.13 (1.14)	7.13 (1.14)	<0.001	-10.5 (2.46)	-72%
Milk (g/day)	213 (11.7)	212 (11.8)	159 (10.3)	99.7 (8.43)	<0.001	-114 (13.6)	-53%	219 (11.5)	207 (11.5)	156 (9.58)	104 (8.56)	<0.001	-114 (14.4)	-53%
Cheese (g/day)	25.5 (1.73)	27.2 (1.59)	31.0 (1.57)	30.6 (1.79)	0.041	5.06 (2.48)	20%	24.9 (1.69)	25.2 (1.45)	32.1 (1.58)	31.9 (1.86)	0.005	6.99 (2.50)	28%
Yogurt and Greek yogurt (g/day)	110 (6.25)	103 (5.74)	94.0 (5.74)	76.9 (5.72)	<0.001	-33.4 (8.49)	-30%	117 (6.88)	101 (5.29)	88.1 (5.19)	77.4 (5.73)	<0.001	-39.6 (8.97)	-34%
Red meat (g/day)	56.2 (3.36)	53.9 (3.50)	44.5 (2.66)	39.8 (2.78)	<0.001	-16.4 (4.35)	-29%	55.3 (3.39)	53.0 (3.54)	44.5 (2.55)	41.8 (2.96)	0.003	-13.5 (4.49)	-24%
Poultry (g/day)	45.3 (3.93)	34.9 (3.28)	24.0 (2.01)	22.8 (2.94)	<0.001	-22.5 (4.92)	-50%	42.8 (3.98)	34.1 (2.95)	26.2 (2.53)	24.5 (3.02)	<0.001	-18.3 (4.99)	-43%
Eggs (g/day)	14.2 (1.92)	10.4 (1.14)	10.3 (1.11)	7.11 (1.26)	0.002	-7.05 (2.29)	-50%	13.7 (1.81)	10.4 (1.25)	10.7 (1.17)	7.23 (1.27)	0.004	-6.45 (2.22)	-47%
Fish and Seafood (g/day)	29.9 (2.75)	19.2 (1.72)	20.9 (2.16)	14.4 (2.03)	<0.001	-15.5 (3.42)	-52%	27.7 (2.61)	23.3 (2.17)	18.8 (1.88)	14.7 (2.05)	<0.001	-12.9 (3.32)	-47%
Processed meat (g/day)	24.8 (1.71)	27.7 (1.92)	36.3 (2.30)	50.4 (2.92)	<0.001	25.6 (3.39)	103%	26.6 (1.81)	28.9 (2.16)	34.8 (2.25)	48.6 (2.85)	<0.001	21.9 (3.38)	83%
Nuts (g/day)	1.29 (0.26)	1.55 (0.30)	1.11 (0.28)	0.58 (0.14)	0.018	-0.71 (0.29)	-55%	1.17 (0.26)	1.48 (0.26)	1.28 (0.33)	0.62 (0.15)	0.068	-0.54 (0.29)	-47%
Sweetened foods (g/day)	94.8 (3.14)	116 (4.11)	141 (3.98)	157 (5.36)	<0.001	61.9 (6.18)	66%	91.6 (3.20)	120 (4.24)	137 (3.76)	158 (5.34)	<0.001	66.6 (6.21)	72%
Vegetable oil (g/day)	9.00 (0.61)	7.60 (0.42)	6.81 (0.39)	6.51 (0.62)	0.004	-2.49 (0.88)	-28%	8.17 (0.58)	7.56 (0.49)	7.45 (0.41)	6.78 (0.62)	0.105	-1.38 (0.85)	-17%
Animal fats (g/day)	12.65 (0.78)	13.3 (0.90)	14.6 (1.17)	11.8 (0.77)	0.461	-0.81 (1.09)	-7%	12.5 (0.75)	12.8 (0.93)	14.9 (1.09)	12.1 (0.83)	0.695	-0.44 (1.12)	-3%
Sweetened beverages (g/day)	87.3 (6.75)	99.5 (9.71)	126 (9.47)	212 (19.4)	<0.001	125 (20.6)	143%	83.4 (6.41)	99.9 (9.17)	136 (9.91)	205 (19.7)	<0.001	122 (20.8)	146%
Artificially sweetened beverages (g/day)	12.9 (3.95)	3.42 (1.14)	12.7 (2.86)	10.3 (3.79)	0.630	-2.63 (5.48)	-20%	12.4 (3.95)	3.37 (1.04)	11.8 (2.77)	11.3 (3.82)	0.840	-1.11 (5.49)	-9%
Fruit and vegetables juices (g/day)	67.1 (5.37)	74.9 (6.78)	74.9 (8.27)	54.1 (6.09)	0.111	-13.0 (8.15)	-19%	73.3 (5.67)	75.1 (6.43)	75.4 (8.66)	47.4 (5.59)	0.001	-25.9 (7.99)	-35%
Water (g/day)	802 (30.7)	777 (42.4)	690 (26.4)	681 (32.2)	0.007	-121 (44.9)	-15%	821 (32.5)	771 (41.7)	698 (27.7)	664 (30.8)	0.001	-157 (45.0)	-19%

Abbreviations: NS-NPM DI: Nutri-Score nutrient profiling model dietary index
Data were expressed as mean (SE) across quartiles.
Deltas expressed as mean difference (SE).
All values were adjusted for total energy intake using the residual method except for energy intake (kcal/day).

Table 4. Nutrient intake assessment across quartiles of NS-NPM DI (2023 and 2015 versions) in children and adolescents of the ESTEBAN study (n=1,279)

	Quartiles of 2023 NS-NPM DI *New version*							Quartiles of 2015 NS-NPM DI *Old version*						
	Q1 *(Highest nutritional quality)*	Q2	Q3	Q4 *(Lowest nutritional quality)*	p-value (Linear contrast)	Δ_{Q4-Q1}	Relative difference (%)	Q1 *(Highest nutritional quality)*	Q2	Q3	Q4 *(Lowest nutritional quality)*	p-value (Linear contrast)	Δ_{Q4-Q1}	Relative difference (%)
Nutrients														
Energy intake (kcal/day)	1675 (29.8)	1913 (40.8)	1855 (34.8)	2051 (47.8)	<0.001	376 (55.9)	22%	1697 (32.4)	1875 (40.9)	1859 (35.4)	2062 (46.3)	<0.001	365 (56.1)	22%
Age-adjusted energy intake (kcal/day)	1658 (31.5)	1904 (38.8)	1858 (35.0)	2049 (46.1)	<0.001	391 (55.4)	24%	1673 (33.4)	1877 (38.7)	1856 (35.9)	2062 (44.3)	<0.001	389 (55.1)	23%
Proteins %	17.8 (0.26)	16.4 (0.22)	15.3 (0.16)	14.3 (0.20)	<0.001	-3.51 (0.33)	-20%	17.7 (0.26)	16.4 (0.21)	15.2 (0.18)	14.4 (0.21)	<0.001	-3.27 (0.33)	-19%
Carbohydrates %	45.4 (0.45)	45.8 (0.40)	44.8 (0.42)	44.7 (0.47)	0.247	-0.75 (0.65)	-2%	46.3 (0.43)	45.7 (0.42)	44.9 (0.41)	43.8 (0.46)	<0.001	-2.49 (0.64)	-5%
Lipids %	36.4 (0.39)	37.5 (0.31)	39.5 (0.35)	40.8 (0.42)	<0.001	4.42 (0.57)	12%	35.5 (0.35)	37.6 (0.33)	39.4 (0.31)	41.5 (0.42)	<0.001	5.93 (0.54)	17%
SFA (g/day)	30.3 (0.38)	33.0 (0.47)	35.6 (0.42)	37.2 (0.48)	<0.001	6.84 (0.62)	23%	29.8 (0.37)	32.8 (0.42)	35.5 (0.41)	37.9 (0.51)	<0.001	8.16 (0.63)	27%
MUFA (g/day)	27.7 (0.41)	28.5 (0.34)	29.7 (0.36)	30.9 (0.49)	<0.001	3.21 (0.63)	12%	27.1 (0.39)	28.7 (0.37)	29.7 (0.33)	31.4 (0.48)	<0.001	4.26 (0.62)	16%
PUFA (g/day)	11.6 (0.33)	10.8 (0.22)	11.1 (0.30)	11.0 (0.28)	0.136	-0.64 (0.43)	-5%	11.2 (0.28)	11.1 (0.28)	11.1 (0.22)	11.2 (0.33)	0.991	0.01 (0.44)	0%
Simple sugars (g/day)	102 (1.86)	107 (1.99)	108 (1.72)	111 (1.91)	<0.001	9.34 (2.66)	9%	103 (1.96)	105 (1.76)	109 (1.87)	109 (1.94)	0.026	6.15 (2.75)	6%
Added sugars (g/day)	47.1 (1.15)	55.7 (1.49)	62.0 (1.31)	74.2 (1.81)	<0.001	27.1 (2.15)	58%	47.2 (1.15)	56.1 (1.52)	61.7 (1.29)	73.9 (1.81)	<0.001	26.6 (2.15)	57%
Fibers (g/day)	16.3 (0.31)	15.1 (0.25)	14.0 (0.23)	12.7 (0.23)	<0.001	-3.31 (0.39)	-22%	16.6 (0.30)	14.9 (0.24)	14.1 (0.24)	12.5 (0.22)	<0.001	-4.08 (0.38)	-25%
Vitamins and minerals														
Calcium (mg/day)	952 (18.5)	933 (16.9)	891 (18.6)	812 (19.1)	<0.001	-139 (26.5)	-15%	972 (20.3)	917 (15.9)	880 (15.8)	819 (19.6)	<0.001	-153 (28.2)	-16%
Potassium (mg/day)	2802 (33.3)	2598 (28.7)	2407 (25.9)	2131 (29.7)	<0.001	-671 (44.5)	-24%	2819 (33.2)	2566 (27.8)	2431 (25.5)	2129 (30.1)	<0.001	-690 (44.9)	-24%

RESULTS

Magnesium (mg/day)	277 (3.19)	258 (2.40)	242 (2.59)	220 (2.76)	<0.001	-57.2 (4.23)	-21%	277 (3.08)	257 (2.56)	245 (2.46)	218 (2.71)	<0.001	-59.5 (4.10)	-21%
Phosphorus (mg/day)	1280 (13.8)	1219 (12.9)	1137 (11.8)	1072 (15.5)	<0.001	-208 (20.8)	-16%	1279 (14.3)	1215 (12.3)	1134 (11.1)	1082 (15.9)	<0.001	-197 (21.5)	-15%
Sodium (mg/day)	2664 (38.1)	2584 (42.1)	2552 (42.5)	2645 (52.9)	0.772	-18.9 (65.2)	-1%	2705 (39.3)	2591 (38.8)	2572 (45.0)	2579 (51.9)	0.054	-125 (65.1)	-5%
Iron (mg/day)	11.6 (0.23)	11.4 (0.19)	10.7 (0.17)	10.6 (0.36)	0.027	-0.95(0.43)	-9%	11.6 (0.20)	11.3 (0.23)	10.9 (0.18)	10.6 (0.35)	0.017	-0.97 (0.41)	-9%
Zinc (mg/day)	10.8 (0.19)	10.2 (0.17)	9.45 (0.14)	8.95 (0.15)	<0.001	-1.82 (0.24)	-17%	10.7 (0.19)	10.1 (0.17)	9.48 (0.15)	9.08 (0.16)	<0.001	-1.64 (0.25)	-15%
Retinol (µg/day)	338 (11.6)	368 (16.9)	408 (16.9)	387 (16.6)	0.015	49.5 (20.3)	14%	346 (18.2)	356 (10.5)	398 (15.6)	399 (17.1)	0.032	53.8 (25.0)	15%
Vitamin B1 (mg/day)	1.22 (0.02)	1.23 (0.03)	1.11 (0.02)	1.11 (0.02)	0.001	-0.11 (0.03)	-9%	1.24 (0.03)	1.19 (0.03)	1.13 (0.02)	1.09 (0.02)	<0.001	-0.14 (0.03)	-12%
Vitamin B2 (mg/day)	1.73 (0.03)	1.72 (0.03)	1.54 (0.03)	1.36 (0.02)	<0.001	-0.37 (0.04)	-21%	1.75 (0.03)	1.67 (0.03)	1.55 (0.03)	1.38 (0.02)	<0.001	-0.37 (0.04)	-21%
Vitamin B9 (µg/day)	268 (5.59)	269 (6.10)	240 (4.21)	211 (4.07)	<0.001	-56.4 (6.95)	-21%	272 (5.63)	259 (5.32)	247 (5.57)	210 (4.00)	<0.001	-62.2 (6.94)	-23%
Vitamin B12 (µg/day)	4.55 (0.15)	3.89 (0.15)	3.89 (0.15)	3.44 (0.11)	<0.001	-1.11 (0.19)	-24%	4.44 (0.15)	3.95 (0.12)	3.82 (0.14)	3.55 (0.12)	<0.001	-0.89 (0.19)	-20%
β-Carotene (µg/day)	2445 (134)	1973 (89.8)	1855 (104)	1487 (97.7)	<0.001	-958 (167)	-39%	2388 (116)	1964 (112)	1963 (107)	1456 (97.9)	<0.001	-932 (152)	-39%
Vitamin C (mg/day)	99.0 (3.89)	97.4 (3.88)	94.1 (4.55)	75.8 (4.14)	<0.001	-23.3 (5.67)	-23%	100 (4.09)	96.0 (3.51)	99.1 (4.84)	71.6 (3.82)	<0.001	-28.5 (5.58)	-28%
Vitamin D (µg/day)	2.32 (0.17)	1.70 (0.07)	1.95 (0.09)	1.13 (0.11)	0.004	-0.59 (0.20)	-51%	2.16 (0.15)	1.87 (0.11)	1.84 (0.07)	1.83 (0.12)	0.089	-0.33 (0.19)	-15%
Vitamin E (mg/day)	11.9 (0.37)	10.9 (0.25)	11.0 (0.36)	10.6 (0.29)	0.005	-1.35 (0.48)	-11%	11.5 (0.33)	11.1 (0.32)	11.1 (0.25)	10.8 (0.38)	0.145	0.74 (0.50)	-6%

Abbreviations: NS-NPM DI: Nutri-Score nutrient profiling model dietary index; SFA: saturated fatty acids; MUFA monounsaturated fatty acids; PUFA: polyunsaturated fatty
Data were expressed as mean (SE) across quartiles.
Deltas expressed as mean difference (SE).
All values were adjusted for total energy intake using the residual method except for energy intake (kcal/day)

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Supplementary Material

Ability of a dietary index based on the updated algorithm underpinning the Nutri-Score to discriminate food consumption and nutrient intake in a French population of children and adolescents

Nadine Khoury[1,2,3], Barthélémy Sarda[4], Mathilde Touvier[4], Emmanuelle Kesse-Guyot[4], Jordi Salas-Salvadó[1,2,3], Nancy Babio[1,2,3], María Ángeles Martínez[1,2,3], Serge Hercberg[4,5], Pilar Galan[4], Pauline Ducrot[6], Valérie Deschamps[7], Chantal Julia[4,5*]

1. Universitat Rovira i Virgili, Departament de Bioquímica i Biotecnologia, Unitat de Nutrició Humana, ANUT-DSM group. Spain.
2. Institut d'Investigació Sanitària Pere Virgili (IISPV), Reus, Spain.
3. Consorcio CIBER, M.P. Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III (ISCIII), Madrid, Spain.
4. Université Sorbonne Paris Nord and Université Paris Cité, INSERM, INRAE, CNAM, Center of Research in Epidemiology and StatisticS (CRESS), Nutritional Epidemiology Research Team (EREN), F-93017 Bobigny, France
5. Public health Department, Hôpital Avicenne, Assistance Publique-Hôpitaux de Paris (AP-HP), Bobigny, France.
6. Santé publique France, French national public health agency, F-94415, Saint-Maurice, France.
7. Nutritional Epidemiology Surveillance Team (ESEN), Santé Publique France, The French Public Health Agency, Bobigny, France.

***Corresponding author:** Chantal Julia, c.julia@eren.smbh.univ-paris13.fr, 74 rue Marcel Cachin, 93017 Bobigny

Supplementary Material File S1. The updated Nutri-Score algorithms

	Nutri-Score Algorithm for general food						
	Unfavorable components				Favorable components		
Points	Energy (kJ per 100g)	Sugars (g per 100g)	Saturated fat (g per 100g)	Salt (g per 100g)	Protein* (g per 100g)	Fiber (g per 100g)	Fruit, vegetables, legumes (%)
0	≤ 335	≤ 3.4	≤ 1.0	≤ 0.2	≤ 2.4	≤ 3.0	≤ 40
1	> 335	> 3.4	> 1.0	> 0.2	> 2.4	> 3.0	> 40
2	> 670	> 6.8	> 2.0	> 0.4	> 4.8	> 4.1	> 60
3	> 1005	> 10	> 3.0	> 0.6	> 7.2	> 5.2	-
4	> 1340	> 14	> 4.0	> 0.8	> 9.6	> 6.3	-
5	> 1675	> 17	> 5.0	> 1.0	> 12	> 7.4	> 80
6	> 2010	> 20	> 6.0	> 1.2	> 14		
7	> 2345	> 24	> 7.0	> 1.4	> 17		
8	> 2680	> 27	> 8.0	> 1.6			
9	> 3015	> 31	> 9.0	> 1.8			
10	> 3350	> 34	> 10	> 2.0			
11		> 37		> 2.2			
12		> 41		> 2.4			
13		> 44		> 2.6			
14		> 48		> 2.8			
15		> 51		> 3.0			
16				> 3.2			
17				> 3.4			
18				> 3.6			
19				> 3.8			
20				> 4.0			

*Red meat products are given maximum 2 protein points.

Nutri-Score total points for general food	Class	Color
≤ 0	A	Dark green
1 to 2	B	Light green
3 to 10	C	Yellow
11 to 18	D	Light orange
≥ 19	E	Dark orange

RESULTS

	Nutri-Score total points for fats, oils, nuts and seeds						
	Unfavorable components				Favorable components		
Points	Energy from saturated fat (kJ per 100g) *	Sugars (g per 100g)	Saturated fat/total fat (%)	Salt (g per 100g)	Protein (g per 100g)	Fiber (g per 100g)	Fruit, vegetables, legumes (%)
0	≤ 120	≤ 3.4	< 10	≤ 0.2	≤ 2.4	≤ 3.0	≤ 40
1	> 120	> 3.4	< 16	> 0.2	> 2.4	> 3.0	> 40
2	> 240	> 6.8	< 22	> 0.4	> 4.8	> 4.1	> 60
3	> 360	> 10	< 28	> 0.6	> 7.2	> 5.2	-
4	> 480	> 14	< 34	> 0.8	> 9.6	> 6.3	-
5	> 600	> 17	< 40	> 1.0	> 12	> 7.4	> 80
6	> 720	> 20	< 46	> 1.2	> 14		
7	> 840	> 24	< 52	> 1.4	> 17		
8	> 960	> 27	< 58	> 1.6			
9	> 1080	> 31	< 64	> 1.8			
10	> 1200	> 34	≥ 64	> 2.0			
11		> 37		> 2.2			
12		> 41		> 2.4			
13		> 44		> 2.6			
14		> 48		> 2.8			
15		> 51		> 3.0			
16				> 3.2			
17				> 3.4			
18				> 3.6			
19				> 3.8			
20				> 4.0			

*Energy from saturated fat = saturated fat (g per 100 grams) x 37

Nutri-Score total points for fats, oils, nuts and seeds	Class	Color
≤ -6	A	Dark green
-5 to 2	B	Light green
3 to 10	C	Yellow
11 to 18	D	Light orange
≥ 19	E	Dark orange

RESULTS

	Nutri-Score total points for beverages							
	Unfavorable components					Favorable components		
Points	Energy (kJ per 100ml)	Sugars (g per 100ml)	Saturated fat (g per 100ml)	Salt (g per 100ml)	Non-nutritive sweeteners (absence/presence)	Protein (g per 100ml)	Fiber (g per 100ml)	Fruit, vegetables, legumes (%)
0	≤ 30	≤ 0.5	≤ 1.0	≤ 0.2		≤ 1.2	≤ 3.0	≤ 40
1	≤ 90	≤ 2.0	> 1.0	> 0.2		> 1.2	> 3.0	-
2	≤ 150	≤ 3.5	> 2.0	> 0.4		> 1.5	> 4.1	> 40
3	≤ 210	≤ 5.0	> 3.0	> 0.6		> 1.8	> 5.2	-
4	≤ 240	≤ 6.0	> 4.0	> 0.8	Presence	> 2.1	> 6.3	> 60
5	≤ 270	≤ 7.0	> 5.0	> 1.0		> 2.4	> 7.4	-
6	≤ 300	≤ 8.0	> 6.0	> 1.2		> 2.7		> 80
7	≤ 330	≤ 9.0	> 7.0	> 1.4		> 3.0		
8	≤ 360	≤ 10	> 8.0	> 1.6				
9	≤ 390	≤ 11	> 9.0	> 1.8				
10	> 390	> 11	> 10	> 2.0				
				> 2.2				
				> 2.4				
				> 2.6				
				> 2.8				
				> 3.0				
				> 3.2				
				> 3.4				
				> 3.6				
				> 3.8				
				> 4.0				

Nutri-Score total points for beverages	Class	Color
Water*	A	Dark green
≤ 2	B	Light green
3 to 6	C	Yellow
7 to 9	D	Light orange
≥ 10	E	Dark orange

*Plain water was automatically given Nutri-Score class A.

Chapter 2: Association between the (Ultra)-processing/formulation dimension of foods and cardiometabolic risk factors

c. Article 3

Title: Ultraprocessed Food Consumption and Cardiometabolic Risk Factors in Children

Nadine Khoury, María Ángeles Martínez, Tany E Garcidueñas-Fimbres, Belén Pastor-Villaescusa, Rosaura Leis, Sara de Las Heras-Delgado, María L Miguel-Berges, Santiago Navas-Carretero, Olga Portoles, Karla Alejandra Pérez-Vega, Jose Manuel Jurado-Castro, Rocío Vázquez-Cobela, Gisela Mimbrero, Raquel Andía Horno, J Alfredo Martínez, Katherine Flores-Rojas, Rosaura Picáns-Leis, Verónica Luque, Luis A Moreno, Cristina Castro-Collado, Mercedes Gil-Campos, Jordi Salas-Salvadó, Nancy Babio

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Summary:

Of 1509 enrolled CORALS participants, 1426 (mean [SD] age, 5.8 [1.1] years; 698 boys [49.0%]) were included in this study. Mothers of children with high UPF consumption were younger, had a higher BMI, were more likely to have overweight or obesity, and had lower education levels and employment rates. Compared with participants in the lowest tertile of energy-adjusted UPF consumption, those in the highest tertile showed higher z scores of BMI (β coefficient, 0.20; 95% CI, 0.05-0.35), waist circumference (β coefficient, 0.20; 95% CI, 0.05-0.35), fat mass index (β coefficient, 0.17; 95% CI, 0.00-0.32), and fasting plasma glucose (β coefficient, 0.22; 95% CI, 0.06-0.37) and lower z scores for HDL cholesterol (β coefficient, -0.19; 95% CI, -0.36 to -0.02). One-SD increments in energy-adjusted UPF consumption were associated with higher z scores for BMI (β coefficient, 0.11; 95% CI, 0.05-0.17), waist circumference (β coefficient, 0.09; 95% CI, 0.02-0.15), fat mass index (β coefficient, 0.11; 95% CI, 0.04-1.18), and fasting plasma glucose (β coefficient, 0.10; 95% CI, 0.03-0.17) and lower HDL cholesterol (β coefficient, -0.07; 95% CI, -0.15 to -0.00). Substituting 100 g of UPFs with 100 g of unprocessed or minimally processed foods was associated with lower z scores of BMI (β coefficient, -0.03; 95% CI, -0.06 to -0.01), fat mass index (β coefficient, -0.03; 95% CI, -0.06 to 0.00), and fasting plasma glucose (β coefficient, -0.04; 95% CI, -0.07 to -0.01).

Conclusions and relevance: These findings suggest that high UPF consumption in young children is associated with adiposity and other cardiometabolic risk factors, highlighting the need for public health initiatives to promote the replacement of UPFs with unprocessed or minimally processed foods.

RESULTS

JAMA Network Open.

Original Investigation | Nutrition, Obesity, and Exercise

Ultraprocessed Food Consumption and Cardiometabolic Risk Factors in Children

Nadine Khoury, MSc; María Ángeles Martínez, PhD; Tany E. Garcidueñas-Fimbres, MD, PhD; Belén Pastor-Villaescusa, PhD; Rosaura Leis, MD, PhD; Sara de Las Heras-Delgado, MSc; María L. Miguel-Berges, PhD; Santiago Navas-Carretero, PhD; Olga Portoles, PhD; Karla Alejandra Pérez-Vega, PhD; Jose Manuel Jurado-Castro, PhD; Rocío Vázquez-Cobela, PhD; Gisela Mimbrero, RN; Raquel Andía Horno, RN; J. Alfredo Martínez, MD, PhD; Katherine Flores-Rojas, PhD; Rosaura Picáns-Leis, MD; Verónica Luque, PhD; Luis A. Moreno, MD, PhD; Cristina Castro-Collado, MSc; Mercedes Gil-Campos, MD, PhD; Jordi Salas-Salvadó, MD, PhD; Nancy Babio, PhD

Abstract

IMPORTANCE High intake of ultraprocessed foods (UPFs) has been associated with higher cardiometabolic risk in adults; however, the evidence in children is limited.

OBJECTIVE To investigate the association between UPF consumption and cardiometabolic risk factors in the Childhood Obesity Risk Assessment Longitudinal Study (CORALS).

DESIGN, SETTING, AND PARTICIPANTS This baseline cross-sectional analysis was conducted using the data of CORALS participants recruited between March 22, 2019, and June 30, 2022. Preschool children (aged 3-6 years) were recruited from schools and centers in 7 cities in Spain. Inclusion criteria included informed consent signed by parents or caregivers and having a completed a set of questionnaires about the child's prenatal history at home. Exclusion criteria included low command of Spanish or unstable residence.

EXPOSURE Energy-adjusted UPF consumption (in grams per day) from food frequency questionnaires and based on the NOVA food classification system.

MAIN OUTCOMES AND MEASURES Age- and sex-specific *z* scores of adiposity parameters (body mass index [BMI], fat mass index, waist-to-height ratio, and waist circumference) and cardiometabolic parameters (diastolic and systolic blood pressure, fasting plasma glucose, homeostasis model assessment for insulin resistance, high-density and low-density lipoprotein cholesterol, and triglycerides) were estimated using linear regression models.

RESULTS Of 1509 enrolled CORALS participants, 1426 (mean [SD] age, 5.8 [1.1] years; 698 boys [49.0%]) were included in this study. Mothers of children with high UPF consumption were younger, had a higher BMI, were more likely to have overweight or obesity, and had lower education levels and employment rates. Compared with participants in the lowest tertile of energy-adjusted UPF consumption, those in the highest tertile showed higher *z* scores of BMI (β coefficient, 0.20; 95% CI, 0.05-0.35), waist circumference (β coefficient, 0.20; 95% CI, 0.05-0.35), fat mass index (β coefficient, 0.17; 95% CI, 0.00-0.32), and fasting plasma glucose (β coefficient, 0.22; 95% CI, 0.06-0.37) and lower *z* scores for HDL cholesterol (β coefficient, −0.19; 95% CI, −0.36 to −0.02). One-SD increments in energy-adjusted UPF consumption were associated with higher *z* scores for BMI (β coefficient, 0.11; 95% CI, 0.05-0.17), waist circumference (β coefficient, 0.09; 95% CI, 0.02-0.15), fat mass index (β coefficient, 0.11; 95% CI, 0.04-1.18), and fasting plasma glucose (β coefficient, 0.10; 95% CI, 0.03-0.17) and lower HDL cholesterol (β coefficient, −0.07; 95% CI, −0.15 to −0.00). Substituting 100 g of UPFs with 100 g of unprocessed or minimally processed foods was associated with lower *z* scores of BMI (β coefficient, −0.03; 95% CI, −0.06 to −0.01), fat mass index (β coefficient, −0.03; 95% CI, −0.06 to 0.00), and fasting plasma glucose (β coefficient, −0.04; 95% CI, −0.07 to −0.01).

(continued)

Key Points

Question What is the association of consuming ultraprocessed foods (UPFs) with cardiometabolic risk factors in children?

Findings In this cross-sectional study of 1426 children, higher consumption of UPFs was positively associated with body mass index, waist circumference, fat mass index, and fasting plasma glucose and negatively associated with high-density lipoprotein cholesterol concentrations.

Meaning These findings highlight the need for public health initiatives to promote the replacement of UPFs with unprocessed or minimally processed foods.

+ Supplemental content

Author affiliations and article information are listed at the end of this article.

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RESULTS

Abstract (continued)

CONCLUSIONS AND RELEVANCE These findings suggest that high UPF consumption in young children is associated with adiposity and other cardiometabolic risk factors, highlighting the need for public health initiatives to promote the replacement of UPFs with unprocessed or minimally processed foods.

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Introduction

The presence of abnormal cardiometabolic risk factors often begins in childhood, highlighting the importance of identifying and controlling them early to delay or prevent cardiovascular disease (CVD) in the future.[1] Modifiable risk factors (eg, diet and physical activity) may contribute to the development of recognized cardiometabolic risk factors.[2-4] Emerging studies have shed light on the potential role of ultraprocessed foods (UPFs) in determining the risk of chronic diseases,[5-7] independent of their nutritional profiles.[8]

Commonly, UPFs represent a category of food products that undergo extensive industrial processing, often containing multiple ingredients, additives, and preservatives to make them not only convenient (ready to eat) but also palatable and appealing. This approach has been used to create the most widely used UPF classification, the NOVA Food Classification system.[9,10] Ultraprocessed foods are typically rich in saturated fats, sugars, sodium, and other substances (eg, additives) and lower in essential nutrients, all of which are associated with cardiometabolic health.[11,12] Due to their high availability and affordability and wide marketing to children, UPFs have become increasingly frequent in modern diets, particularly among children, adolescents,[13-15] and their families, and especially among individuals and families with low socioeconomic status and educational levels in which obesity is more prevalent.[16] Additionally, the habits established during early childhood often track to later ages[17] and carry into adulthood, compounding the risk of CVD and other noncommunicable diseases.[18-20]

Previous observational studies in adults have reported positive associations between UPF consumption and obesity,[21] type 2 diabetes,[22] CVD,[23] and all-cause mortality[24]; however, the epidemiologic evidence in children remains limited and controversial.[25] While the majority of studies have reported unfavorable associations with body mass index (BMI), others did not find this association, and few have focused on cardiometabolic risk factors.[25]

Given the public health burden of CVDs and the increasing availability of UPFs, having a better understanding of potential associations between UPF consumption and cardiometabolic risk factors in children is essential. Therefore, the aim of this study was to examine the associations between UPF consumption and cardiometabolic risk factors in a population of Spanish preschool children (aged 3-6 years).

Methodology

Population and Study Design

This cross-sectional study was conducted using data from the Childhood Obesity Risk Assessment Longitudinal Study (CORALS), which followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline. The ethics committee of each of recruitment center approved the study protocol, which was conducted following the standards of the Declaration of Helsinki.[26] Parents or caregivers provided written informed consent.

CORALS is an ongoing prospective multicenter study conducted in 7 Spanish centers aiming to identify potential risk factors for childhood obesity over a 10-year follow-up period. A detailed description of the CORALS is published elsewhere.[27] Between March 22, 2019, and June 30, 2022,

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eligible participants aged 3 to 6 years at enrollment were recruited from schools across 7 cities in Spain. To be enrolled in the study, parents or caregivers had to sign a consent form, attend the inclusion face-to-face visit and complete several questionnaires at home for data collection on leisure time physical activity, 3-day food consumption, and sociodemographics. The exclusion criteria included belonging to a family with difficulty collaborating due to low command of Spanish or unstable residence.

Dietary Intake of UPFs and NOVA Food Classification System

To estimate the dietary intake of UPFs, trained dietitians (B.P.-V., S.d.L.H.D., M.L.M.-B., K.A.P.-V., and R.V.-C.) used the validated, semiquantitative, 125-item food and beverage frequency COME-Kids questionnaire.[28] Participants with energy intake below the first percentile or above the 99th percentile were excluded to minimize misreporting (details are provided in the eAppendix in Supplement 1). The NOVA Food Classification system was used to determine the consumption of food depending on its degree of processing[27] (details are provided in the eAppendix and eTable 1 in Supplement 1).

Outcomes

Adiposity Measurements

Adiposity measurements and cardiometabolic risk factor assessments were conducted in health care centers. Weight and body fat mass were measured using a precision scale and an octopolar multifrequency bioelectrical impedance device (MC780MAS; Tanita). Height was measured using a portable seca 213 stadiometer according to standard procedures. Body mass index was calculated and categorized as underweight or normal weight or as overweight or obesity based on pediatric cutoffs.[29] Waist circumference was determined using a flexible, nonextensible measuring tape. The fat mass index was estimated by dividing body fat mass in kilograms by height in meters squared.[30] Waist-to-height ratio was estimated by dividing waist circumference in centimeters by height in centimeters.[31]

Cardiometabolic Risk Factors Assessment

Blood pressure was measured in the nondominant arm 3 times, with a 5-minute gap between each measurement, using an automatic oscillometer (M3 Intelligence HEM-75051-EV; OMRON Healthcare) equipped with a child-sized cuff. Eight-hour fasting blood samples were collected from participants, and serum total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, plasma glucose, and insulin concentrations were measured using standard procedures. Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated as fasting insulin (μIU/mL) × fasting glucose (mmol/L) / 22.5.

Covariates

Parents or caregivers were provided with a set of questionnaires to complete at home, gathering information on early life factors, maternal characteristics, and lifestyle patterns. To assess physical activity, the total time (in hours) of engagement in sports and physical activities per week was estimated using a previously validated questionnaire.[32] An 18-item questionnaire for children was used to assess adherence to the Mediterranean diet, an indicator of diet quality.[27]

Statistical Analysis

The current analysis was conducted using the CORALS database updated through January 20, 2023. Analysis of descriptive baseline characteristics are reported as mean (SD) or median (IQR) for continuous variables and as numbers with percentages for categorical variables using one-way analysis of variance and χ^2 test, respectively.

Consumption of UPFs (in grams per day) was adjusted for total energy intake using the residual regression method.[33] Intake of UPFs in grams per day was calculated instead of energy percentage

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to account for foods with no energy content (eg, artificially sweetened beverages) and for nonnutritional concerns associated with food processing (eg, food additives). Participants were categorized by tertiles of energy-adjusted UPF consumption, ranging from tertile 1 for the lowest intake to tertile 3 for the highest intake.

Age- and sex-specific *z* scores of each outcome were estimated from standardized residuals conducted using linear regression models. Missing data of less than 5% for covariates were imputed to the mean and to the highest frequency category for quantitative and qualitative confounders, respectively.[34] Multivariable linear regression models were fitted to assess the associations (β coefficient and 95% CI) between tertiles of energy-adjusted UPFs and *z* scores of cardiometabolic risk factors. The first tertile (lowest intake) was considered the reference. Models were adjusted for maternal education level (primary or lower, secondary or university), maternal BMI (underweight, normal overweight, obesity), physical activity (minutes per week), exclusive breastfeeding (yes or no), recruitment center size (<200, 200-400, >400 participants), and NOVA group 1, 2, or 3 (as detailed in eTable 1 in Supplement 1). To assess the linear trend, the median value of each tertile of UPF consumption was modeled as a continuous variable. The analysis was also conducted in a continuous form, with a 1-SD increment and using the same confounders.

Additionally, a simulation model was fitted to substitute 100 g of consumed UPFs with 100 g of unprocessed or minimally processed food to examine the association of healthier food consumption with the outcomes. The theoretical impact of substituting 1 food group for another was assessed by introducing both variables simultaneously as continuous variables into the model. Differences in the β coefficients, variances, and covariance were used to estimate the β coefficients and 95% CIs for the substitution association. Sensitivity analyses were conducted to assess associations according diet quality, maternal education, and socioprofessional level (details provided in the eAppendix in Supplement 1).

Data were analyzed using Stata, version 14 software (StataCorp LLC). All statistical tests were 2-sided, and $P < .05$ was deemed statistically significant.

Results

A total of 1426 participants (mean [SD] age, of 5.8 [1.1] years; 698 boys [49.0%] and 728 girls [51.0%]) were included in this study after excluding 54 participants lacking the food and beverage frequency questionnaire and 29 with missing data or implausible reported energy intake (eFigure 1 in Supplement 1). The characteristics of the study population across tertiles of energy-adjusted UPF consumption are shown in **Table 1**. Children in the third tertile (highest UPF consumption) had a higher BMI *z* score, waist-to-height ratio, fat mass index, systolic blood pressure, and overweight or obesity prevalence and lower HDL and LDL cholesterol. Mothers whose children were categorized in the highest tertile of energy-adjusted UPF consumption were younger, had a higher BMI, were more prone to be living with overweight or obesity, were less likely to have exclusively breastfed their children, and had lower educational achievement and employment rates.

General dietary characteristics of participants are shown in **Table 2**. Children in the top tertile were more likely to consume higher amounts of total energy, carbohydrates, yogurt, other dairy products, sugar and candy, and sugary beverages and lower amounts of protein, fat, monounsaturated and polyunsaturated fatty acids, fiber, milk, cheese, white meat, unprocessed red meat, eggs, fish, seafood, vegetables, fruits, nuts, legumes, whole and refined cereals, and oils and fat.

Cross-sectional associations between energy-adjusted UPF consumption across tertiles and by 1-SD increment (in grams per day) and cardiometabolic risk factors are shown in **Table 3**. Compared with participants in the lowest tertile, those in the top tertile had higher *z* scores of waist circumference (β coefficient, 0.20; 95% CI, 0.05-0.35), BMI (β coefficient, 0.20; 95% CI, 0.05-0.35), fat mass index (β coefficient, 0.17; 95% CI, 0.00-0.32), and fasting plasma glucose (β coefficient, 0.22; 95% CI, 0.06-0.37). Additionally, participants in the highest tertile had a lower *z* score of HDL

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cholesterol (β coefficient, −0.19; 95% CI, −0.36 to −0.02). After adjusting for the Mediterranean diet score (12 points), the associations were maintained for the *z* scores of fasting plasma glucose (β coefficient, 0.17; 95% CI, 0.03-0.31) and HDL cholesterol (β coefficient, −0.20; 95% CI, −0.36 to −0.05) (eTable 2 in Supplement 1). Positive associations were also observed between 1-SD increments of UPF consumption and *z* scores of waist circumference (β coefficient, 0.09; 95% CI, 0.02-0.15), fat mass index (β coefficient, 0.11; 95% CI, 0.04-1.18), BMI (β coefficient, 0.11; 95% CI, 0.05-0.17), and fasting plasma glucose (β coefficient, 0.10; 95% CI, 0.03-0.17) and negatively associated with HDL cholesterol (β coefficient, −0.07; 95% CI, −0.15 to 0.00) (Table 3). Likewise,

Table 1. General Characteristics of Study Participants Across Tertiles of Energy-Adjusted Ultraprocessed Food Consumption

Characteristic	Tertiles of energy-adjusted ultraprocessed food consumption			
	1 (Lowest) (n = 476)	2 (n = 475)	3 (Highest) (n = 475)	*P* value[a]
Child				
Age, mean (SD), y	4.8 (1.1)	5.0 (1.1)	5.2 (1.1)	<.001
Sex, No. (%)				
Girls	237 (49.8)	247 (52)	244 (51.4)	.78
Boys	239 (50.2)	228 (48)	231 (48.6)	
Adiposity, mean (SD)				
BMI	16.1 (1.85)	16.3 (2.02)	16.6 (2.21)	.002
BMI *z* score	−0.11 (0.91)	0.00 (1.01)	0.10 (1.07)	.01
Weight status, No. (%)				
Underweight or normal weight	381 (82.5)	370 (79.1)	351 (75.0)	.02
Overweight or obesity	81 (17.5)	98 (20.9)	115 (25.0)	
Waist circumference, mean (SD), cm	51.7 (5.93)	52.3 (6.41)	52.0 (7.43)	.43
Waist-to-height ratio, mean (SD)	0.48 (0.05)	0.48 (0.05)	0.47 (0.06)	.01
Fat mass index, mean (SD)	3.72 (1.11)	3.88 (1.25)	3.97 (1.31)	.02
Lifestyle				
Physical activity, mean (SD), min/wk	178 (110)	181 (111)	184 (121)	.69
Cardiometabolic risk factors				
Systolic blood pressure, mean (SD), mm Hg	102 (12.3)	103 (12.7)	104 (13.5)	.003
Diastolic blood pressure, mean (SD), mm Hg	63.9 (11.6)	65.2 (11.5)	64.3 (12.0)	.26
Fasting plasma glucose, mean (SD), mg/dL	76.8 (9.24)	77.6 (9.39)	77.0 (8.85)	.50
HDL cholesterol, median (IQR), mg/dL	58.0 (50.0-66.7)	58.0 (50.0-66.2)	55.0 (47.0-64.0)	.01
LDL cholesterol, median (IQR), mg/dL	96.0 (84.0-113)	96.0 (83.0-112)	93.0 (78.0-110)	.04
Triglycerides, median (IQR), mg/dL	51.0 (42.0-62.5)	51.5.0 (43.0-63.0)	56.0 (45.0-69.0)	.04
Maternal				
Age, mean (SD), y	39.5 (4.69)	38.7 (4.73)	37.6 (5.14)	<.001
BMI, mean (SD)	24.1 (4.25)	24.4 (4.46)	25.9 (5.49)	<.001
Exclusive breastfeeding, No. (%)	194 (40.8)	37.9 (180)	157 (33.1)	.046
Weight status				
Underweight or normal weight	322 (67.6)	310 (65.3)	273 (57.5)	.003
Overweight or obesity	154 (32.3)	165 (34.7)	202 (42.5)	
Educational level				
Primary or lower	24 (5.04)	39 (8.21)	72 (15.2)	<.001
Secondary	157 (32.9)	161 (33.9)	235 (49.5)	
University	281 (59.0)	263 (55.4)	155 (32.6)	
Not reported	14 (2.9)	13 (2.5)	13 (2.7)	
Socioprofessional category				
Homemaker, student, retired, or unemployed	122 (25.6)	115 (24.2)	163 (34.3)	.005
Employed	354 (74.4)	360 (75.8)	312 (65.7)	

Abbreviations: BMI, body mass index (as measured by weight in kilograms divided by height in meters squared); HDL, high-density lipoprotein; LDL, low-density lipoprotein.

SI conversions: To convert glucose to mmol/L, multiply by 0.0555; to convert HDL and LDL to mmol/L, multiply by 0.0259; to convert triglycerides to mmol/L, multiply by 0.0113.

[a] *P* values for comparisons were tested by 1-way analysis of variance or χ^2 test, as appropriate, according to tertiles of energy-adjusted ultraprocessed food consumption.

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after further adjusting for the Mediterranean diet score, the associations between 1-SD increments remained significant for the *z* scores of fasting plasma glucose (β coefficient, 0.08; 95% CI, 0.02-0.13) and HDL cholesterol (β coefficient, −0.07; 95% CI, −0.13 to −0.01) (eTable 2 in Supplement 1).

Table 2. Baseline Dietary Characteristics of Participants Across Tertiles of Energy-Adjusted Ultraprocessed Food Consumption in the Diet

Characteristic	Tertiles of energy-adjusted ultraprocessed food consumption, mean (SD)			
	1 (Lowest) (n = 476)	2 (n = 475)	3 (Highest) (n = 475)	*P* value[a]
Energy-adjusted ultraprocessed food (n = 1426), g/d	192.8 (76.7)	354.8 (39.9)	593.4 (167)	<.001
Dietary intake contribution				
Total energy intake, kcal/d	1867.3 (420.1)	1626.4 (388.3)	1780.8 (470.7)	<.001
Carbohydrates, % of total energy intake	40.9 (5.7)	42.9 (4.9)	46.1 (5.1)	<.001
Proteins, % of total energy intake	14.9 (2.3)	15.1 (2.2)	14.9 (2.1)	.19
Protein intake per body weight, g/kg/d	3.8 (1.2)	3.2 (0.9)	3.3 (1.1)	<.001
Total fat, % of total energy intake	44.1 (6.4)	41.9 (5.1)	38.9 (5.1)	<.001
Saturated fatty acids, % of total energy intake	13.9 (2.3)	13.9 (2.0)	13.8 (2.0)	.71
Monounsaturated fatty acids, % of total energy intake	20.6 (4.8)	18.7 (3.8)	16.3 (3.5)	<.001
Polyunsaturated fatty acids, % of total energy intake	6.2 (1.7)	6.3 (1.6)	6.0 (1.8)	.01
Fiber, g/d	17.1 (5.3)	13.6 (3.8)	13.1 (3.7)	<.001
≥14 g/1000 kcal, No. (%)	43 (9.0)	12 (2.5)	3 (0.6)	<.001
Sodium, mg/d	138.8 (1.9)	138.5 (7.9)	139.3 (1.9)	.08
Dairy products, g/d				
Milk	384.3 (280.6)	298.2 (197.7)	286.8 (213/7)	<.001
Yogurt	92.7 (80.4)	96.5 (67.5)	151.7 (130.9)	<.001
Cheese	16.0 (17.5)	11.8 (10.6)	11.6 (14.9)	<.001
Other dairy products	50.1 (53.9)	70.9 (66.2)	155.4 (150.1)	<.001
Protein foods, g/d				
White meat	26.7 (14.9)	24.7 (9.81)	24.2 (13.3)	.01
Unprocessed red meat	22.3 (23.6)	19.9 (14.5)	18.1 (13.1)	.001
Processed and derivatives meat products	25.8 (16.6)	24.1 (13.9)	28.4 (19.0)	.001
Egg	26.7 (21.1)	23.7 (9.6)	22.5 (17.3)	<.001
Fish and seafood	36.0 (19.3)	33.4 (17.5)	33.1 (21.3)	.04
Vegetables and fruits, g/d				
Vegetables	101 (59.4)	68.3 (43.7)	58.8 (44.0)	<.001
Tubers	42.7 (22.8)	41.2 (19.0)	40.9 (20.8)	.35
Fruits	253.9 (160.4)	180.2 (105.9)	150.6 (108.5)	<.001
Nuts, g/d	5.1 (7.6)	3.2 (4.4)	2.7 (3.9)	<.001
Cereals and legumes, g/d				
Legumes	15.5 (11.9)	13.4 (6.9)	13.8 (7.3)	.001
Refined cereals	81.6 (44.2)	73.9 (34.8)	70.8 (35.7)	<.001
Whole cereals	14.1 (23.9)	6.59 (12.8)	4.65 (10.7)	<.001
Miscellaneous, g/d				
Oil and fats	34.5 (18.3)	24.4 (13.5)	19.7 (12.9)	<.001
Pastries	42.0 (36.9)	41.5 (33.4)	43.6 (32.5)	.60
Sugars and candies	6.2 (7.2)	5.7 (6.1)	6.7 (10.7)	.13
Beverages, mL/d				
Water	895.2 (350.5)	871.9 (385.2)	840.0 (377.7)	.07
Sugary beverages	92.8 (126.4)	81.1 (94.2)	192.0 (191.3)	<.001
Tea and infusions	6.7 (26.2)	7.6 (30.0)	6.2 (35.1)	.76

[a] *P* values for comparisons were tested by one-way analysis of variance or χ^2 test, as appropriate, according to energy-adjusted ultraprocessed food consumption.

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RESULTS

Similar positive associations among fat mass index, BMI, and plasma glucose were observed, irrespective of the animal or vegetable origin of the UPFs consumed. Substitution of 100 g of UPFs with 100 g of unprocessed or minimally processed foods was associated with a decrease in *z* scores of fat mass index (β coefficient, −0.03; 95% CI, −0.06 to 0.00) and BMI (β coefficient, −0.03; 95% CI, −0.06 to −0.01), and fasting plasma glucose (β coefficient, −0.04; 95% CI, −0.07 to −0.01) (**Figure**). The same models adjusted for the Mediterranean diet score showed positive associations for *z* scores of fasting plasma glucose (β coefficient, −0.04; 95% CI, −0.06 to −0.01) and inverse association for *z* score of HDL cholesterol (β coefficient, 0.03; 95% CI, 0.00-0.07) (eFigure 2 in Supplement 1). No associations were shown for the other outcomes.

In children whose mothers were unemployed, a positive association was found between energy-adjusted UPF consumption and *z* scores of waist circumference (β coefficient, 0.26; 95% CI, 0.14-0.39), fat mass index (β coefficient, 0.20; 95% CI, 0.07-0.34), waist-to-height ratio (β coefficient, 0.21; 95% CI, 0.09-0.34), BMI (β coefficient, 0.18; 95% CI, 0.04-0.31), fasting plasma

Table 3. Association Between Energy-Adjusted Ultraprocessed Food Consumption in Tertiles and 1-SD Increments and Cardiometabolic Risk Factor *z* Scores

Model	Tertiles of energy-adjusted ultraprocessed food consumption, β coefficient (95% CI)			*P* value for trend	Continuous (1-SD increment), β coefficient (95% CI)
	1 (Lowest)	2	3 (Highest)		
Waist circumference (n = 1390)					
Crude	1 [Reference]	0.05 (−0.07 to 0.18)	−0.04 (−0.17 to 0.08)	.43	−0.02 (−0.08 to 0.03)
Fully adjusted[a]	1 [Reference]	0.11 (−0.02 to 0.24)	0.20 (0.05 to 0.35)	.01	0.09 (0.02 to 0.15)
Fat mass index (n = 1219)					
Crude	1 [Reference]	0.09 (−0.04 to 0.22)	0.16 (0.03 to 0.29)	.02	0.08 (0.03 to 0.14)
Fully adjusted[a]	1 [Reference]	0.13 (−0.00 to 0.27)	0.17 (0.00 to 0.32)	.04	0.11 (0.04 to 1.18)
Waist-to-height ratio (n = 1389)					
Crude	1 [Reference]	0.03 (−0.09 to 0.16)	−0.10 (−0.23 to 0.03)	.09	−0.04 (−0.09 to 0.01)
Fully adjusted[a]	1 [Reference]	0.07 (−0.06 to 0.19)	0.12 (−0.03 to 0.27)	.12	0.06 (−0.01 to 0.12)
BMI (n = 1398)					
Crude	1 [Reference]	0.12 (−0.01 to 0.24)	0.21 (0.09 to 0.34)	.001	0.10 (0.05 to 0.15)
Fully adjusted[a]	1 [Reference]	0.14 (0.01 to 0.27)	0.20 (0.05 to 0.35)	.009	0.11 (0.05 to 0.17)
LDL cholesterol (n = 1120)					
Crude	1 [Reference]	0.03 (−0.11 to 0.17)	−0.13 (−0.27 to 0.01)	.054	−0.05 (−0.11 to 0.00)
Fully adjusted[a]	1 [Reference]	0.01 (−0.13 to 0.16)	−0.06 (−0.23 to 0.11)	.47	−0.03 (−0.09 to 0.04)
HDL cholesterol (n = 1174)					
Crude	1 [Reference]	−0.00 (−0.14 to 0.14)	−0.23 (−0.37 to −0.09)	.001	−0.09 (−0.15 to −0.03)
Fully adjusted[a]	1 [Reference]	−0.01 (−0.16 to 0.14)	−0.19 (−0.36 to −0.02)	.02	−0.07 (−0.15 to 0.00)
Triglycerides (n = 1175)					
Crude	1 [Reference]	0.09 (−0.05 to 0.23)	0.21 (0.07 to 0.34)	.004	0.07 (0.02 to 0.13)
Fully adjusted[a]	1 [Reference]	0.07 (−0.07 to 0.22)	0.14 (−0.03 to 0.30)	.10	0.04 (−0.03 to 0.11)
Fasting plasma glucose (n = 1191)					
Crude	1 [Reference]	0.02 (−0.11 to 0.17)	−0.02 (−0.17 to 0.12)	.69	−0.01 (−0.07 to 0.04)
Fully adjusted[a]	1 [Reference]	0.06 (−0.08 to 0.19)	0.22 (0.06 to 0.37)	.01	0.10 (0.03 to 0.17)
HOMA-IR (n = 933)					
Crude	1 [Reference]	0.03 (−0.13 to 0.19)	−0.02 (−0.18 to 0.14)	.78	−0.01 (−0.07 to 0.06)
Fully adjusted[a]	1 [Reference]	0.06 (−0.10 to 0.22)	0.02 (−0.16 to 0.21)	.69	0.02 (−0.06 to 0.10)
Diastolic blood pressure (n = 1348)					
Crude	1 [Reference]	0.10 (−0.03 to 0.23)	0.05 (−0.08 to 0.18)	.55	0.01 (−0.04 to 0.07)
Fully adjusted[a]	1 [Reference]	0.08 (−0.05 to 0.21)	−0.01 (−0.16 to 0.14)	.81	−0.01 (−0.08 to 0.05)
Systolic blood pressure (n = 1346)					
Crude	1 [Reference]	0.14 (0.01 to 0.27)	0.19 (0.06 to 0.32)	.01	0.08 (0.02 to 0.13)
Fully adjusted[a]	1 [Reference]	0.12 (−0.02 to 0.25)	0.12 (−0.03 to 0.28)	.14	0.05 (−0.01 to 0.11)

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment for insulin resistance; LDL, low-density lipoprotein.

[a] Fully adjusted for maternal education level, maternal BMI, total minutes of physical activity per week, exclusive breastfeeding, center size, and NOVA classification system groups 1, 2, and 3.

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glucose (β coefficient, 0.14; 95% CI, 0.03-0.25), and diastolic blood pressure (β coefficient, 0.14; 95% CI, 0.0-0.27). In children with employed mothers, a positive association was observed between 1-SD increments in energy-adjusted UPF consumption and *z* scores of fasting plasma glucose (β coefficient, 0.09; 95% CI, 0.01-0.17) and a negative association in case of the HDL cholesterol (β coefficient, −0.09; 95% CI, −0.18 to −0.01) (eTable 3 in Supplement 1).

Children whose mothers had a low education level had higher *z* scores of waist circumference (β coefficient, 0.14; 95% CI, 0.05-0.23), fat mass index (β coefficient, 0.15; 95% CI, 0.06-0.25), BMI (β coefficient, 0.15; 95% CI, 0.06-0.24), and fasting plasma glucose (β coefficient, 0.11; 95% CI, 0.02-0.19). Children whose mothers had a high education level had a lower HDL cholesterol *z* score (β coefficient, −0.15; 95% CI, −0.26 to −0.03) (eTable 4 in Supplement 1).

Discussion

To our knowledge, this study is the first to assess the associations between UPF consumption and various cardiometabolic risk factors in young children. In this large cross-sectional study, UPF consumption was positively associated with *z* scores of BMI, waist circumference, fat mass index, and fasting plasma glucose concentration and inversely associated with HDL cholesterol concentration.

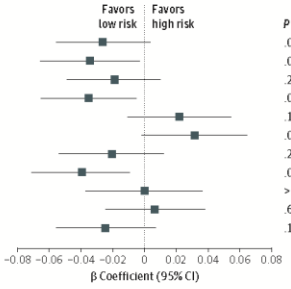
Our findings are in line with those of previous studies.[35-37] Consumption of UPFs at age 4 years was associated with increased BMI *z* scores at age 10 years in the Generation XXI cohort, while no association was found at age 7 years.[35] Another study showed that high UPF consumption in children aged 7 to 13 years was associated with increased BMI growth trajectories.[36] Similarly, lower UPF intake in Spanish children aged 4 to 7 years was associated with lower BMI *z* scores at age 7 years, though this association became nonsignificant after adjusting for maternal factors.[37]

A global study by Neri et al[38] revealed that increased UPF consumption was associated with higher dietary energy density and intake of free sugars, alongside decreased total fiber intake, potentially contributing to childhood obesity. Additionally, findings from the Avon Longitudinal Study of Parents and Children showed that high UPF consumption was associated with unfavorable fat mass index trajectories from age 7 to 24 years.[36] Similarly, in a Brazilian cohort, UPF consumption during preschool years was associated with increases in waist circumference from preschool to school age.[39] Other studies found no significant association between UPF consumption and HDL cholesterol and fasting plasma glucose concentrations.[37,40] Therefore, to our knowledge, our study is the first in children to find significant associations with the aforementioned risk factors and is in line with other studies assessing adult populations.[41]

Our results provide new insight into the association between UPF consumption and health and the importance of recognizing that early dietary habits in childhood might have future implications

Figure. Substitution Analysis of 100 g of Ultraprocessed Food With 100 g of Unprocessed or Minimally Processed Foods and Cardiometabolic Risk Factors

Cardiometabolic risk factors	No. of participants	β Coefficient (95% CI)	*P* value
Waist circumference	1390	−0.03 (−0.05 to 0)	.09
Fat mass index	1219	−0.03 (−0.06 to 0)	.03
Waist-to-height ratio	1389	−0.02 (−0.05 to 0.01)	.20
BMI	1407	−0.03 (−0.06 to −0.01)	.02
LDL cholesterol	1120	0.02 (−0.01 to 0.05)	.19
HDL cholesterol	1174	0.03 (0 to 0.06)[a]	.07
Triglycerides	1175	−0.02 (−0.05 to 0.01)	.21
Fasting plasma glucose	1191	−0.04 (−0.07 to −0.01)	.01
HOMA-IR	933	0 (−0.04 to 0.04)	>.99
Diastolic blood pressure	1348	0.01 (−0.02 to 0.04)	.67
Systolic blood pressure	1346	−0.02 (−0.05 to 0.01)	.13



Linear regression models were fitted and adjusted for maternal education level, maternal body mass index (BMI), total minutes of physical activity per week, breastfeeding, center size, and NOVA classification system groups 2 and 3. HDL indicates high-density lipoprotein; HOMA-IR, homeostasis model assessment for insulin resistance; LDL, low-density lipoprotein.

[a] For HDL cholesterol, a positive β coefficient signifies low risk.

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on cardiometabolic health. While the magnitude of the associations reported in our study may be considered of limited clinical relevance, it is important to note that our study consisted of young children. Therefore, if such minimal differences can reveal a significant association, they may serve as an early warning of future cardiometabolic conditions.

Our results are in line with previous studies showing that the main UPF products consumed are pastries, sweet beverages, cookies, and candies.[42-44] In addition, our results support the findings of other European studies that have shown that children of mothers with lower education or with lower socioeconomic status are more likely to consume UPFs. These findings suggest that educational and socioeconomic factors may contribute to the purchase of low-cost and unhealthy foods, such as UPFs, increasing the risk of health disorders.[37,45,46]

Several possible mechanisms could explain our results. First, UPFs contain higher amounts of sodium, energy, fat, and sugar and lower amounts of fiber, which are well recognized as contributors to cardiometabolic risk factors.[47] In addition, some UPFs may be linked to a higher glycemic response, and it has been shown that high consumption of sugar-sweetened beverages may delay the internal satiety signal, leading to excessive calorie intake and higher glycemic load.[48-50] Moreover, excessive consumption of energy, saturated fat, and sugar contributes to weight gain and a higher risk of obesity, which is an important risk factor in CVD.[51] Furthermore, our study showed that children who consumed high amounts of UPFs tended to have lower intakes of fruits and vegetables, which, along with a healthy dietary pattern, are known to be beneficial for cardiometabolic health.[52]

Most of the associations were maintained in our study after further adjusting the models to Mediterranean diet adherence, suggesting that other intrinsic UPF factors may play an important role in determining these associations (eg, additives). Animal and cellular studies revealed potential cardiovascular risks from authorized additives such as sulfites, monosodium glutamate, and emulsifiers.[53-58] Food processing generates contaminants such as acrylamide and acrolein, which have been linked, respectively, to increased odds and risk of cardiovascular disease.[59,60] Ultraprocessed foods may contain chemicals such as bisphenols and perfluoroalkyl substances that have been associated with a higher risk of cardiometabolic outcomes in children.[61,62]

The NOVA Food Classification system has sparked debate among researchers due to disagreements over UPF definitions, bias concerns, and the system's contribution to dietary guidelines.[63,64] The NOVA system itself has some limitations, as it does not consider that certain minimal processing could improve the final product (eg, fermentation in milk) and adopts a vague definition of what is considered a cosmetic additive, which has led to considering carotenoids as an additive that increases the potential harmfulness of a product.[65] Despite these limitations, NOVA categories have consistently shown associations with cardiometabolic health in adults.

Strengths and Limitations

This study has several strengths. Most importantly, the study was conducted in a large sample size from 7 different geographic areas of Spain. The study also assessed cardiometabolic risk factors not considered in other similar studies.[21-24]

Our study also has several limitations. First, because the study is observational, we cannot draw conclusions on cause and effect. Second, our study involved preschool children from Spain, which means that the generalization of our findings to different populations is not appropriate. Third, some grade of misclassification could be present in our study since UPF consumption was estimated from a food and beverage frequency questionnaire that was not specifically developed to assess this type of food, which could result in either an overestimation or underestimation of consumption within various NOVA categories. Additionally, imprecise estimations could also arise from the use of a food and beverage frequency questionnaire, which may be influenced by social desirability bias. Finally, we cannot dismiss that associations may be due to residual confounding or that undetected cardiometabolic disorders in our study population may exist due to age.

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Conclusions

In this large cross-sectional study, UPFs consumption was positively associated with fasting plasma glucose levels, BMI, waist circumference, and fat mass index and inversely associated with HDL cholesterol concentration. These findings highlight the importance of promoting unprocessed or minimally processed foods and reducing UPF consumption, particularly starting from early ages. However, further prospective studies are warranted to validate our findings.

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Corresponding Author: Nancy Babio, PhD, Unitat de Nutrició Humana, Departament de Bioquímica i Biotecnologia, Universitat Rovira i Virgili, c/Sant Llorenç 21, 43201 Reus, Spain (nancy.babio@urv.cat).

Author Affiliations: Universitat Rovira i Virgili Departament de Bioquímica i Biotecnologia, Unitat de Nutrició Humana, ANUT-DSM group, Spain (Khoury, M. Á. Martínez, Garcidueñas-Fimbres, de Las Heras-Delgado, Mimbrero, Salas-Salvadó, Babio); Institut d'Investigació Sanitària Pere Virgili, Reus, Spain (Khoury, M. Á. Martínez, Garcidueñas-Fimbres, de Las Heras-Delgado, Luque, Salas-Salvadó, Babio); Consorcio Centro de Investigación Biomédica en Red, M. P. Fisiopatología de la Obesidad y Nutrición, Instituto de Salud Carlos III, Madrid, Spain (Khoury, M. Á. Martínez, Garcidueñas-Fimbres, Leis, de Las Heras-Delgado, Miguel-Berges, Navas-Carretero, Portoles, Pérez-Vega, Jurado-Castro, Vázquez-Cobela, J. A. Martínez, Moreno, Gil-Campos, Salas-Salvadó, Babio); Metabolism and Investigation Unit, Maimónides Institute of Biomedicine Research of Córdoba, Reina Sofia University Hospital, University of Córdoba, Córdoba, Spain (Pastor-Villaescusa, Jurado-Castro, Flores-Rojas, Castro-Collado, Gil-Campos); Primary Care Interventions to Prevent Maternal and Child Chronic Diseases of Perinatal and Developmental Origin, Instituto de Salud Carlos III, Madrid, Spain (Pastor-Villaescusa); Unit of Pediatric Gastroenterology, Hepatology and Nutrition, Pediatric Service, Hospital Clínico Universitario de Santiago, Santiago de Compostela, Spain (Leis, Vázquez-Cobela); Pediatric Nutrition Research Group, Health Research Institute of Santiago de Compostela, Unit of Investigation in Nutrition, Growth and Human Development of Galicia-Universidad de Santiago de Compostela, Santiago de Compostela, Spain (Leis, Vázquez-Cobela, Picáns-Leis); Growth, Exercise, Nutrition and Development Research Group, University of Zaragoza, Spain (Miguel-Berges, Andía Horno, Moreno); Instituto Agroalimentario de Aragón, University of Zaragoza, Spain (Miguel-Berges, Andía Horno, Moreno); Instituto de Investigación Sanitaria de Aragón, Zaragoza, Spain (Miguel-Berges, Moreno); Center for Nutrition Research, Faculty of Pharmacy and Nutrition, Department of Nutrition, Food Science and Physiology, University of Navarra, Pamplona, Spain (Navas-Carretero, J. A. Martínez); Navarra Medical Research Institute, Pamplona, Spain (Navas-Carretero); Department of Preventive Medicine and Public Health, University of Valencia, Spain (Portoles); Hospital del Mar Medical Research Institute, Barcelona, Spain (Pérez-Vega); Centre d'Atenció Primària, Institut Català de la Salut, Reus, Spain (Mimbrero); Pediatrics, Nutrition, and Development Research Unit, Universitat Rovira I Virgili, Reus, Spain (Luque).

Author Contributions: Ms Khoury and Dr Babio had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Ms Khoury and Dr M. Á. Martínez contributed equally to this work as co-first authors. Prof Salas-Salvadó and Dr Babio contributed equally to this work as co-senior authors.

Concept and design: Khoury, M. Á. Martínez, Miguel-Berges, Navas-Carretero, Portoles, Leis, J. F. Martínez, Flores-Rojas, Moreno Aznar, Gil-Campos, Salas-Salvadó, Babio.

Acquisition, analysis, or interpretation of data: Khoury, M. Á. Martínez, Garcidueñas-Fimbres, Pastor-Villaescusa, Leis, Pérez-Vega, Jurado-Castro, Vázquez-Cobela, Andía Horno, J. A. Martínez, Picáns-Leis, Luque, Moreno Aznar, Castro-Collado, Gil-Campos, Salas-Salvadó, Babio.

Drafting of the manuscript: Khoury, Leis, de las Heras Delgado, Vázquez-Cobela, Andía Horno, J. A. Martínez, Flores-Rojas, Salas-Salvadó, Babio.

Critical review of the manuscript for important intellectual content: Khoury, M. Á. Martínez, Garcidueñas-Fimbres, Pastor-Villaescusa, Leis, Miguel-Berges, Navas-Carretero, Portoles, Pérez-Vega, Jurado-Castro, Vázquez-Cobela, J. A. Martínez, Picáns-Leis, Luque, Moreno Aznar, Castro-Collado, Gil-Campos, Salas-Salvadó, Babio.

Statistical analysis: Khoury, Garcidueñas-Fimbres, de las Heras Delgado, Vázquez-Cobela.

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Administrative, technical, or material support: Leis, Pérez-Vega, Vázquez-Cobela, Andía Horno, J. A. Martínez, Picáns-Leis, Luque, Gil-Campos, Salas-Salvadó, Babio.

Supervision: M. Á. Martínez, Leis, Portoles, Vázquez-Cobela, J. A. Martínez, Picáns-Leis, Luque, Moreno Aznar, Gil-Campos, Babio.

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SUPPLEMENT 1.

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SUPPLEMENT 2.

Data Sharing Statement

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Supplemental Online Content

Khoury N, Martínez MÁ, Garcidueñas-Fimbres TE, et al. Ultraprocessed food consumption and cardiometabolic risk factors in children from the CORALS cohort. *JAMA Netw Open*. 2024;7(5):e2411852. doi:10.1001/jamanetworkopen.2024.11852

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This supplemental material has been provided by the authors to give readers additional information about their work.

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eAppendix. Additional Study Assessments

Dietary intake

Parents or caregivers were asked to indicate, on average, how frequently the participant had consumed the specified serving of each item over the past year, rating their consumption frequency on a scale of 9 options ranging from "never" to "more than 6 times per day ". This questionnaire was designed in machine-readable format to be later scanned using the Evaldara® software. This software efficiently extracts the reported consumption, which was then integrated into the program e-Diet Base URV[1] to obtain the nutrient and energy values.

NOVA classification

The categorization of food items was carried out by two separate dietitians. Additionally, to guarantee precise accuracy, an additional dietitian conducted a thorough revision. Any discrepancies in the classification of particular food items were resolved through extensive discussions among the researchers, ultimately resulting in decisions based on consensus.

Sensitivity analyses

Sensitivity analyses were conducted to assess the potential influence of the nutritional quality of the diet by adjusting for the adherence to Mediterranean diet score. We took into consideration 12 out of 18 points of the score, excluding the points related to UPFs consumption.

Likelihood ratio test was performed to examine the interaction between maternal education level (low education/high education) and socio-professional status (not employed/employed) in relation to the association between UPFs consumption and each of the outcomes. In addition, two stratified analyses by the maternal socio-professional (not employed or employed mothers) and by the maternal education level (low or high education level) were performed to assess potential modifications. Models were adjusted for the aforementioned confounding factors.

eReference

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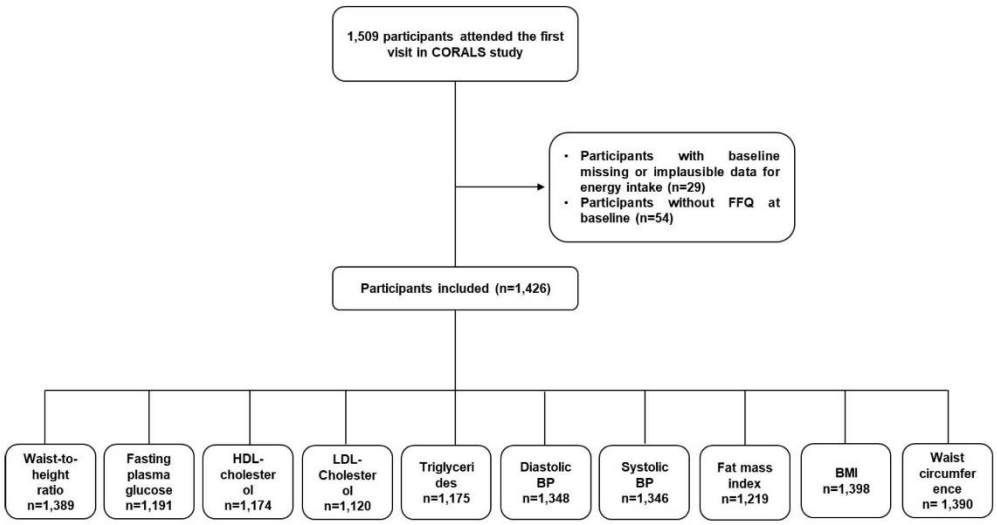
eTable 1. Distribution of FFQ Items Into 4 Groups According to the Degree of Their Processing Established by NOVA Classification System

Group 1: Unprocessed or minimally processed foods	**Milk (whole, semi-skimmed, skimmed), yogurt (without added sugar, skimmed), eggs, chicken (with and without skin), fresh meat (cow, turkey, pork, lamb, rabbit), liver meat, offal meat, fresh fish, oysters, calamari, fresh shrimps, fresh vegetables, gazpacho, fruits, dates, nuts, legumes, rice (white and whole), pasta (white and whole), water, fresh juices, coffee, tea and infusions.**
Group 2: Processed culinary ingredients	Olive oil (extra virgin and refined), corn oil, sunflower oil, soybean oil, butter, salt, cacao and sugar.
Group 3: Processed foods	Condensed milk, lactose free yogurt, cooking cream, curd, cheeses (white cheese), ham serrano, bacon, salted fish, canned fish, homemade fries, fruits in syrup, olives, bread (white and whole), cereals (with no added sugars and whole) and non-dairy milk.
Group 4: Ultra-processed food and drink products	Milkshakes, yogurt (whole and with added sugars), fermented milk, cheese (Petite Suisse, soft cheese), custard, ice cream, ham, processed meat, pate, hamburger, surimi, potato chips, loaf of bread, bread sticks, cereals (with added sugar, flavored-stuffed cereals), pizza, margarine, biscuits (plain, whole, with chocolate), pastries, donuts, muffins, cakes, churros, chocolate, butter cookies, croquettes, ready-to-eat soups, mustard, mayonnaise, ketchup, jams, chocolate cream, snacks, candies, sparkling water, flavored water, bottled juices, soft drinks

Group 1 includes unprocessed or minimally processed foods consisting of natural and whole foods that have undergone little to no processing. Group 2 includes processed culinary ingredients, this group comprises substances extracted from Group 1 foods or nature, used to season and cook foods. Group 3 includes processed food that are manufactured by combining Group 1 or 2 ingredients and may involve some processing methods. Group 4 includes ultra-processed foods (UPF), this group consists of industrial formulations that are characterized by a high energy content, rich in saturated fats, added sugars, salt, with a low amount of fiber and vitamins, which may also contain additives like artificial colors and flavors or stabilizers. Ultra-processed foods are typically ready-to-eat or require minimal preparation (sugary drinks, packaged snacks, fast food, frozen pizzas, and many convenience foods).

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eFigure 1. Flow Chart of the Children Included in the Analysis

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eTable 2. Association Between Energy-Adjusted Ultraprocessed Food Consumption in Tertiles (in g/day) and CVD Risk Factors at Baseline

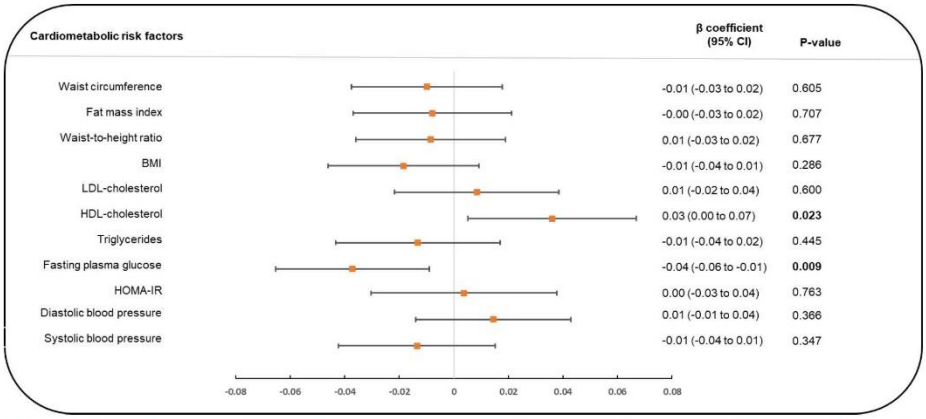
	Tertiles of energy-adjusted UPF consumption in g/day β coefficient (95% confidence interval)				Continuous (1SD increment)
	T1	T2	T3	p-trend	
Waist circumference (z-score) n= 1,390	0 (ref)	0.04 (-0.09 to 0.17)	0.08 (-0.06 to 0.21)	0.26	0.02 (-0.03 to 0.08)
Fat mass index (z-score) n= 1,219	0 (ref)	0.04 (-0.10 to 0.17)	-0.01 (-0.14 to 0.15)	0.97	0.02 (-0.03 to 0.08)
Waist-to-height ratio (z-score) n= 1,389	0 (ref)	0.03 (-0.10 to 0.15)	0.05 (-0.08 to 0.18)	0.49	0.02 (-0.03 to 0.07)
BMI (z-score) n=1,398	0 (ref)	0.07 (-0.06 to 0.20)	0.08 (-0.05 to 0.22)	0.25	0.04 (-0.01 to 0.09)
LDL-cholesterol (z-score) n= 1,120	0 (ref)	0.02 (-0.12 to 0.17)	-0.03 (-0.18 to 0.12)	0.63	-0.01 (-0.07 to 0.05)
HDL-cholesterol (z-score) n= 1,174	0 (ref)	-0.03 (-0.18 to 0.12)	**-0.20 (-0.36 to -0.05)**	**0.01**	**-0.07 (-0.13 to -0.01)**
Triglycerides (z-score) n= 1,175	0 (ref)	0.04 (-0.10 to 0.18)	0.09 (-0.05 to 0.25)	0.20	0.03 (-0.03 to 0.08)
Fasting plasma glucose (z-score) n=1,191	0 (ref)	0.02 (-0.12 to 0.16)	**0.17 (0.03 to 0.31)**	**0.01**	**0.08 (0.02 to 0.13)**
HOMA-IR (z-score) n=933	0 (ref)	0.03 (-0.13 to 0.20)	-0.01 (-0.18 to 0.16)	0.86	-0.00 (-0.07 to 0.06)
Diastolic blood pressure (z-score) n= 1,348	0 (ref)	0.05 (-0.08 to 0.19)	-0.05 (-0.19 to 0.09)	0.41	-0.03 (-0.08 to 0.03)
Systolic blood pressure (z-score) n= 1,346	0 (ref)	0.08 (-0.05 to 0.22)	0.07 (-0.07 to 0.21)	0.36	0.03 (-0.03 to 0.08)

Abbreviations: UPF: ultra-processed food; BMI: Body Mass Index; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance.

Linear regression models were fitted [β coefficient (95% Confidence Interval)] adjusted for maternal education level, maternal BMI, total minutes of physical activity per week, breastfeeding, center, total energy intake and adherence to Mediterranean diet score (12 points).

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eFigure 2. Linear Regression Models Replacing 100 g of UPF With 100 g of Unprocessed/Minimally Processed Foods

Abbreviations: BMI: Body Mass Index; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein.
All outcomes are assessed as z-scores
Linear regression models were fitted [β coefficient (95 % Confidence Interval)] adjusted for maternal education level, maternal BMI, total minutes of physical activity per week, breastfeeding, center and adherence to Mediterranean score (12 points)
Fasting plasma glucose n=1,191; HDL-cholesterol n= 1,174; LDL-Cholesterol n= 1,120; Triglycerides n= 1,175; Diastolic BP n= 1,348; Systolic BP n= 1,346; Fat mass index n= 1,219; Waist-to-height ratio n= 1,389; z-BMI n= 1,407; Waist circumference n= 1,390

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eTable 3. Maternal Socioprofessional Stratified Regression Association Between 1-SD Increment of Energy-Adjusted Ultraprocessed Food Consumption (in g/day) and CVD Risk Factors at Baseline

	Energy-adjusted UPF consumption (in g/day, 1SD increment) β coefficient (95% CI)	p-value
Waist circumference (z-score)		
Employed (n=1,028)	0.01 (-0.06 to 0.08)	0.80
Not employed (n= 362)	**0.26 (0.14 to 0.39)**	**<0.001**
Fat mass index (z-score)		
Employed (n=894)	0.06 (-0.02 to 0.14)	0.12
Not employed (n= 325)	**0.20 (0.07 to 0.34)**	**0.004**
Waist-to-height ratio (z-score)		
Employed (n=1,027)	-0.01 (-0.09 to 0.06)	0.70
Not employed (n=362)	**0.21 (0.09 to 0.34)**	**0.001**
BMI (z-score)		
Employed (n=1,032)	0.07 (-0.00 to 0.15)	0.05
Not employed (n=366)	**0.18 (0.04 to 0.31)**	**0.01**
LDL-cholesterol (z-score)		
Employed (n=815)	-0.05 (-0.13 to 0.04)	0.26
Not employed (n=305)	0.02 (-0.10 to 0.16)	0.70
HDL-cholesterol (z-score)		
Employed (n=857)	**-0.09 (-0.18 to -0.01)**	**0.03**
Not employed (n=317)	-0.03 (-0.17 to 0.11)	0.64
Triglycerides (z-score)		
Employed (n=858)	0.03 (-0.05 to 0.12)	0.47
Not employed (n=317)	0.08 (-0.04 to 0.20)	0.18
Fasting plasma glucose (z-score)		
Employed (n=871)	**0.09 (0.01 to 0.17)**	**0.03**
Not employed (n=320)	**0.14 (0.03 to 0.25)**	**0.01**
HOMA-IR (z-score)		
Employed (n=677)	-0.00 (-0.10 to 0.09)	0.96
Not employed (n=256)	0.09 (-0.03 to 0.20)	0.14
Diastolic blood pressure (z-score)		
Employed (n=994)	-0.07 (-0.14 to 0.01)	0.09
Not employed (n=354)	**0.14 (0.00 to 0.27)**	**0.04**

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Systolic blood pressure (z-score)		
Employed (n=993)	0.02 (-0.06 to 0.10)	0.53
Not employed (n=353)	0.12 (-0.01 to 0.25)	0.07

Abbreviations: BMI: Body Mass Index; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein.

Linear regression models were fitted [β coefficient (95 % Confidence Interval)] adjusted for maternal education level, maternal BMI, total minutes of physical activity per week, exclusive breastfeeding, center size and NOVA group 1,2 and 3.

Z-score of: Fasting plasma glucose n=1,191; HDL-cholesterol n= 1,174; LDL-Cholesterol n= 1,120; Triglycerides n= 1,175; Diastolic BP n= 1,348; Systolic BP n= 1,346; Fat mass index n= 1,219; Waist-to-height ratio n= 1,389; BMI n= n=1,398; Waist circumference n= 1,390

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eTable 4. Maternal Education Level Stratified Regression Association Between 1-SD Increment of Energy-Adjusted Ultraprocessed Food Consumption (in g/day) and CVD Risk Factors at Baseline

	Energy-adjusted UPF consumption (in g/day, 1SD increment) β coefficient (95% CI)	p-value
Waist circumference (z-score)		
Low education level (n=670)	**0.14 (0.05 to 0.23)**	**<0.01**
High education level (n=720)	0.01 (-0.08 to 0.10)	0.81
Fat mass index (z-score)		
Low education level (n=583)	**0.15 (0.06 to 0.25)**	**<0.01**
High education level (n=636)	0.04 (-0.06 to 0.14)	0.42
Waist-to-height ratio (z-score)		
Low education level (n=669)	0.09 (-0.00 to 0.19)	**0.04**
High education level (n=720)	0.00 (-0.10 to 0.09)	0.99
BMI (z-score)		
Low education level (n=678)	**0.15 (0.06 to 0.24)**	**<0.01**
High education level (n=720)	0.05 (-0.45 to 0.14)	0.28
LDL-cholesterol (z-score)		
Low education level (n=571)	-0.02 (-0.10 to 0.07)	0.69
High education level (n=594)	-0.03 (-0.15 to 0.09)	0.64
HDL-cholesterol (z-score)		
Low education level (n=595)	-0.03 (-0.12 to 0.07)	0.58
High education level (n=579)	**-0.15 (-0.26 to -0.03)**	**0.02**
Triglycerides (z-score)		
Low education level (n=597)	0.05 (-0.04 to 0.14)	0.32
High education level (n=578)	0.02 (-0.09 to 0.13)	0.78
Fasting plasma glucose (z-score)		
Low education level (n=603)	**0.11 (0.02 to 0.19)**	**0.01**
High education level (n=588)	0.09 (-0.01 to 0.20)	0.09
HOMA-IR (z-score)		
Low education level (n=496)	0.03 (-0.10 to 0.15)	0.63
High education level (n=437)	0.01 (-0.08 to 0.09)	0.89
Diastolic blood pressure (z-score)		
Low education level (n=651)	-0.01 (-0.09 to 0.08)	0.89
High education level (n=697)	-0.03 (-0.14 to 0.07)	0.51

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Systolic blood pressure (z-score)		
Low education level (n=650)	0.08 (-0.01 to 0.17)	0.08
High education level (n=696)	0.02 (-0.09 to 0.12)	0.74

Abbreviations: BMI: Body Mass Index; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein.

Linear regression models were fitted [β coefficient (95 % Confidence Interval)] adjusted for maternal BMI, total minutes of physical activity per week, exclusive breastfeeding, center size and NOVA group 1, 2 and 3.

Z-score of: Fasting plasma glucose n=1,191; HDL-cholesterol n= 1,174; LDL-Cholesterol n= 1,120; Triglycerides n= 1,175; Diastolic BP n= 1,348; Systolic BP n= 1,346; Fat mass index n= 1,219; Waist-to-height ratio n= 1,389; BMI n= n=1,398; Waist circumference n= 1,390

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Chapter 3: Association between the dietary intake of chemical contaminants, adiposity, obesity status and diabetes

d. Article 4:

Title: Dietary intake of polychlorinated dibenzo-p-dioxins and furans, adiposity and obesity status

Nadine Khoury , María Ángeles Martínez, Indira Paz-Graniel, Miguel Ángel Martínez-González, Dolores Corella, Olga Castañer, J Alfredo Martínez, Ángel M Alonso-Gómez, Julia Wärnberg, Jesús Vioqu, Dora Romaguera, José López-Miranda, Ramon Estruch, Francisco J Tinahones, José Lapetra, J Lluís Serra-Majem, Aurora Bueno-Cavanillas, Josep A Tur, Sergio Cinza Sanjurjo, Xavier Pintó, José Juan Gaforio, Pilar Matía-Martín, Josep Vidal, Clotilde Vázquez, Lidia Daimiel, Emilio Ros, Carmen Sayon-Orea, Jose V Sorlí, Karla-Alejandra Pérez-Vega, Antonio Garcia-Rios, Nuria Gómez Bellvert, Enrique Gómez-Gracia, M A Zulet, Alice Chaplin, Rosa Casas, Inmaculada Salcedo-Bellido, Lucas Tojal-Sierra, Maria-Rosa Bernal-Lopez, Zenaida Vázquez-Ruiz, Eva M Asensio, Albert Goday, Patricia J Peña-Orihuela, Antonio J Signes-Pastor, Ana Garcia-Arellano, Montse Fitó, Nancy Babio, Jordi Salas-Salvadó

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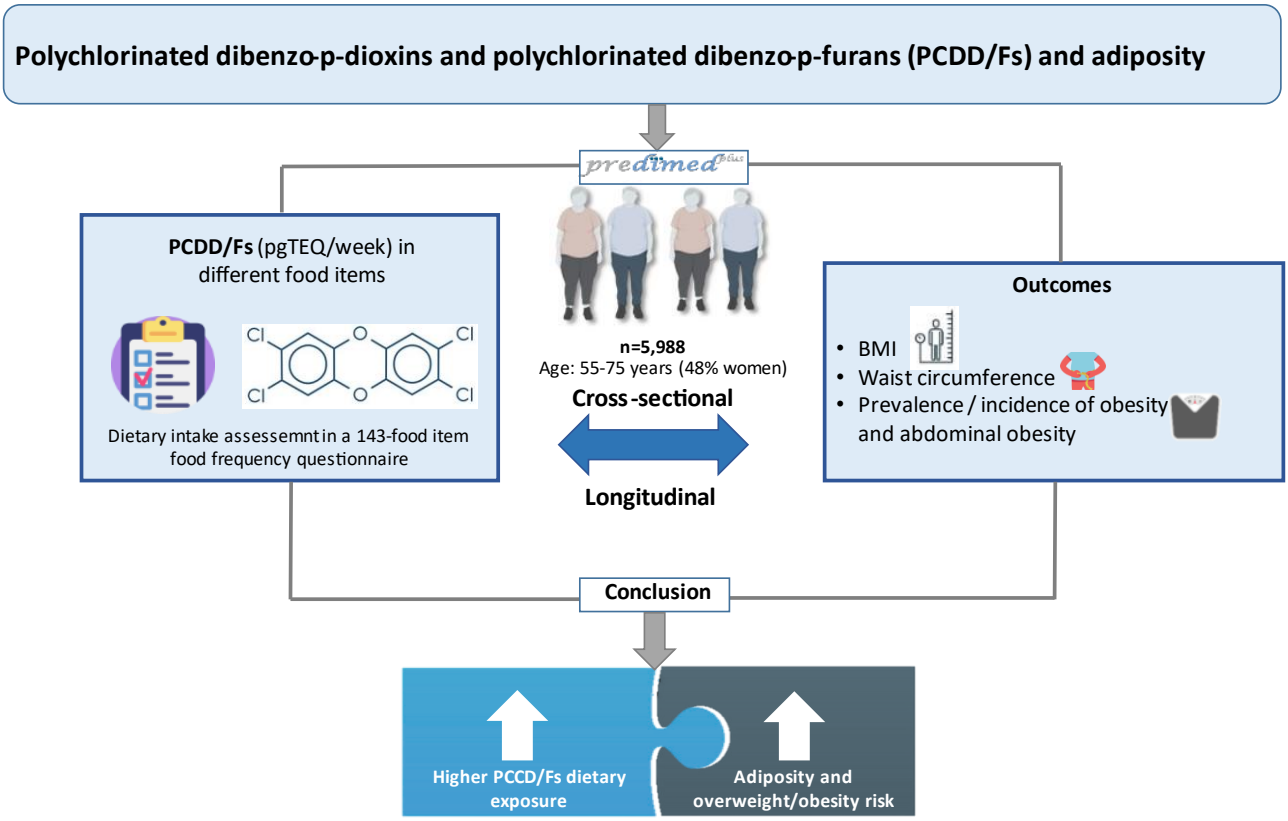
DOI: 10.1016/j.envres.2023.115697

Summary:

Results: Compared to participants in the first PCDD/F dietary intake tertile, those in the highest tertile presented a higher BMI (β-coefficient [confidence interval]) (0.43kg/m2 [0.22; 0.64]; P-trend <0.001), a higher waist circumference (1.11 cm [0.55; 1.66]; P-trend <0.001), and a higher prevalence of obesity and abdominal obesity (1.05 [1.01; 1.09] and 1.02 [1.00; 1.03]; P-trend = 0.09 and 0.027, respectively). In the prospective analysis, participants in the top PCDD/F baseline dietary intake tertile showed an increase in waist circumference compared with those in the first tertile after 1-year of follow-up (β-coefficient 0.37 cm [0.06; 0.70]; P-trend = 0.015).

Conclusion: Higher dietary intake of PCDD/Fs was positively associated with adiposity parameters and obesity status at baseline and with changes in waist circumference after 1-year of follow-up in subjects living with overweight/obesity. Further large prospective studies using a different population with longer follow-up periods are warranted in the future to strengthen our results.

RESULTS


Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzo-p-furans (PCDD/Fs) and adiposity
PREDIMED Plus
PCDD/Fs (pgTEQ/week) in different food items
Dietary intake assessemnt in a 143-food item food frequency questionnaire
n=5,988
Age: 55-75 years (48% women)
Cross-sectional
Longitudinal
Outcomes
BMI
Waist circumference
Prevalence / incidence of obesity and abdominal obesity
Conclusion
Higher PCCD/Fs dietary exposure
Adiposity and overweight/obesity risk

Environmental Research 227 (2023) 115697

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Dietary intake of polychlorinated dibenzo-p-dioxins and furans, adiposity and obesity status.

Nadine Khoury [a,b,c,1], María Ángeles Martínez [a,b,c,1,*], Indira Paz-Graniel [a,b,c], Miguel Ángel Martínez-González [a,d,e], Dolores Corella [a,f], Olga Castañer [a,g], J. Alfredo Martínez [a,h,i], Ángel M. Alonso-Gómez [a,j], Julia Wärnberg [a,k], Jesús Vioque [l,m], Dora Romaguera [a,n], José López-Miranda [a,o], Ramon Estruch [a,p], Francisco J. Tinahones [a,q], José Lapetra [a,r], J. Lluís Serra-Majem [a,s], Aurora Bueno-Cavanillas [l,t,aj], Josep A. Tur [a,u], Sergio Cinza Sanjurjo [v,w,x], Xavier Pintó [a,y], José Juan Gaforio [l,z], Pilar Matía-Martín [aa], Josep Vidal [ab,ac], Clotilde Vázquez [a,ad], Lidia Daimiel [a,ae,ai], Emilio Ros [a,af], Carmen Sayon-Orea [a,d], Jose V. Sorlí [a,f], Karla-Alejandra Pérez-Vega [a,g], Antonio Garcia-Rios [a,o], Nuria Gómez Bellvert [ag], Enrique Gómez-Gracia [a,k], M.A. Zulet [a,h,i], Alice Chaplin [a,n], Rosa Casas [a,p], Inmaculada Salcedo-Bellido [l,t,aj], Lucas Tojal-Sierra [a,j], Maria-Rosa Bernal-Lopez [a,ah], Zenaida Vázquez-Ruiz [a,d], Eva M. Asensio [a,f], Albert Goday [a,g], Patricia J. Peña-Orihuela [a,o], Antonio J. Signes-Pastor [l,m], Ana Garcia-Arellano [a,d], Montse Fitó [a,g], Nancy Babio [a,b,c,2], Jordi Salas-Salvadó [a,b,c,2,**]

[a] Centro de Investigación Biomédica en Red dePisiopatología de la Obesidad y Nutrición, Instituto deSalud Carlos III, Spain
[b] Universitat Rovira i Virgili, Departament de Bioquímica i Biotecnologia, Alimentació, Nutrició, Desenvolupament i Salut Mental ANUT-DSM, Reus, Spain
[c] Institut D'Investigació Sanitària Pere Virgili (IISPV), Reus, Spain
[d] University of Navarra, Department of Preventive Medicine and Public Health, IDISNA, Pamplona, Spain
[e] Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA, USA
[f] Department of Preventive Medicine, University of Valencia, Valencia, Spain
[g] Unit of Cardiovascular Risk and Nutrition, Institut Hospital Del Mar de Investigaciones Médicas Municipal D'Investigació Médica (IMIM), Barcelona, Spain
[h] Department of Nutrition, Food Sciences, and Physiology, Center for Nutrition Research, University of Navarra, Pamplona, Spain
[i] Precision Nutrition and Cardiometabolic Health Program. IMDEA Food, CEI UAM + CSIC, Madrid, Spain
[j] Bioaraba Health Research Institute, Cardiovascular, Respiratory and Metabolic Area; Osakidetza Basque Health Service, Araba University Hospital; University of the Basque Country UPV/EHU, Vitoria Gasteiz, Spain
[k] EpiPHAAN Research Group, School of Health Sciences, University of Málaga - Instituto de Investigación Biomédica en Málaga (IBIMA), Málaga, Spain
[l] CIBER de Epidemiología y Salud Pública (CIBERESP), Instituto de Salud Carlos III, Madrid, Spain
[m] Instituto de Investigación Sanitaria y Biomédica de Alicante. Universidad Miguel Hernández (ISABIAL-UMH). Alicante, Spain
[n] Health Research institute of the Balearic Islands (IdISBa), Palma de Mallorca, Spain
[o] Department of Internal Medicine, Maimonides Biomedical Research Institute of Cordoba (IMIBIC), Reina Sofia University Hospital, University of Cordoba, Cordoba, Spain
[p] Department of Internal Medicine, Hospital Clinic, Institut D'Investigacions Biomèdiques August Pi I Sunyer (IDIBAPS), Institut de Recerca en Nutrició i Seguretat Alimentària (INSA), University of Barcelona, Barcelona, Spain
[q] Virgen de La Victoria Hospital, Department of Endocrinology, Instituto de Investigación Biomédica de Málaga (IBIMA). University of Málaga, Málaga, Spain
[r] Department of Family Medicine, Research Unit, Distrito Sanitario Atención Primaria Sevilla, Sevilla, Spain
[s] Research Institute of Biomedical and Health Sciences (IUIBS), University of Las Palmas de Gran Canaria & Centro Hospitalario Universitario Insular Materno Infantil (CHUIMI), Canarian Health Service, Las Palmas de Gran Canaria, Spain

Abbreviations: CVD, Cardiovascular diasease; EDC, Endocrine disrupting chemicals; EFSA, European Food Safety Authority; er-MedDiet, energy-reduced Mediterranean diet; FFQs, food frequency questionnaires; OR, odds ratios; PCDD/Fs, polychlorinated dibenzo-p-furans; PREDIMED-Plus, PREvención con Dieta MEDiterránea; PRs, prevalence ratios; TCDD/F, 2,3,7,8-tetrachlorodibenzo-p-dioxin/furan; TEQ, Toxic Equivalents; TWI, Tolerable weekly intake; WHO, World Health Organization; BMI, Body Mass Index; MET, Metabolic Equivalent of Task.

* Corresponding author. Institut D'Investigació Sanitària Pere Virgili (IISPV).Hospital Universitari Sant Joan de Reus Avda. Josep Laporte, 2Planta 0 - E2 color taronja, 43204 Reus (Tarragona), Spain.

** Corresponding author. Universitat Rovira i Virgili, Departament de Bioquímica i Biotecnologia, Alimentació, Nutrició, Desenvolupament i Salut Mental ANUT-DSM. Sant Llorenç 21, 43201 Reus (Tarragona), Spain.

E-mail addresses: mariaangeles.martinez@iispv.cat (M.Á. Martínez), jordi.salas@urv.cat (J. Salas-Salvadó).

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RESULTS

[t] Department of Preventive Medicine and Public Health, University of Granada, Granada, Spain
[u] Research Group on Community Nutrition & Oxidative Stress, University of Balearic Islands, Palma de Mallorca, Spain
[v] CS Milladoiro, Área Sanitaria de Santiago de Compostela, Spain
[w] Instituto de Investigación de Santiago de Compostela (IDIS), Spain
[x] Centro de Investigación Biomédica en Red-Enfermedades Cardiovasculares (CIBERCV) , Spain
[y] Lipids and Vascular Risk Unit, Internal Medicine, Hospital Universitario de Bellvitge-IDIBELL, Hospitalet de Llobregat, Barcelona Spain
[z] Departamento de Ciencias de La Salud, Instituto Universitario de Investigación en Olivar y Aceites de Oliva, Universidad de Jaén, Jaén, Spain
[aa] Department of Endocrinology and Nutrition, Instituto de Investigación Sanitaria Hospital Clínico San Carlos (IdISSC), Madrid, Spain
[ab] CIBER Diabetes y Enfermedades Metabólicas (CIBERDEM), Instituto de Salud Carlos III (ISCIII), Madrid, Spain
[ac] Department of Endocrinology, Institut D'Investigacions Biomèdiques August Pi Sunyer (IDIBAPS), Hospital Clínic, University of Barcelona, Barcelona, Spain
[ad] Department of Endocrinology and Nutrition, Hospital Fundación Jimenez Díaz. Instituto de Investigaciones Biomédicas IISFJD. University Autonoma, Madrid, Spain
[ae] Nutritional Control of the Epigenome Group. Precision Nutrition and Obesity Program. IMDEA Food, CEI UAM + CSIC, Madrid, Spain
[af] Lipid Clinic, Department of Endocrinology and Nutrition, Institut D'Investigacions Biomèdiques August Pi Sunyer (IDIBAPS), Hospital Clínic, Barcelona, Spain
[ag] Centro Salud San Vicente Del Raspeig, Alicante, Spain
[ah] Internal Medicine Clinical Management Unit, Hospital Regional Universitario de Málaga, Instituto de Investigación Biomédica de Málaga (IBIMA-Plataforma BIONAND), Universidad de Málaga, Spain
[ai] Departamento de ciencias farmacéuticas y de la salud. Facultad de Farmacia. Universidad San Pablo CEU. CEU Universities. Urbanización Montepríncipe, 28660 Boadilla del Monte, Spain
[aj] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain

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ABSTRACT

Introduction: The principal source of exposure to Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzo-p-furans (PCDD/Fs) in humans comes from food intake. PCDD/Fs, are a family of potential endocrine disruptors and have been associated with different chronic diseases such as diabetes and hypertension. However, studies assessing the relationship between dietary exposure to PCDD/Fs and adiposity or obesity status in a middle-aged population are limited.

Objective: To assess cross-sectionally and longitudinally the associations between estimated dietary intake (DI) of PCDD/Fs and body mass index (BMI), waist circumference, and the prevalence/incidence of obesity and abdominal obesity in a middle-aged population.

Methods: In 5899 participants aged 55–75 years (48% women) living with overweight/obesity from the PREDIMED-plus cohort, PCDD/Fs DI was estimated using a 143-item validated food-frequency questionnaire, and the levels of food PCDD/F expressed as Toxic Equivalents (TEQ). Consequently, cross-sectional and prospective associations between baseline PCDD/Fs DI (in pgTEQ/week) and adiposity or obesity status were assessed at baseline and after 1-year follow-up using multivariable cox, logistic or linear regression models.

Results: Compared to participants in the first PCDD/F DI tertile, those in the highest tertile presented a higher BMI (β-coefficient [confidence interval]) (0.43kg/m2 [0.22; 0.64]; P-trend <0.001), a higher waist circumference (1.11 cm [0.55; 1.66]; P-trend <0.001), and a higher prevalence of obesity and abdominal obesity (1.05 [1.01; 1.09] and 1.02 [1.00; 1.03]; P-trend 0.09 and 0.027, respectively). In the prospective analysis, participants in the top PCDD/F DI baseline tertile showed an increase in waist circumference compared with those in the first tertile after 1-year of follow-up (β-coefficient 0.37 cm [0.06; 0.70]; P-trend 0.015).

Conclusion: Higher DI of PCDD/Fs was positively associated with adiposity parameters and obesity status at baseline and with changes in waist circumference after 1-year of follow-up in subjects living with overweight/obesity. Further large prospective studies using a different population with longer follow-up periods are warranted in the future to strengthen our results.

1. Introduction

Nowadays, overweight and obesity are a major public health concern worldwide, with nearly one out of every three adults (30.7%) are living with overweight and more than two out of five adults (42.4%) are living with obesity (Ogden et al., 2021). In addition, in the next two decades, a potential 120% increase in the prevalence of severe obesity is estimated to occur (Finkelstein et al., 2012). Studies also have shown that obesity is associated with the onset of chronic diseases, such as cardiovascular diseases (CVD) and some types of cancer, but also premature death (Matsunaga et al., 2022; Célind et al., 2022; MacMahon et al., 2009).

It is well established that obesity is a complex and multidimensional condition caused by the interaction of several factors such as diet, physical activity and other behavioral factors, genetic predisposition and environmental exposures (Qasim et al., 2018; Sun et al., 2017; Ramos-Lopez et al., 2021). While some factors are already long studied and well-known, those concerning environmental exposure, specifically endocrine disrupting chemicals (EDCs) in our daily life have just started to gain important consideration (Street et al., 2018).

EDCs are synthetic compounds capable of binding to cell receptors and mimicking or blocking the functioning of natural hormones, thus could cause adverse health effects (Lauretta et al., 2019; Diamanti-Kandarakis et al., 2009). They can be bio-accumulated in the human body from the food chain. Consequently, they can be also referred to as persistent organic pollutants (Van den Berg et al., 1994). Among the most hazardous ones, the Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzo-p-furans (PCDD/Fs) are considered to pose serious risks for humans (Srogi, 2008). The sources of PCDD/Fs were described either from their natural occurrence in some food manufacture, agricultural practices, industrial applications and atmospheric deposition into the food chain. The most relevant reported exposure to PCDD/Fs worldwide comes from the consumption of food, being meat, dairy, and fish products as the main contributors, accounting for almost 98% of human exposure (Air Quality Guidelines for Europe Second Edition, 2000; Fries, 1995). These EDCs could reach the human body and become significant because of chronic exposure and the tendency of these contaminants to accumulate due to their high metabolic stability, lipid solubility in the body, and resistance to degradation (Srogi, 2008; Schuhmacher et al., 1999).

There is a growing body of evidence coming from the literature

[1] These authors have contributed equally to this work.
[2] These authors share senior authorship.

examining the associations between exposure to PCDD/Fs and the risk of insulin resistance (Chang et al., 2016a), diabetes (Fierens et al., 2003), metabolic syndrome (Uemura et al., 2009a) or CVD (Donat-Vargas et al., 2020). However, the epidemiological evidence in middle-aged people is limited and mostly cross-sectionally analysed. Moreover, most of the studies conducted until now have not specifically assessed dietary exposure to PCDD/Fs.

Given the public health burden of the obesity epidemic and the increasing health effects of dietary exposure to these EDCs could cause, it is essential to understand and better delimitate potential associations between these compounds in everyday life and the risk of obesity or other related non-communicable diseases. We hypothesized that high exposure to dioxins and furans through the diet may have a relationship with adiposity and obesity development, therefore, the aim of the present study was to cross-sectionally and prospectively assess the associations between the estimated dietary intake of PCDD/Fs and adiposity or obesity risk in a middle-aged Spanish Mediterranean cohort. This will provide new insights to grow the evidence and to implement public health strategies in the future.

2. Materials and methods

2.1. Study population and design: the PREDIMED-plus study

A cross-sectional and prospective analysis was conducted using the data of the PREDIMED-Plus (PREvención con DIeta MEDiterránea) cohort. The PREDIMED-Plus study is a randomized and ongoing controlled trial, conducted in 23 Spanish centres with the objective to compare the effect of an intensive weight loss intervention based on an energy-reduced Mediterranean diet -er-MedDiet-, physical activity promotion, and behavioral support (intervention group), to an intervention consisting in recommending a non-caloric reduced MedDiet following usual care advice (control group), on the incidence of CVD and mortality. More available details of the PREDIMED-Plus study can be found at https://www.predimedplus.com and have been described elsewhere (Sayón-Orea et al., 2019; Salas-Salvadó et al., 2019). Participants were recruited (between October 2013 and December 2016) in Spain. A total of 6874 participants were randomly assigned to the intervention group or the control group in a 1:1 ratio. Eligible participants are living with overweight or obesity (BMI = 27–40 kg/m^2), men and women aged 55–75 years without documented history of CVD, who met at least three of the following metabolic syndrome criteria: a) waist circumference of more than 102 cm in men and more than 88 cm in women; b) serum triglycerides equal to or more than 150 mg/dl or drug treatment for elevated triglycerides; c) HDL-c less than 40 mg/dl in men and less than 50 mg/dl in women or drug treatment for low HDL-cholesterol; d) blood pressure equal or higher than 130/85 mmHg or antihypertensive drug treatment; e) and fasting plasma glucose level equals or more than 100 mg/dl or hypoglycemic treatment. Extensive descriptions of inclusion and exclusion criteria can be found elsewhere (Martínez-González et al., 2019). All subjects provided written informed consent, and the final project protocol was approved by the ethical review boards of each centre involved. Participants without complete food frequency questionnaires (FFQs) at baseline and after 1-year of follow-up or those with an energy intake outside the pre-specified energy limits (less than 500 and more than 3500 kcal/day, and less than 800 and higher than 4000 kcal/day in women and men respectively) and participants without data for BMI and waist circumference after 1 year of follow-up n = 22 were excluded (Supplementary Material, File S1). Participants included (n = 5899) in the present study showed no differences in sociodemographic, dietary, and biomedical characteristics compared to the total randomized PREDIMED-plus participants (n = 6874) (Supplementary Material, Table S1).

2.2. Theory and calculation of the dietary intake assessment

Validated face-to-face baseline FFQs were administrated to our study cohort population by trained staff (De La Fuente-Arrillaga et al., 2010). The participants documented their average frequency and quantity of consumption for 143 food and beverage items over the preceding year. The frequency of consumption was shown through nine categories ranging from never or almost never, one to three times per month, once per week, two to four times per week, five to six times per week, once per day, two to three times per day, four to six and more than 6 times per day.

The dietary intake was estimated using the frequency of consumption previously mentioned and the levels of the PCDD/F expressed as Toxic Equivalents (TEQ). The TEQ is established by the World Health Organization (WHO) (Van den Berg et al., 2006) to assess total PCDD/F exposure by the sum of an individual's congener concentration multiplied by their relative toxicity factor. This factor's scale ranges from [0 to 1], where 0 corresponds to the less toxic compound and the value of 1 corresponds to 2,3,7,8-tetrachlorodibenzo-p-dioxin/furan (TCDD/F) which is the most toxic PCDD/F known (Van den Berg et al., 2006).

The TEQ for each food item was obtained from the most updated published studies with a first priority for studies from the Mediterranean region since our participants are from Spain and they technically follow a Mediterranean diet. Therefore, the hierarchy rule followed to gather the TEQ for each food item was: data from Spain > data from other Mediterranean countries > Europe > other available data (Supplementary Material, Table S2).

TEQ data was available for most products and reported as pg TEQ/g (on a fresh weight basis). For some particular food products, the TEQ/g was estimated (as an average) from related food items as previously performed by other studies such as Kiviranta et al., 2001 (Supplementary material, Table S2). Alcoholic beverages and coffee were excluded from the dietary intake assessment since they generally contain very low levels of PCDD/Fs and will, therefore, add little to the total dietary TEQ intake (Air Quality Guidelines for Europe Second Edition, 2000; Kiviranta et al., 2001).

The estimated dietary intake of PCDD/F (expressed in TEQ pg/week) was the sum of each TEQ (in TEQ pg/day) from a specific food item multiplied by the amount of each specific food consumed for each participant (in g/day) multiplied by seven considering the following equation:

$$\textit{Dietary intake of PCDD/F}_{(pg\ /week)} = (\sum TEQ_i * A_i) * 7$$

Where TEQ_i *represents the toxic equivalency of a specific food item (in pg PCDD/F/g),* A_i *represents the amount of a specific food consumed (in g/day) and it was multiplied by 7 to obtain the weekly dietary intake of PCDD/F in pg TEQ/week.*

2.3. Estimation of dietary exposure to PCDD/Fs

Dietary exposure to dioxins and furans was estimated according to the following equation:

$$\text{Dietary exposure to PCDD/F}_{(pg/kg\ of\ body\ weight/week)} = (\sum TEQ_i * A_i * 7) / BW_i$$

Where TEQi represents the toxic equivalency of a specific food item (in pg PCDD/F/g), Ai represents the amount of a specific food consumed (in g/day), BWi represents the body weight (kg) of each participant, and the result was multiplied by 7 to obtain the weekly dietary exposure of PCDD/F in pg TEQ/Kg of body weight/week.

3. Outcomes

Measurements of different anthropometric variables at baseline and 1 year of follow-up were assessed by PREDIMED-Plus staff. Body weight was measured twice with light clothes using high-quality electronic

calibrated scales, and the mean of both measurements were used. Height was measured using a wall-mounted stadiometer. Waist circumference was measured midway between the lowest rib and the iliac crest using an anthropometric tape. Body mass index (BMI) was calculated by dividing the weight in kilograms by the square of height in meters. On one hand, obesity was defined when BMI was higher than or equal to 30 kg/m^2. On the other hand, abdominal obesity was defined when waist circumference was higher than or equal to 88 cm for women and higher than or equals to 102 cm for men (Alberti et al., 2009). Therefore, the prevalence of obesity and abdominal obesity were defined as participants having higher levels of BMI and waist circumference at baseline than those indicated above. In addition, the incidence of obesity and abdominal obesity were defined when participants did not present these conditions (obesity and abdominal obesity) at baseline but showed higher levels of BMI and waist circumference than those indicated above after 1-year of follow-up.

4. Assessment of covariates

Covariates were evaluated using general face-to-face questionnaires that collect information on lifestyle (smoking habits and physical activity), socio-demographics (sex, marital status, age, and level of education), history of type 2 diabetes, hypertension, hypercholesterolemia, and medication use. A validated Minnesota-REGICOR Short Physical Activity questionnaire was used to assess the physical activity (Molina et al., 2017). In addition, adherence to the traditional MedDiet was using a validated 14-item MEDAS questionnaire was provided (Schröder et al., 2011).

4.1. Statistical analyses

For the statistical analyses, the PREDIMED-Plus database updated in December 2020 was used. Descriptive baseline sociodemographic, dietary, and biomedical characteristics by tertiles of energy-adjusted PCDD/F intake are shown in Table 1, and expressed as mean ± SD for continuous variables and percentages (number) for categorical variables.

The dietary intake of PCDD/F (expressed in TEQ pg/week) was adjusted for total energy intake using the residual-regression method (Willett and Stampfer, 1986) and categorized in tertiles of energy-adjusted (TEQ pg/week) corresponding to lower (first tertile), intermediate (second tertile), and higher (third tertile) dietary intake of PCDD/F. The Chi-square test for categorical variables and one-way ANOVA for continuous variables were used to compare the baseline characteristics across the tertiles.

Linear regression models were fitted to cross-sectionally assess the associations [β-coefficient (95% confidence interval (CI)] between energy-adjusted dietary intake of PCDD/F and adiposity parameters (BMI and waist circumference) at baseline. We also used linear regression models to explore the prospective associations between baseline energy-adjusted PCDD/F and changes in BMI and waist circumference after 1 year of follow-up.

Cox proportional hazards regression with a robust variance estimator and constant follow-up time (t = 1) were fitted to estimate prevalence ratios (PRs) and 95% confidence interval (95% CI) for the prevalence of obesity and abdominal obesity across tertiles of energy-adjusted dietary intake of PCDD/F. This method has been described to be more appropriate than logistic regression in cross-sectional studies when the outcome prevalence is >10%, as odds ratios (OR) could overestimate or underestimate the risk in logistic regression (Barros and Hirakata, 2003).

Logistic regression models were also fitted to estimate the Odd Ratio (OR) and 95% confidence interval (95% CI) for the incidence of obesity and abdominal obesity across tertiles of energy-adjusted dietary intake of PCDD/F. To assess the linear trend, the median values for each tertile of PCDD/F dietary intake were assigned and used as continuous

Table 1
Characteristics of included participants overall and across energy-adjusted total PCDD/F dietary intake tertiles.

	All	Energy-adjusted total PCDD/F dietary intake – (TEQ pg/week)			
		T1 n=1,970	T2 n=1,964	T3 n=1,965	P-value
Energy adjusted total PCDD/F dietary intake (TEQ pg/week) **range**	73.6 - 496	73.6 - 223	224 - 271	272 - 496	<0.001
Sex					
Women	48.3 (2,847)	39.6 (781)	49.1 (965)	56.0 (1,101)	<0.001
Age (years) (n=5,899)	65.0 ± 4.90	65.0 ± 5.00	65.1 ± 4.90	64.9 ± 4.80	0.72
Educational level (n=5,899)					
Up to primary	49.7 (2,931)	48.0 (945)	50.0 (981)	51.1 (1,005)	0.196
Secondary	28.8 (1,696)	29.0 (572)	29.4 (578)	27.8 (546)	
University	21.5 (1,272)	23.0 (453)	20.6 (405)	21.1 (414)	
Marital status (n=5,899)					
Single	12.5 (738)	14.8 (291)	11.9 (234)	10.8 (213)	0.001
Married	77.1 (4,546)	76.0 (1,498)	77.4 (1,520)	77.8 (1,528)	
Widowed	10.4 (615)	9.20 (181)	10.7 (210)	11.4 (224)	
Smoking status (n=5,899)					
Never	44.7 (2,636)	40.1 (791)	45.3 (890)	48.6 (955)	<0.001
Former	43.0 (2,535)	46.0 (905)	42.2 (829)	40.8 (801)	
Current	12.3 (728)	14.0 (274)	12.5 (245)	10.6 (209)	
Physical activity (MET min/week) (n=5,899)	2509 ± 2325	2454 ± 2306	2464 ± 2291	2610 ± 2375	0.06
Waist circumference (cm) (n=5,899)					
Men	110 ± 8.70	110 ± 8.70	111 ± 9.00	111 ± 8.40	0.06
Women	103 ± 9.20	103 ± 9.20	103 ± 8.90	104 ± 9.30	0.06
BMI (kg/m^2) (n=5,899)	32.5 ± 3.40	32.2 ± 3.30	32.5 ± 3.50	32.7 ± 3.50	<0.001
Intervention group (%)	48.9	49.6	49.6	47.7	0.34
Energy-reduced Mediterranean diet adherence (0-14 points) (n=5,899)	8.18 ± 1.94	7.88 ± 1.97	8.24 ± 1.83	8.42 ± 1.96	<0.001
Dietary assessment (n=5,899)					
Fruits and vegetables (g/d)	666 ± 260	605 ± 194	676 ± 266	747 ± 315	<0.001
Legumes (g/d)	20.6 ± 11.0	20.1 ± 11.5	20.3 ± 10.2	21.3 ± 11.4	0.001
Total cereals (g/d)	151 ± 78.0	170 ± 87.5	144 ± 71.0	136 ± 70.5	<0.001
Unrefined cereals (g/d)	41.0 ± 63.2	38.3 ± 68.9	40.3 ± 60.1	44.4 ± 60.2	0.009
Dairy products (g/d)	345 ± 201	345 ± 207	334 ± 188	353 ± 205	0.009
Red meat and derivatives (g/d)	82.3 ± 44.8	73.7 ± 43.8	77.2 ± 39.2	95.9 ± 47.8	<0.001
White meat (g/d)	62.0 ± 34.0	47.6 ± 28.0	63.9 ± 30.5	74.4 ± 37.6	<0.001
Fish and shellfish (g/d)	102 ± 47.4	82.5 ± 40.4	102 ± 42.2	122 ± 50.0	<0.001
Virgin olive oil (g/d)	31.9 ± 20.8	30.9 ± 20.8	32.4 ± 20.7	32.6 ± 20.5	0.02
Total energy (Kcal/d)	2367 ± 550	2422 ± 578	2293 ± 522	2386 ± 541	<0.001

(continued on next page)

Table 1 (*continued*)

	All	Energy-adjusted total PCDD/F dietary intake – (TEQ pg/week)			
		T1 n=1,970	T2 n=1,964	T3 n=1,965	P-value
Proteins (g/d)	97.7 ± 22.0	90.2 ± 20.5	95.4 ± 19.6	107 ± 22.0	<0.001
Saturated fatty acids (g/d)	26.2 ± 8.40	26.4 ± 8.90	25.4 ± 7.80	26.8 ± 8.49	<0.001
Polyunsaturated fatty acids (g/d)	18.0 ± 6.5	17.8 ± 6.86	17.6 ± 6.24	18.5 ± 6.46	<0.001
Carbohydrates (g/d)	241 ± 72.7	257 ± 76.3	231 ± 66.9	234 ± 71.4	<0.001
Dietary fibre (g/d)	26.1 ± 8.7	25.2 ± 8.73	25.6 ± 8.17	27.6 ± 9.05	<0.001
Total sugar (g/d)	6.70 ± 12.0	8.63 ± 13.3	6.04 ± 11.2	5.56 ± 11.0	<0.001
Obesity prevalence					
Yes	72.7 (4,286)	71.6 (1,411)	71.5 (1,405)	74.8 (1,470)	0.03
Abdominal obesity prevalence					
Yes	93.1 (5,490)	92.0 (1,812)	92.4 (1,815)	94.8 (1,863)	0.001
Hormonal treatment (n=5,899)					
Yes	2.23 (131)	1.94 (38)	2.55 (50)	2.19 (43)	0.42

Abbreviations: PCDD/F: Polychlorinated dibenzo-p-dioxins or dibenzofurans; TEQ: Toxic Equivalency; BMI: body mass index; MET: Metabolic Equivalent of Task.Data are expressed as mean ± SD and percentages (number) for continuous and categorical variables, respectively.P-values for comparisons were tested by one-way ANOVA or Chi-square test, as appropriate across tertiles.

variables in the models. Additionally, statistical analyses were conducted to evaluate whether the associations observed could be modified by the intervention group. Therefore, interaction analyses were conducted by intervention group using the likelihood ratio test.

Three multivariable-adjusted models with progressive adjustment for covariates that may confound the association were used. 1) Model 1: adjusted for age and sex; 2) Model 2: model 1 additionally adjusted for physical activity (METs min/week), marital status (married, widowed, single or divorced or separated, or religious), smoking status (never, former, or current), education level (primary or lower, secondary or academic, or graduate) and the size of the recruitment centres representing the total field centre workload (<250, 250 to <300, 300 to <400, ≥400 randomized participants); 3) Fully adjusted: Model 2 additionally adjusted for adherence to Mediterranean diet using the 14-item MEDAS score, hormonal treatment, and prevalence of having diabetes (yes/no), hypertension (yes/no) and hypercholesterolemia (yes/no) or taking medications for their control. In the prospective analysis, each adiposity parameter was further adjusted for its baseline measurement (in the fully adjusted model) and the intervention group.

The statistical significance threshold for the results was set at $p < 0.05$. All analyses were conducted with robust estimates of the variance to correct for intra-cluster correlation and using the Stata 14 software program (StataCorp).

5. Results

5899 participants (52% men and 48% women, with a mean age of 65 years) were included in this study after excluding 953 participants lacking FFQ and with an energy intake out of the specified limits. The baseline characteristics of the participants overall and according to tertiles of total energy-adjusted dietary PCDD/F intake (TEQ pg/week) are shown in Table 1. A total of 72.7% of participants had obesity and 93.1% had abdominal obesity at baseline.

In this cohort, the participants' PCDD/Fs dietary intake ranged between 73.6 and 496 pg/week and the leading food groups responsible for PCDD/F intake were red meat (28%), fish and seafood (18%), and fruits and vegetables (17%), followed by white meat (11%) and oils and fats (10%) (Fig. 1).

Compared to participants with lower dietary intake of PCDD/Fs (Tertile 1, range between 73.6 and 223 pg/week), those participants with highest PCDD/Fs intake (Tertile 3, range between 272 and 496 pg/week) were more frequently women, more physically active and were less frequently smokers and consume higher amounts of fruits and vegetables, legumes, unrefined cereals, dairy products, red meat and derivatives, white meat, fish and shellfish, virgin olive oil, dietary fibre, protein, and saturated and polyunsaturated fatty acids. On the other hand, they consumed lower amounts of refined cereals, biscuits, and sugar.

In the cross-sectional analysis (Table 2), positive and linear associations were observed between the energy-adjusted dietary intake of PCDD/F and BMI or waist circumference and significantly increased through models 1 and 2 after controlling for different confounders reaching the most significant association in the fully-adjusted model (β coefficient [95%CI]) (0.43 kg/m^2 [0.22 to 0.64] and 1.11 cm [0.55 to 1.66], P for trend <0.001 for BMI and waist circumference respectively).

Compared to those participants in the lowest tertile of energy-adjusted dietary intake of PCDD/F, those in the highest tertile showed a higher prevalence of obesity and abdominal obesity PRs [95% CI] = 1.05 [1.01 to 1.09] and 1.02 [1.00 to 1.03], respectively.

In the prospective analysis (Table 3), participants in the top tertile of baseline energy-adjusted dietary intake of PCDD/F (pg/week) showed an increase in waist circumference (β coefficient [95%CI]; 0.37 cm [0.06 to 0.70]; P for trend = 0.01) compared to those in the lowest tertile after 1-year of follow-up. No other significant associations were observed for BMI nor for obesity or abdominal obesity incidence, although the direction of these associations remains as those observed in the cross-sectional analysis. In addition, cross-sectional and prospective associations between dietary intake of PCDD/Fs in each food group and adiposity parameters, prevalence/incidence of obesity, and abdominal obesity are found in the Supplementary Material (Tables S3-S12).

Interactions of the likelihood ratio tests between tertiles of PCDD/Fs and the stratified subgroup variable (intervention and control groups) were not significant ($p > 0.05$).

6. Discussion

In this large cohort of middle-aged subjects at high cardiovascular risk, higher dietary exposure to PCDD/Fs was shown to be cross-sectionally associated with higher measures of adiposity parameters and a higher prevalence of obesity and abdominal obesity. In addition, a higher dietary intake of PCDD/Fs at baseline was positively associated with changes in waist circumference after 1 year of follow-up. No other significant associations were observed for BMI nor for obesity or abdominal obesity incidence, although the direction of these associations was the same as those observed in the cross-sectional analysis. Consequently, our results support the hypothesis that there is a positive relationship between the dietary intake of PCDD/Fs and adiposity and obesity parameters.

Current results are in line with other epidemiological and mechanistic studies (Windal et al., 2010b; Fattore et al., 2006). The present study provides data concerning the associations between dietary intake of PCDD/Fs and adiposity parameters and prevalence/incidence of obesity in a large cohort study population, being to the best of our knowledge, the first article on this topic. However, some studies have also investigated the association between similar chemical substances such as dioxin-like polychlorinated biphenyls (DL-PCBs) and adiposity through the dietary intake (Windal et al., 2010b; Fattore et al., 2006). For example, in a prospective Spanish study conducted with healthy participants, a positive association between higher dietary intake of DL-PCBs and obesity risk was found (Donat-Vargas et al., 2014). As reviewed by Hassan et al., 2021), excessive exposure to PCB in humans was also related to different metabolic disorders including hepatic steatosis, obesity, diabetes mellitus, endocrine metabolism, thyroid

RESULTS

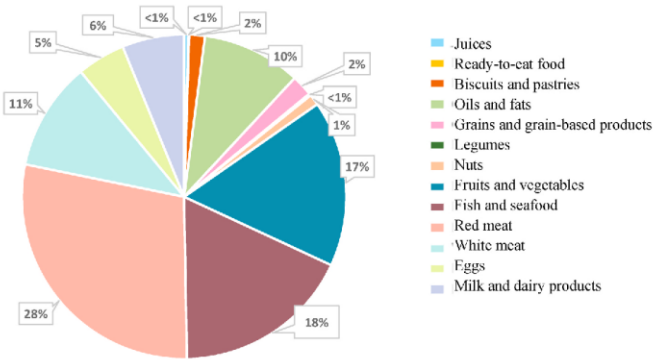


Fig. 1. Percentage of dietary PCDD/Fs intake coming from the different food groups consumed by the PREDIMED-Plus participants.

metabolism, and dyslipidaemia (Hassan et al., 2021).

It was determined that red meat (28%), fish and seafood (18%) and fruits and vegetables (17%) were the main food groups contributing to the total PCDD/Fs dietary intake in our study (Fig. 1), being also the main food groups contributors in each PCDD/Fs dietary intake tertile (data not shown). Our results showed a higher dietary intake of PCDD/Fs in women compared to men ($p < 0.001$), this could be explained because women present a higher consumption of fish, seafood and fruits and vegetables. This is in line with other studies reporting that red meat and seafood were the most contributors to the total intake of PCDD/Fs (Schwarz et al., 2014; Törnkvist et al., 2011; Windal et al., 2010a). The high dietary intake of PCDD/Fs coming from fruits and vegetables could be explained due to the high consumption of these food items in our study population and not by the high levels of TEQ detected in this food category. According to the literature, it is important to consider the concentration levels of these chemicals and the specific frequency of consumption of each food item from each population profile to estimate the exposure (Barone et al., 2021; Sirot et al., 2012; Windal et al., 2010b). In fact, the major contributors detected here could be different in other cohort study populations depending on their lifestyle and dietary patterns. In our PREDIMED-plus study, participants have a high adherence to the Mediterranean diet. Because of that, fruits and vegetables (instead of dairy products) are one of the main contributors to total dioxin exposure along with meat and fish.

Our findings are also in line with other studies measuring PCDD/F in blood. Fattore et al., 2006 reported higher serum PCDD/F levels in middle-aged women than in men (Fattore et al., 2006). Beyond the dietary intake, it is reasonable to expect that women present higher PCDD/Fs serum levels than men since PCDD/Fs are principally stored in adipose tissue, and women tend to have naturally greater percentage of body fat (Van den Berg et al., 1994). Moreover, in the cross-sectional analyses of Chang et al., 2016b, it has been found a positive and significant association between serum PCDD/Fs concentrations and the prevalence of abdominal obesity. They also found that participants with abdominal obesity had significantly higher serum dioxin levels compared to those participants without abdominal obesity (Chang et al., 2016b). In addition, a study conducted by Lee et al., 2007, found a positive relationship between serum concentrations of dioxins and waist circumference and prevalence of metabolic syndrome (Lee et al., 2007). Furthermore, Uemura et al., 2009b reported that body levels of dioxins and related compounds, particularly those of DL-PCBs, were cross-sectionally associated with BMI and the risk of metabolic syndrome (Uemura et al., 2009b).

Animal studies also suggest that these compounds can have potential effects on adiposity. Hoyeck et al., 2020 demostrated in animals that low-dose TCDD exposure in female mice impair metabolic adaptability to a high fat diet, indicating that dioxin exposure may be a causative factor of obesity and diabetes pathogenesis in females (Hoyeck et al., 2020). Brulport et al., 2017 also provided new evidence that dioxins, could present obesogenic effects and metabolic disturbances in adult mice (Brulport et al., 2017).

According to the European Food Safety Authority (EFSA), the tolerable weekly intake (TWI) of dioxins and furans in food (established in 2018) is 2.0 pgTEQ/kg of body weight/week (Knutsen et al., 2018). The TWI set by the European Commission's Scientific Committee on Food in 2001 was seven times higher than the current EFSA TWI (EFSA, 2018). It is important to consider that in this current study the 87% of our population exceeded the PCDD/Fs TWI with a mean of 2.99 pg TEQ/kg of body weight/week. The current TWI mean is similar to other studies conducted around the world and in accordance, even slightly above, with a previous study carried out in Spain (Llobet et al., 2008) in which dietary mean exposure also exceeded the EFSA TWI limits. Compared to other countries, the mean level of exposure of our participants is similar to other studies conducted in Netherlands or Ireland (De Mul et al., 2008). In contrast, studies from Italy (Diletti et al., 2018), France (Sirot et al., 2012) and Ireland (Tlustos et al., 2014) reported lower PCDD/Fs exposure compared to our participants. On the other hand, in countries such as Germany (Schwarz et al., 2014), the United Kingdom (Bramwell et al., 2016) and Taiwan (Wang et al., 2009) a higher PCDD/Fs exposure has been detected. It has been observed that the PCDD/Fs dietary exposure in healthy general populations of numerous countries in Europe has been declining in the last years (Llobet et al., 2008; Arisawa, 2018). Dioxin emissions have been decreased as a result of demanding laws governing waste treatment and disposal, which may account for the majority of this decline. According to several studies, food consumption of PCDDs/PCDFs decreased to a greater extent than DL-PCBs (González and Domingo, 2021; Tard et al., 2007). The improvements and efforts made in the waste incineration process concerning PCDDs/PCDFs emissions in recent years could be the reason for these decreases (González and Domingo, 2021; Tard et al., 2007). However, the problem is that due to the large discharges produced and the lack of periodic review of the legislation, we still have high levels of dioxins remaining, especially in the trophic chain (Arisawa, 2018). Dioxins can be bioaccumulated in the human body for almost a decade, and it is known that between 90 and 95% of dioxin absorption occurs via dietary sources (WHO, 2016).

Our study has several limitations that deserve to be discussed. The results cannot be generalized to other populations since participants included in the analysis were middle-aged Mediterranean individuals with overweight/obesity and metabolic syndrome. In addition, the

Table 2
Multivariable-adjusted β-coefficients or prevalence ratios and its 95% confidence interval for adiposity parameters and obesity prevalence, respectively, across baseline energy-adjusted total PCDD/F dietary intake tertiles.

	Energy-adjusted total PCDD/F dietary intake tertiles (TEQ pg/week)				
	Tertile 1 n=1,970	**Tertile 2** n=1,964	**Tertile 3** n=1,965	***P-trend***	**Continuous n=5,899**
Energy adjusted total PCDD/F dietary intake (TEQ pg/week), mean ± SD	189 ± 27	247 ± 14	324 ± 59		
β-coefficients (95%CI)					
BMI (kg/m^2)					
Model 1	**0 (Ref.)**	0.21 (-0.01 to 0.42)	**0.33 (0.12 to 0.55)**	**0.003**	**0.002 (0.00 to 0.002)**
Model 2	**0 (Ref.)**	0.21 (-0.00 to 0.43)	**0.38 (0.16 to 0.59)**	**0.001**	**0.002 (0.00 to 0.003)**
Fully-adjusted model	**0 (Ref.)**	**0.25 (0.03 to 0.46)**	**0.43 (0.22 to 0.64)**	**<0.001**	**0.002 (0.00 to 0.003)**
Waist circumference (cm)					
Model 1	**0 (Ref.)**	0.37 (-0.19 to 0.94)	**0.86 (0.29 to 1.42)**	**0.003**	**0.01 (0.00 to 0.02)**
Model 2	**0 (Ref.)**	0.47 (-0.09 to 1.04)	**1.06 (0.5 to 1.62)**	**<0.001**	**0.01 (0.00 to 0.01)**
Fully-adjusted model	**0 (Ref.)**	0.51 (-0.05 to 1.06)	**1.11 (0.55 to 1.66)**	**<0.001**	**0.01 (0.00 to 0.01)**
Prevalence ratios (95%CI)					
Obesity					
Prevalence, % (n)	71.6 (1,411)	71.5 (1,405)	74.8 (1,470)		72.7 (4,286)
Model 1	**1 (Ref.)**	0.99 (0.96 to 1.03)	1.04 (0.99 to 1.08)	**0.049**	1.00 (0.099 to 1.00)
Model 2	**1 (Ref.)**	0.99 (0.95 to 1.03)	**1.04 (1.00 to 1.08)**	**0.030**	**1.00 (1.00 to 1.00)**
Fully -adjusted model	**1 (Ref.)**	1.00 (0.96 to 1.04)	**1.05 (1.01 to 1.09)**	**0.009**	**1.00 (1.00 to 1.001)**
Abdominal obesity					
Prevalence, % (n)	92.0 (1,812)	92.4 (1,815)	94.8 (1,863)		93.1 (5,490)
Model 1	**1 (Ref.)**	0.99 (0.98 to 1.01)	1.01 (0.99 to 1.03)	0.065	**1.00 (1.00 to 1.00)**
Model 2	**1 (Ref.)**	0.99 (0.98 to 1.01)	1.02 (0.99 to 1.03)	**0029**	**1.00 (1.00 to 1.00)**
Fully -adjusted model	**1 (Ref.)**	0.99 (0.98 to 1.01)	**1.02 (1.00 to 1.03)**	**0.027**	**1.00 (1.00 to 1.00)**

Abbreviations: PCCD/F: Polychlorinated dibenzo-p-dioxins or dibenzofurans; TEQ: Toxic Equivalency; CI: confidence interval; BMI: body mass index. SD: Standard deviation.
Linear and cox regression models were fitted to assess the β-coefficients or prevalence ratio, and its 95%CI for adiposity parameters and obesity/overweight prevalence, respectively, across energy-adjusted total dietary PCDD/F intake. Model 1: adjusted for age and sex. Model 2: Model 1 additionally adjusted for physical activity (MET min/week), marital status (single, married, widowed), smoking status (current, former, never), education level (up to primary, secondary, university) and the size of the recruitment center (<250, 250 to <300, 300 to <400, ≥400). Fully adjusted: Model 2 additionally adjusted for adherence to Mediterranean diet MEDAS score (0-14 points) and type 2 diabetes (yes/no), hypertension (yes/no), and hypercholesterolemia (yes/no), or prevalence or taking medications for their control, and hormonal treatment. The robust estimate of variance was used in all analyses to account for the intra-cluster correlation.

limitation of the food intake assessment through FFQ (Food Frequency Questionnaire) is that it is susceptible to measurement errors. However, despite this drawback, FFQs have been widely adopted as a tool in epidemiological studies since the 1990s (Shim et al., 2014). The present study only assessed exposure to PCDDs/PCDFs through dietary sources, but not from other sources such as inhalation or dermal contact. However, the exposure to these chemicals occurs largely (90%) through the diet (Air Quality Guidelines for Europe Second Edition, 2000). Nowadays, estimation of PCDD/F from food sources is feasible. However, more detailed information in the literature on different foods, including their origin, production methods, content and packaging, would be helpful for more accurate estimations. Additionally, it was challenging to compare current results with other articles based on dietary exposure estimations, due to the limited literature data available on that topic. Moreover, we lacked information about the food preparation or cooking techniques that could change PCDD/Fs levels in the finished food product.

7. Conclusion

In conclusion, to our knowledge, this is the first study to explore the association between dietary intake of PCDD/Fs and adiposity. We found that in this middle-aged population, a higher dietary intake of PCDD/Fs was associated with a higher prevalence of obesity and higher adiposity parameters at baseline. In addition, a higher baseline dietary intake of PCDD/Fs was positively associated with changes in waist circumference after 1 year of follow-up. These findings support the growing epidemiological data linking the current exposure to PCDD/Fs with adiposity. Even though the production of PCDD/Fs has stopped in the 80s, PCDD/Fs continue to pose a serious health risk since the DI of our population and of other countries are still above the levels recommended by the EFSA. It is therefore very important to assess the unintentionally exposed population and to keep establishing awareness and prophylactic measures to reduce these levels. Further large prospective studies using different population and with longer follow-up period are warranted in the future to strengthen our results.

Credit author statement

NK, MÁM, NB, and JS-S conceived and designed the study. MÁM-G, DC, MF, JAM, ÁA-G, JW, JV, DR, JL-M, RE, FJT, JL, LS-M, AB-C, JAT, VMS, XP, MD-R, PM-M, JVi, CV, LD, and JS-S, conducted data acquisition. NK, MÁ.M, IP and JS-S performed statistical analyses. NK, MÁ.M, NB, and JS-S carried out interpretation of the data for the study. NK, MÁM, NB and JS-S conducted the first draft. All authors were involved in draft redaction, revision for important intellectual content, read, and approved the final manuscript.

Ethics statement

The studies involving human participants were reviewed and approved by JW: CEI Provincial de Málaga-Servicio Andaluz de Salud (O01_feb_PR2), José Lapetra: CEI de los Hospitales Universitarios Virgen Macarena y Virgen del Rocío-Servicio Andaluz de Salud (PI13/00673), JAM: CEIC Universidad de Navarra (053/2013), DR: CEI de las Illes Balears – Conselleria de Salut Direcció General de Salut Publica i Consum (IB 2242/14 PI), MF: CEIC Parc de Salut Mar y IDIAP Jordi Gol (PI13/120), JSS: CEIC del Hospital Universitari Sant Joan de Reus y IDIAB Jordi Gol (13-07-25/7proj2), Aurora Bueno: CEI de la Provincia de Granada- Servicio Andaluz de Salud (MAB/BGP/pg), CV: CEIC de la Fundacion Jiménez Díaz (EC 26–14/IIS-FJD), MM-G: CEIC Universidad de Navarra (053/2013), Fernando Aros: CEIC Euskadi (PI2014044), DC: CEIC Corporativo de Atención Primaria de la Comunitat Valenciana

Table 3
Multivariable-adjusted β-coefficients or Odds ratios and its 95% confidence intervals for adiposity changes or obesity incidence, respectively, after 1-year of follow-up across baseline energy-adjusted total dietary PCDD/F intake.

	Energy-adjusted total PCDD/F dietary intake tertiles (TEQ pg/week)				
	Tertile 1	**Tertile 2**	**Tertile 3**	***P- trend***	**Continuous**
β-coefficients (95%CI)					
BMI changes **(kg/m^2)**	(n=1,970)	(n=1,964)	(n=1,965)		(n=5,899)
Energy adjusted total PCDD/F dietary intake **(TEQ pg/week), mean ± SD**	189 ± 27	247 ± 14	324 ± 59		
Model 1	**0 (Ref.)**	0.21 (-0.08 to 0.12)	0.09 (-0.00 to 0.20)	0.050	**0.001 (0.000 to 0.001)**
Model 2	**0 (Ref.)**	0.04 (-0.04 to 0.13)	0.09 (-0.01 to 0.19)	0.053	**0.001 (0.000 to 0.001)**
Fully- adjusted model	**0 (Ref.)**	0.03 (-0.05 to 0.13)	0.08 (-0.01 to 0.17)	0.113	0.00 (-0.000 to 0.001)
Waist circumference changes (cm)	(n=1,967)	(n=1,966)	(n=1,966)		(n=5,5899)
Energy adjusted total PCDD/F dietary intake **(TEQ pg/week), mean ± SD**	189 ± 27	247 ± 14	324 ± 59		
Model 1	**0 (Ref.)**	0.11 (-0.22 to 0.44)	**0.36 (0.09 to 0.69)**	**0.029**	0.001 (-0.000 to 0.003)
Model 2	**0 (Ref.)**	0.16 (-0.14 to 0.46)	**0.38 (0.07 to 0.69)**	**0.013**	**0.002 (0.000 to 0.004)**
Fully-adjusted model	**0 (Ref.)**	0.15 (-0.15 to 0.45)	**0.37 (0.06 to 0.70)**	**0.015**	0.002 (-0.000 to 0.004)
Odds ratios (95%CI)					
Obesity	(n= 538)	(n= 538)	(n= 537)		(n=1,613)
Incidence, % (n)	6.8 (38)	8.59 (48)	8.08 (40)		
Energy adjusted total PCDD/F dietary intake **(TEQ pg/week) mean ± SD**	188 ± 27.0	244 ± 13.1	318 ± 56.3		
Model 1	**1 (Ref.)**	1.17 (0.74 to 1.85)	1.28 (0.80 to 2.01)	0.310	1.00 (0.998 to 1.003)
Model 2	**1 (Ref.)**	1.17 (0.73 to 1.86)	1.24 (0.78 to 1.97)	0.651	1.00 (0.997 to 1.003)
Fully-adjusted model	**1 (Ref.)**	1.19 (0.74 to 1.91)	1.25 (0.77 to 2.02)	0.635	1.00 (0.997 to 1.003)
Abdominal obesity	(n=137)	(n=136)	(n=136)		(n=409)
Incidence, % (n)	15.1 (24)	18.2 (27)	20.59 (21)		
Energy adjusted total PCDD/F dietary intake **(TEQ pg/week) mean ± SD**	184 ± 26	236 ± 11	300 ± 42		
Model 1	**1 (Ref.)**	1.20 (0.64 to 2.26)	1.15 (0.60 to 2.19)	0.759	1.00 (0.995 to 1.005)
Model 2	**1 (Ref.)**	1.28 (0.64 to 2.52)	1.11 (0.57 to 2.14)	0.485	0.99 (0.994 to 1.004)
Fully-adjusted model	**1 (Ref.)**	1.28 (0.66 to 2.51)	1.08 (0.56 to 2.08)	0.571	0.99 (0.994 to 1.004)

Abbreviations: PCCD/F: Polychlorinated dibenzo-p-dioxins or dibenzofurans; TEQ: Toxic Equivalency; CI: confidence interval; BMI: body mass index; SD: Standard Deviation.
Linear regression models and logistic regression models were fitted to assess the β-coefficients or odd ratio, and its 95%CI for adiposity parameters and obesity/overweight incidence, respectively across energy-adjusted total dietary PCDD/F intake. Model 1: adjusted for age, sex. Model 2: Model 1 additionally adjusted for physical activity (MET min/week), marital status (single, married, widowed), smoking status (current, former, never), education level (up to primary, secondary, university) and the size of the recruitment centres (<250, 250 to <300, 300 to <400, ≥400). Fully adjusted: Model 2 additionally adjusted to adherence to Mediterranean diet MEDAS score (0–14 points) and the prevalence of having and type 2 diabetes (yes/no), hypertension (yes/no), and hypercholesterolemia (yes/no), or prevalence or taking medications for their control, and hormonal treatment, intervention group and outcome variable at baseline. The robust estimate of variance was used in all analyses to account for the intra-cluster correlation.

(2011-005398-22), LS-M: CEI Humana de la Universidad de las Palmas de Gran Canaria (CEIH-2013-07), XP: CEIC del Hospital de Bellvitge (PR240/13), José López Miranda: CEI de Cordoba-Junta de Salud (3078), José María Ordovás: CEI de la Fundación IMDEA Alimentación (PI-012), PM-M: CEIC Hospital Clínico San Carlos de Madrid-Piloto-CEIC Servicio Madrileño de salud-General (30/15), FT: CEI Provincial de Málaga-Servicio Andaluz de Salud, JT: CEI de las Illes Balears – Conselleria de Salut Direcció General de Salut Publica i Consum (IB 2251/14 PI), JoV: CEIC del Hospital Clínic de Barcelona (HCB/2017/0351), JeV: CEIC del Hospital General Universitario de Alicante (CEIC PI 2017/02), M-DR: CEIC de la Investigación Biomédica de Andalucía (CCEIBA), VM: CEI de la Universidad de León (TICA-ULE-014-2015), and Ramon Estruch: CEIC del Hospital Clínic de Barcelona (HCB/2016/0287). The participants of the current project research provided their written informed consent to join the study.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envres.2023.115697.

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Supplementary Material

Dietary intake of polychlorinated dibenzo-p-dioxins and furans, adiposity and obesity status.

Running title: PCDD/F, adiposity and obesity

Nadine Khoury[1,2,3 †], María Ángeles Martínez[1,2,3*†], Indira Paz-Graniel[1,2,3], Miguel Ángel Martínez-González[1,4,5], Dolores Corella[1,6], Olga Castañer[1,7], J. Alfredo Martínez[1,8,9], Ángel M. Alonso-Gómez[1,10], Julia Wärnberg[1,11], Jesús Vioque[12,13], Dora Romaguera[1,14], José López-Miranda[1,15], Ramon Estruch[1,16], Francisco J Tinahones[1,17], José Lapetra[1,18], J. Lluís Serra-Majem[1,19], Aurora Bueno-Cavanillas[12,20], Josep A. Tur[1,21], Sergio Cinza Sanjurjo[22,23,24], Xavier Pintó[1,25], José Juan Gaforio[12,26], Pilar Matía-Martín[27], Josep Vidal[28,29], Clotilde Vázquez[1,30], Lidia Daimiel[31], Emili Ros[1,32]Carmen Sayon-Orea[1,4], Jose V Sorli[1,6], Karla-Alejandra Pérez-Vega[1,7]; Antonio Garcia-Rios[1,15], Nuria Gómez Bellvert[33]; Enrique Gómez-Gracia[1,11], MA Zulet[1,8,9], Alice Chaplin[1,14], Rosa Casas[1,16], Inmaculada Salcedo-Bellido[12,20], Lucas Tojal-Sierra[1,10], Maria-Rosa Bernal-Lopez[1,34], Zenaida Vazquez[1,4], Eva M. Asensio[1,6], Albert Goday[1,7], Patricia J. Peña-Orihuela[1,15], Antonio Signes Pastor[12,13], Ana Garcia-Arellano[1,4], Montse Fitó[1,7], Nancy Babio[1,2,3 †], Jordi Salas-Salvadó[1,2,3* †]

1. Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y la Nutrición (CIBEROBN), Institute of Health Carlos III, Madrid, Spain
2. Universitat Rovira i Virgili, Departament de Bioquímica i Biotecnologia, Unitat de Nutrició, Reus, Spain
3. Institut d'Investigació Sanitària Pere Virgili (IISPV), Reus, Spain
4. University of Navarra, Department of Preventive Medicine and Public Health, IDISNA, Pamplona, Spain
5. Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA, USA
6. Department of Preventive Medicine, University of Valencia, Valencia, Spain
7. Unit of Cardiovascular Risk and Nutrition, Institut Hospital del Mar de Investigaciones Médicas Municipal d`Investigació Médica (IMIM), Barcelona, Spain.
8. Department of Nutrition, Food Sciences, and Physiology, Center for Nutrition Research, University of Navarra, Pamplona, Spain
9. Precision Nutrition and Cardiometabolic Health Program. IMDEA Food, CEI UAM + CSIC, Madrid, Spain
10. Bioaraba Health Research Institute, Cardiovascular, Respiratory and Metabolic Area; Osakidetza Basque Health Service, Araba University Hospital; University of the Basque Country UPV/EHU, Vitoria-Gasteiz, Spain.
11. EpiPHAAN research group, School of Health Sciences, University of Málaga - Instituto de Investigación Biomédica en Málaga (IBIMA), Málaga, Spain
12. CIBER de Epidemiología y Salud Pública (CIBERESP), Instituto de Salud Carlos III, Madrid, Spain
13. Instituto de Investigación Sanitaria y Biomédica de Alicante. Universidad Miguel Hernández (ISABIAL-UMH). Alicante, Spain
14. Health Research Institute of the Balearic Islands (IdISBa), Palma de Mallorca, Spain.
15. Department of Internal Medicine, Maimonides Biomedical Research Institute of Cordoba (IMIBIC), Reina Sofia University Hospital, University of Cordoba, Cordoba, Spain
16. Department of Internal Medicine, Hospital Clinic, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Institut de Recerca en Nutrició i Seguretat Alimentària (INSA), University of Barcelona, Barcelona, Spain
17. Virgen de la Victoria Hospital, Department of Endocrinology, Instituto de Investigación Biomédica de Málaga (IBIMA). University of Málaga, Málaga, Spain.

1

18. Department of Family Medicine, Research Unit, Distrito Sanitario Atención Primaria Sevilla, Sevilla, Spain
19. Research Institute of Biomedical and Health Sciences (IUIBS), University of Las Palmas de Gran Canaria & Centro Hospitalario Universitario Insular Materno Infantil (CHUIMI), Canarian Health Service, Las Palmas de Gran Canaria, Spain.
20. Department of Preventive Medicine and Public Health, University of Granada, Granada, Spain
21. Research Group on Community Nutrition & Oxidative Stress, University of Balearic Islands, Palma de Mallorca, Spain
22. CS Milladoiro, Área Sanitaria de Santiago de Compostela
23. Instituto de Investigación de Santiago de Compostela (IDIS)
24. Centro de Investigación Biomédica en Red-Enfermedades Cardiovasculares (CIBERCV)
25. Lipids and Vascular Risk Unit, Internal Medicine, Hospital Universitario de Bellvitge-IDIBELL, Hospitalet de Llobregat, Barcelona Spain
26. Departamento de Ciencias de la Salud, Instituto Universitario de Investigación en Olivar y Aceites de Oliva, Universidad de Jaén, Jaén, Spain
27. Department of Endocrinology and Nutrition, Instituto de Investigación Sanitaria Hospital Clínico San Carlos (IdISSC), Madrid, Spain
28. CIBER Diabetes y Enfermedades Metabólicas (CIBERDEM), Instituto de Salud Carlos III (ISCIII), Madrid, Spain
29. Department of Endocrinology, Institut d'Investigacions Biomédiques August Pi Sunyer (IDIBAPS), Hospital Clinic, University of Barcelona, Barcelona, Spain.
30. Department of Endocrinology and Nutrition, Hospital Fundación Jimenez Díaz. Instituto de Investigaciones Biomédicas IISFJD. University Autonoma, Madrid, Spain
31. Nutritional Control of the Epigenome Group. Precision Nutrition and Obesity Program. IMDEA Food, CEI UAM + CSIC, Madrid, Spain
32. Lipid Clinic, Department of Endocrinology and Nutrition, Institut d'Investigacions Biomèdiques August Pi Sunyer (IDIBAPS), Hospital Clínic, Barcelona, Spain
33. Centro Salud San Vicente del Raspeig, Alicante, Spain
34. Internal Medicine Clinical Management Unit, Hospital Regional Universitario de Málaga, Instituto de Investigación Biomédica de Málaga (IBIMA-Plataforma BIONAND). Universidad de Málaga

***Correspondence:** María Ángeles Martínez: mariaangeles.martinez@iispv.cat; Jordi Salas-Salvadó: jordi.salas@urv.cat

ǂ These authors have contributed equally to this work.

† These authors share senior authorship.

RESULTS

Supplementary Material, File S1

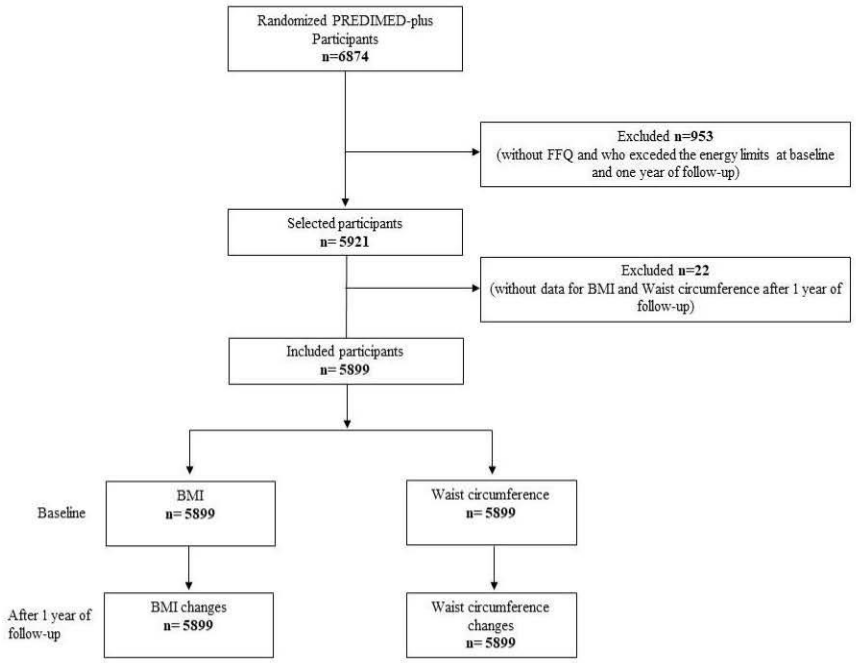


Figure 1. Flow chart of participant´s inclusion

3

Supplementary material, Table S1

Table S1. Comparison of the included and the total randomized PREDIMED-plus participants

	Randomized PREDIMED-Plus participants (n=6,874)	Included participants (n=5,899)	P-value
Sex			
Women	48.52 (3,335)	48.26 (2,847)	0.775
Age (years)	64.9 ± 4.91	65.0 ± 4.89	0.367
Educational level (n=5,899)			0.607
Up to primary	48.91 (3,362)	49.69 (2,931)	
Secondary	28.89 (1,986)	28.75 (1,696)	
University	22.20 (1,526)	21.56 (1,272)	
Marital status			0.666
Single	13.05 (897)	12.51 (738)	
Married	76.53 (5,261)	77.07 (4,546)	
Widowed	10.42 (716)	10.43 (615)	
Smoking status			0.824
Never	44.14 (3,034)	44.69 (2,636)	
Former	43.40 (2,983)	42.97 (2,535)	
Current	12.47 (857)	12.34 (728)	
Physical activity (MET min/week)	2463 ± 2301	2509 ± 2323	0.253
Waist circumference (cm)			
Men	111 ± 8.79	111 ± 8.72	0.213
Women	104 ± 9.24	104 ± 9.15	0.644
BMI (kg/m^2)	32.6 ± 3.46	32.5 ± 3.43	0.134
Intervention group (%)	49.55	48.97	0.517
Energy-reduced Mediterranean diet adherence (0-14 points)	8.15 ± 1.94	8.18 ± 1.94	0.283
Dietary assessment			
Fruits (g/d)	362 ± 210	359 ± 205	0.459
Vegetables (g/d)	329 ± 141	329 ± 137	0.768
Legumes (g/d)	20.9 ± 11.5	20.6 ± 11.0	0.124
Red meat and derivatives (g/d)	150 ± 61.1	148 ± 58.2	0.052
Fish and shellfish (g/d)	103 ± 48.3	102 ± 47.4	0.514
Virgin olive oil (g/d)	31.9 ± 20.9	31.9 ± 20.7	0.893
Total sugar (g/d)	6.92 ± 12.2	6.74 ± 11.9	0.384
Obesity prevalence			
Yes	73.42 (5,047)	72.7 (4,286)	0.331
Abdominal obesity prevalence			
Yes	93.28 (6,412)	93.1 (5,490)	0.635

Data are expressed as means ± SD and percentages (number) for continuous and categorical variables, respectively.

4

Supplementary material, Table S2

Table S2. TEQ levels of PCDD/Fs in food samples collected from different locations in pgTEQ/g

Food products	TOTAL pg TEQ/g	Unit	Source	Country
Whole Milk	$7.1 \cdot 10^{-3}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Semi-skimmed milk	$3.94 \cdot 10^{-3}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Skimmed milk*	$4.76 \cdot 10^{-3}$	pg/g FW	**Average** of whole, semi-skimmed milk, milkshakes and ice cream (González et al., 2018) (Hulin et al., 2020)	
Condensed milk*	$4.76 \cdot 10^{-3}$	pg/g FW	**Average** of whole, semi-skimmed milk, milkshakes and ice cream (González et al., 2018) (Hulin et al., 2020)	
Heavy cream*	$4.76 \cdot 10^{-3}$	pg/g FW	**Average** of whole, semi-skimmed milk, milkshakes and ice cream (González et al., 2018) (Hulin et al., 2020)	
Milk shakes	$4.00 \cdot 10^{-3}$	pg/g FW	(Hulin et al., 2020)	France
Ice creams	$4.00 \cdot 10^{-3}$	pg/g FW	(Hulin et al., 2020)	France
Yogurt	$7.10 \cdot 10^{-3}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Nonfat yogurt *	$7.10 \cdot 10^{-3}$	pg/g FW	**Average** of yogurt, petit Suisse and flan (González et al., 2018)	
Petit suisse	$7.10 \cdot 10^{-3}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Custard, flam*	$7.10 \cdot 10^{-3}$	pg/g FW	**Average** of yogurt, petit Suisse and flan (González et al., 2018)	
Flan, pudding	$7.10 \cdot 10^{-3}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Cottage or curd cheese	$2.00 \cdot 10^{-2}$	pg/g FW	(Barone et al., 2021)	Italy
White cheese (goat…)*	$2.00 \cdot 10^{-2}$	pg/g FW	**Average** of cottage or curd cheese (Barone et al., 2021)	
Cream cheese	$3.00 \cdot 10^{-2}$	pg/g FW	(Barone et al., 2021)	Italy
Other cheeses: cured, semi-cured (Manchego, Bola, Emmental…)	$2.92 \cdot 10^{-2}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Chicken eggs	$7.03 \cdot 10^{-2}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Chicken or turkey with skin	$7.03 \cdot 10^{-2}$	pg/g FW	**Average** of chicken without skin (González et al., 2018)	
Chicken or turkey WITHOUT skin	$7.03 \cdot 10^{-2}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Rabbit or hare*	$7.03 \cdot 10^{-2}$	pg/g FW	**Average** of chicken without skin (González et al., 2018)	

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Cow or beef meat	$1.50 \cdot 10^{-2}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Pork meat	$9.02 \cdot 10^{-3}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Lamb meat	$1.23 \cdot 10^{-2}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Liver (beef, pork, chicken)	$1.91 \cdot 10^{-1}$	pg/g FW	(Bramwell et al., 2016)	UK
Other organs (brain, heart…)*	$1.91 \cdot 10^{-1}$	pg/g FW	**Average** of liver meat (Bramwell et al., 2016)	
Serrano ham*	$4.00 \cdot 10^{-1}$	pg/g FW	**Average** of cooked ham (Barone et al., 2021)	
Cooked ham	$4.00 \cdot 10^{-1}$	pg/g FW	(Barone et al., 2021)	Italy
Cured/cold meat (salchichón, chorizo, black pudding, mortadella, sausages)	$2.30 \cdot 10^{-2}$	pg/g FW	(Llobet et al., 2008)	Spain, Catalonia
Patés- foie-gras	$5.70 \cdot 10^{-1}$	pg/g FW	(Bergkvist et al., 2008)	Sweden
hamburger, meatballs	$3.70 \cdot 10^{-2}$	pg/g FW	(Llobet et al., 2008)	Spain, Catalonia
Bacon, pancetta	$3.70 \cdot 10^{-2}$	pg/g FW	(Llobet et al., 2008)	Spain, Catalonia
White fish	$1.30 \cdot 10^{-2}$	pg/g FW	(Bramwell et al., 2016)	UK
Blue fish: sardines, tuna, bonito, salmon	$2.10 \cdot 10^{-1}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Salted fish (cod)	$1.58 \cdot 10^{-1}$	pg/g FW	(Llobet et al., 2008)	Spain, Catalonia
Oysters, clams, octopus, baby squid, cuttlefish	$1.20 \cdot 10^{-1}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Calamari, octopus, cuttlefish	$4.31 \cdot 10^{-2}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Prawns	$9.95 \cdot 10^{-2}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Canned fish and shellfish WITHOUT oil (sardines, anchovies, bonito, tuna)	$8.20 \cdot 10^{-3}$	pg/g FW	(Food Standards Australia New Zealand, 2004)	Australia, New Zealand
Canned fish and shellfish WITH oil (sardines, anchovies, bonito, tuna)	$1.27 \cdot 10^{-1}$	pg/g FW	(Diletti et al., 2018)	Italy
Chard, spinach, cauliflower	$3.00 \cdot 10^{-3}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Cabbage	$3.00 \cdot 10^{-3}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Lettuce, endives	$2.30 \cdot 10^{-2}$	pg/g FW	(González et al., 2018)	Spain, Tarragona

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Raw tomato	$3.00 \cdot 10^{-3}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Carrot, pumpkin	$2.54 \cdot 10^{-3}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Green beans	$3.00 \cdot 10^{-3}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Onion	$2.00 \cdot 10^{-5}$	pg/g FW	(De Mul et al., 2008)	Netherlands
Andalusian gazpacho	$2.51 \cdot 10^{-3}$	pg/g FW	**Average** of tomato, onion, pepper and garlic (González et al., 2018) (De Mul et al., 2008) (Esposito et al., 2017)	
Aubergines, courgettes, cucumbers	$7.40 \cdot 10^{-4}$	pg/g FW	(De Mul et al., 2008)	Netherlands
Peppers	$4.00 \cdot 10^{-3}$	pg/g FW	(Esposito et al., 2017)	Italy
Asparagus	$3.00 \cdot 10^{-3}$	pg/g FW	(Esposito et al., 2017)	Italy
Other vegetables (artichoke, leek, celery)	$3.00 \cdot 10^{-5}$	pg/g FW	(De Mul et al., 2008)	Netherlands
Garlic	$3.00 \cdot 10^{-3}$	pg/g FW	(Esposito et al., 2017)	Italy
Parsley, thyme, bay leaf, oregano, etc.	$1.86 \cdot 10^{-3}$	pg/g FW	(Esposito et al., 2017)	Italy
Baked potatoes	$1.10 \cdot 10^{-2}$	pg/g FW	(Esposito et al., 2017)	Italy
Potato chips	$3.17 \cdot 10^{-2}$	pg/g FW	(Lin et al., 2011)	Taiwan
Homemade fries	$1.67 \cdot 10^{-2}$	pg/g FW	(Lin et al., 2011)	Taiwan
Mushrooms	$8.30 \cdot 10^{-4}$	pg/g FW	(De Mul et al., 2008)	Netherlands
Orange, grapefruit, tangerines	$3.12 \cdot 10^{-3}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Orange	$3.12 \cdot 10^{-3}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Banana	$3.12 \cdot 10^{-3}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Apple or pear	$3.12 \cdot 10^{-3}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Strawberries, cherries	$4.00 \cdot 10^{-3}$	pg/g FW	(Esposito et al., 2017)	Italy
Plums, peach, apricot, nectarine	$4.50 \cdot 10^{-2}$	pg/g FW	(Esposito et al., 2017)	Italy
Watermelon	$3.20 \cdot 10^{-2}$	pg/g FW	Average of (Esposito et al., 2017)	
Cantaloupe	$3.20 \cdot 10^{-2}$	pg/g FW	(Esposito et al., 2017)	Italy
Kiwi	$2.37 \cdot 10^{-3}$	pg/g FW	**Average** of (González et al., 2018)	
Grapes	$9.00 \cdot 10^{-3}$	pg/g FW	(Esposito et al., 2017)	Italy
Fruits in syrup or in its juice	$1.00 \cdot 10^{-3}$	pg/g FW	(Hulin et al., 2020)	France
Dates, dried figs, raisins, prunes	$1.16 \cdot 10^{-2}$	pg/g FW	**Average** of all fruits (González et al., 2018) (Esposito et al., 2017) (Hulin et al., 2020)	
Almonds	$1.50 \cdot 10^{-2}$	pg/g FW	**Average** of Walnuts and other types of nuts	

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			(Bramwell et al., 2016) (Esposito et al., 2017)	
Pistachios	$1.50 \cdot 10^{-2}$	pg/g FW	**Average** of Walnuts and other types of nuts (Bramwell et al., 2016) (Esposito et al., 2017)	
Walnuts	$1.00 \cdot 10^{-2}$	pg/g FW	(Bramwell et al., 2016)	UK
Other types of nuts	$2.00 \cdot 10^{-2}$	pg/g FW	(Esposito et al., 2017)	Italy
Olives	$2.40 \cdot 10^{-2}$	pg/g FW	(Esposito et al., 2017)	Italy
Lentils	$2.84 \cdot 10^{-3}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Beans	$2.84 \cdot 10^{-3}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Peas	$2.84 \cdot 10^{-3}$	pg/g FW	**Average** of lentils (González et al., 2018)	
Chickpeas	$2.84 \cdot 10^{-3}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
White bread	$5.41 \cdot 10^{-3}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Whole wheat bread	$5.41 \cdot 10^{-3}$	pg/g FW	Average of white bread (González et al., 2018)	
Breakfast cereals*	$5.41 \cdot 10^{-3}$	pg/g FW	Average of white bread (González et al., 2018)	
Whole grains: muesli, oat flakes, all-bran	$5.41 \cdot 10^{-3}$	pg/g FW	Average of white bread (González et al., 2018)	
White rice	$5.41 \cdot 10^{-3}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Whole grain rice	$5.41 \cdot 10^{-3}$	pg/g FW	Average of white rice (González et al., 2018)	
Pasta	$5.41 \cdot 10^{-3}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Whole grain pasta	$5.41 \cdot 10^{-3}$	pg/g FW	Average of pasta (González et al., 2018)	
Pizza	$2.30 \cdot 10^{-2}$	pg/g FW	(Sirot et al., 2012)	France
Olive oil	$5.40 \cdot 10^{-2}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Virgin olive oil	$5.40 \cdot 10^{-2}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Aceite de oliva de orujo	$5.40 \cdot 10^{-2}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Corn oil	$5.40 \cdot 10^{-2}$	pg/g FW	Average of sunflower oil (González et al., 2018)	
Sunflower oil	$5.40 \cdot 10^{-2}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Soy oil	$5.40 \cdot 10^{-2}$	pg/g FW	Average of sunflower oil (González et al., 2018)	
Mix of the above	$5.40 \cdot 10^{-2}$	pg/g FW	Average of sunflower oil (González et al., 2018)	
Margarine	$5.40 \cdot 10^{-2}$	pg/g FW	(González et al., 2018)	Spain, Tarragona

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Butter	$5.40 \cdot 10^{-2}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Maria cookies	$5.97 \cdot 10^{-3}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Whole grain or fiber crackers	$5.97 \cdot 10^{-3}$	pg/g FW	Average of maria cookies (González et al., 2018)	
Chocolate chip cookies	$1.40 \cdot 10^{-2}$	pg/g FW	(Llobet et al., 2008)	Spain, Catalonia
Homemade cakes and pastries	$5.97 \cdot 10^{-3}$	pg/g FW	Average of croissant (González et al., 2018)	
Croissant	$5.97 \cdot 10^{-3}$	pg/g FW	(González et al., 2018)	Spain, Tarragona
Donuts	$1.20 \cdot 10^{-2}$	pg/g FW	Average of cakes (Sirot et al., 2012)	
Muffin	$1.20 \cdot 10^{-2}$	pg/g FW	Average of cakes (Sirot et al., 2012)	
Cakes	$1.20 \cdot 10^{-2}$	pg/g FW	(Sirot et al., 2012)	France
Churros	$2.35 \cdot 10^{-2}$	pg/g FW	Average of potato chips (Lin et al., 2011)	
Chocolates and sweets	$2.35 \cdot 10^{-2}$	pg/g FW	(Food Standards Australia New Zealand, 2004)	
Cocoa powder-soluble cocoa	$2.35 \cdot 10^{-2}$	pg/g FW	Average of chocolates (Food Standards Australia New Zealand, 2004)	
Nougat	$2.35 \cdot 10^{-2}$	pg/g FW	Average of chocolates (Food Standards Australia New Zealand, 2004)	
Mantecados, marzipan	$2.35 \cdot 10^{-2}$	pg/g FW	Average of chocolates (Food Standards Australia New Zealand, 2004)	
Soups and sachet creams	$2.00 \cdot 10^{-3}$	pg/g FW	(Hulin et al., 2020)	France
Mustard	$1.00 \cdot 10^{-2}$	pg/g FW	(Sirot et al., 2012)	France
Mayonnaise	$1.90 \cdot 10^{-1}$	pg/g FW	(Barone et al., 2021)	Italy
Fried tomato sauce, ketchup	$1.00 \cdot 10^{-2}$	pg/g FW	(Sirot et al., 2012)	France
Spicy: tabasco, bell pepper, paprika	$1.00 \cdot 10^{-2}$	pg/g FW	(Sirot et al., 2012)	France
Salt	$1.00 \cdot 10^{-2}$	pg/g FW	(Sirot et al., 2012)	France
Jam	$2.40 \cdot 10^{-2}$	pg/g FW	Average of sugar (Tsutsumi et al., 2018)	
Sugar	$2.40 \cdot 10^{-2}$	pg/g FW	(Tsutsumi et al., 2018)	Japan
Honey	$2.40 \cdot 10^{-2}$	pg/g FW	Average of sugar (Tsutsumi et al., 2018)	
Carbonated drinks with sugar: cola drinks, lemonades, tonics, etc.	0	pg/g FW	(Kiviranta et al., 2001)	Finland

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Low calorie carbonated drinks, diet drinks	0	pg/g FW	(Kiviranta et al., 2001)	Finland
Natural orange juice	$3.55 \cdot 10^{-3}$	pg/g FW	Average of bottled fruit juices (Food Standards Australia New Zealand, 2004)	
Natural juices from other fruits	$3.55 \cdot 10^{-3}$	pg/g FW	Average of bottled fruit juices (Food Standards Australia New Zealand, 2004)	
Bottled or canned fruit juices	$3.55 \cdot 10^{-3}$	pg/g FW	(Food Standards Australia New Zealand, 2004)	Australia, New Zealand
Decaffeinated coffee	0	pg/g FW	(Kiviranta et al., 2001)	Finland
Coffee	0	pg/g FW	(Kiviranta et al., 2001)	Finland
Tea	0	pg/g FW	(Kiviranta et al., 2001)	Finland
Must	0	pg/g FW	(Kiviranta et al., 2001)	Finland
Rosé wine	0	pg/g FW	(Kiviranta et al., 2001)	Finland
Moscatel wine	0	pg/g FW	(Kiviranta et al., 2001)	Finland
Young red wine	0	pg/g FW	(Kiviranta et al., 2001)	Finland
Old red wine	0	pg/g FW	(Kiviranta et al., 2001)	Finland
White wine	0	pg/g FW	(Kiviranta et al., 2001)	Finland
Catalonian wine	0	pg/g FW	(Kiviranta et al., 2001)	Finland
Beer	0	pg/g FW	(Kiviranta et al., 2001)	Finland
Liqueurs, anise	0	pg/g FW	(Kiviranta et al., 2001)	Finland
Spirits: whiskey, vodka, gin, cognac	0	pg/g FW	(Kiviranta et al., 2001)	Finland

Abbreviations: PCCD/F: Polychlorinated dibenzo-p-dioxins or dibenzofurans; TEQ: Toxic Equivalency; FW: Fresh weight.

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Supplementary material, Table S3-S13

Table S3. Multivariable-adjusted β-coefficients or prevalence ratios and its 95% confidence interval for adiposity parameters and obesity prevalence, respectively, across baseline energy-adjusted total PCDD/F dietary intake tertiles from red meat.

		Energy-adjusted total PCDD/F dietary intake tertiles (TEQ pg/week)-Red meat			
		Tertile 1 n=1,970	**Tertile 2** n=1,964	**Tertile 3** n=1,965	***P-trend***
		β-coefficients (95%CI)			
BMI (kg/m²)					
	Model 1	**0 (Ref.)**	0.21 (0.00 to 0.43)	0.38 (0.17 to 0.59)	<0.001

Model 2	**0 (Ref.)**	0.19 (-0.02 to 0.41)	0.39 (0.19 to 0.61)	<0.001
Full-adjusted model	**0 (Ref.)**	0.18 (-0.03 to 0.39)	0.36 (0.15 to 0.58)	0.001
Waist circumference (cm)				
Model 1	**0 (Ref.)**	0.23 (-0.33 to 0.79)	0.92 (0.36 to 1.48)	0.001
Model 2	**0 (Ref.)**	0.21 (-0.35 to 0.77)	1.04 (0.48 to 1.59)	<0.001
Full-adjusted model	**0 (Ref.)**	0.12 (-0.44 to 0.67)	0.88 (0.33 to 1.43)	0.002
		Prevalence ratios (95%CI)		
Obesity				
Prevalence, % (n)		71.6 (1,411)	71.5 (1,405)	74.8 (1,470)	
Model 1	**1 (Ref.)**	1.03 (0.99 to 1.07)	1.04 (1.00 to 1.08)	0.038
Model 2	**1 (Ref.)**	1.03 (0.99 to 1.07)	1.04 (1.00 to 1.08)	0.032
Full-adjusted model	**1 (Ref.)**	1.03 (0.98 to 1.07)	1.04 (1.00 to 1.08)	0.041
Abdominal obesity				
Prevalence, % (n)		92.0 (1,812)	92.4 (1,815)	94.8 (1,863)	
Model 1	**1 (Ref.)**	0.99 (0.98 to 1.01)	1.01 (0.99 to 1.03)	0.094
Model 2	**1 (Ref.)**	0.99 (0.98 to 1.01)	1.01 (0.99 to 1.03)	0.075
Full-adjusted model	**1 (Ref.)**	0.99 (0.98 to 1.01)	1.01 (0.99 to 1.03)	0.073

Abbreviations: PCCD/F: Polychlorinated dibenzo-p-dioxins or dibenzofurans; TEQ: Toxic Equivalency; CI: confidence interval; BMI: body mass index. SD: Standard deviation.

Linear and cox regression models were fitted to assess the β-coefficients or prevalence ratio, and its 95%CI for adiposity parameters and obesity/overweight prevalence, respectively, across energy-adjusted total dietary PCDD/F intake. Model 1: adjusted for age and sex. Model 2: Model 1 additionally adjusted for physical activity (MET min/week), marital status (single, married, widowed), smoking status (current, former, never), education level (up to primary, secondary, university) and the size of the recruitment center (<250, 250 to <300, 300 to <400, ≥400). Fully adjusted: Model 2 additionally adjusted for adherence to Mediterranean diet MEDAS score (0-14 points) and type 2 diabetes (yes/no), hypertension (yes/no), and hypercholesterolemia (yes/no), or prevalence or taking medications for their control, and hormonal treatment. The robust estimate of variance was used in all analyses to account for the intra-cluster correlation.

Table S4. Multivariable-adjusted β-coefficients or Odds ratios and its 95% confidence intervals for adiposity changes or obesity incidence, respectively, after 1-year of follow-up across baseline energy-adjusted total dietary PCDD/F intake from red meat.

	Energy-adjusted total PCDD/F dietary intake tertiles (TEQ pg/week) Red meat			
	Tertile 1	**Tertile 2**	**Tertile 3**	***P- trend***
		β-coefficients (95%CI)		
BMI changes (kg/m²)	(n=1,970)	(n=1,964)	(n=1,965)	
Model 1	**0 (Ref.)**	-0.03 (-0.13 to 0.07)	0.02 (-0.08 to 0.12)	0.718
Model 2	**0 (Ref.)**	0.00 (-0.09 to 0.09)	0.04 (-0.01 to 0.13)	0.420
Full- adjusted model	**0 (Ref.)**	-0.00 (-0.09 to 0.09)	0.03 (-0.06 to 0.13)	0.461
Waist circumference changes (cm)	(n=1,967)	(n=1,966)	(n=1,966)	
Model 1	**0 (Ref.)**	0.16 (-0.16 to 0.49)	0.38 (0.06 to 0.71)	0.021

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Model 2	**0 (Ref.)**	0.22 (-0.08 to 0.53)	0.45 (0.04 to 0.76)	0.004
Full-adjusted model	**0 (Ref.)**	0.20 (-0.10 to 0.51)	0.39 (0.09 to 0.70)	0.011
		Odds ratios (95%CI)		
Obesity	(n= 538)	(n= 538)	(n= 537)	
Incidence, % (n)	6.8 (38)	8.59 (48)	8.08 (40)	
Model 1	**1 (Ref.)**	0.69 (0.44 to 1.10)	1.04 (0.68 to 1.59)	0.862
Model 2	**1 (Ref.)**	0.67 (0.42 to 1.06)	1.01 (0.65 to 1.55)	0.982
Full-adjusted model	**1 (Ref.)**	0.68 (0.43 to 1.09)	1.02 (0.66 to 1.58)	0.932
Abdominal obesity	(n=137)	(n=136)	(n=136)	
Incidence, % (n)	15.1 (24)	18.2 (27)	20.59 (21)	
Model 1	**1 (Ref.)**	0.82 (0.45 to 1.51)	0.80 (0.41 to 1.56)	0.506
Model 2	**1 (Ref.)**	0.76 (0.40 to 1.42)	0.79 (0.41 to 1.57)	0.498
Full-adjusted model	**1 (Ref.)**	0.74 (0.39 to 1.39)	0.75 (0.38 to 1.49)	0.401

Abbreviations: PCCD/F: Polychlorinated dibenzo-p-dioxins or dibenzofurans; TEQ: Toxic Equivalency; CI: confidence interval; BMI: body mass index; SD: Standard Deviation.

Linear regression models and logistic regression models were fitted to assess the β-coefficients or odd ratio, and its 95%CI for adiposity parameters and obesity/overweight incidence, respectively across energy-adjusted total dietary PCDD/F intake. Model 1: adjusted for age, sex. Model 2: Model 1 additionally adjusted for physical activity (MET min/week), marital status (single, married, widowed), smoking status (current, former, never), education level (up to primary, secondary, university) and the size of the recruitment centres (<250, 250 to <300, 300 to <400, ≥400). Fully adjusted: Model 2 additionally adjusted to adherence to Mediterranean diet MEDAS score (0-14 points) and the prevalence of having and type 2 diabetes (yes/no), hypertension (yes/no), and hypercholesterolemia (yes/no), or prevalence or taking medications for their control, and hormonal treatment, intervention group and outcome variable at baseline. The robust estimate of variance was used in all analyses to account for the intra-cluster correlation.

Table S5. Multivariable-adjusted β-coefficients or prevalence ratios and its 95% confidence interval for adiposity parameters and obesity prevalence, respectively, across baseline energy-adjusted total PCDD/F dietary intake tertiles from white meat.

		Energy-adjusted total PCDD/F dietary intake tertiles (TEQ pg/week)-White meat			
		Tertile 1 n=1,970	**Tertile 2** n=1,964	**Tertile 3** n=1,965	***P-trend***
		β-coefficients (95%CI)			
BMI (kg/m²)					
Model 1	**0 (Ref.)**	0.27 (0.06 to 0.49)	0.28 (0.06 to 0.49)	0.005
Model 2	**0 (Ref.)**	0.27 (0.06 to 0.48)	0.26 (0.04 to 0.47)	0.008
Full-adjusted model	**0 (Ref.)**	0.32 (0.11 to 0.53)	0.33 (0.11 to 0.54)	0.001

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RESULTS

Waist circumference (cm)					
	Model 1	**0 (Ref.)**	0.79 (0.23 to 1.36)	0.63 (0.06 to 1.20)	0.011
	Model 2	**0 (Ref.)**	0.80 (0.25 to 1.36)	0.67 (0.11 to 1.24)	0.007
	Full-adjusted model	**0 (Ref.)**	0.93 (0.38 to 1.48)	0.77 (0.20 to 1.34)	0.002
			Prevalence ratios (95%CI)		
Obesity					
Prevalence, % (n)		71.6 (1,411)	71.5 (1,405)	74.8 (1,470)	
	Model 1	**1 (Ref.)**	1.01 (0.98 to 1.05)	1.02 (0.98 to 1.06)	0.317
	Model 2	**1 (Ref.)**	1.01 (0.98 to 1.05)	1.01 (0.97 to 1.05)	0.452
	Full-adjusted model	**1 (Ref.)**	1.02 (0.98 to 1.06)	1.02 (0.98 to 1.06)	0.220
Abdominal obesity					
Prevalence, % (n)		92.0 (1,812)	92.4 (1,815)	94.8 (1,863)	
	Model 1	**1 (Ref.)**	1.01 (0.99 to 1.03)	1.01 (0.99 to 1.03)	0.148
	Model 2	**1 (Ref.)**	1.01 (0.99 to 1.03)	1.01 (0.99 to 1.03)	0.154
	Full-adjusted model	**1 (Ref.)**	1.01 (0.99 to 1.03)	1.01 (0.99 to 1.03)	0.146

Abbreviations: PCCD/F: Polychlorinated dibenzo-p-dioxins or dibenzofurans; TEQ: Toxic Equivalency; CI: confidence interval; BMI: body mass index. SD: Standard deviation.

Linear and cox regression models were fitted to assess the β-coefficients or prevalence ratio, and its 95%CI for adiposity parameters and obesity/overweight prevalence, respectively, across energy-adjusted total dietary PCDD/F intake. Model 1: adjusted for age and sex. Model 2: Model 1 additionally adjusted for physical activity (MET min/week), marital status (single, married, widowed), smoking status (current, former, never), education level (up to primary, secondary, university) and the size of the recruitment center (<250, 250 to <300, 300 to <400, ≥400). Fully adjusted: Model 2 additionally adjusted for adherence to Mediterranean diet MEDAS score (0-14 points) and type 2 diabetes (yes/no), hypertension (yes/no), and hypercholesterolemia (yes/no), or prevalence or taking medications for their control, and hormonal treatment. The robust estimate of variance was used in all analyses to account for the intra-cluster correlation.

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Table S6. Multivariable-adjusted β-coefficients or Odds ratios and its 95% confidence intervals for adiposity changes or obesity incidence, respectively, after 1-year of follow-up across baseline energy-adjusted total dietary PCDD/F intake from white meat.

	Energy-adjusted total PCDD/F dietary intake tertiles (TEQ pg/week) White meat			
	Tertile 1	**Tertile 2**	**Tertile 3**	***P- trend***
	β-coefficients (95%CI)			
BMI changes (kg/m²)	(n=1,970)	(n=1,964)	(n=1,965)	
Model 1	**0 (Ref.)**	0.07 (-0.02 to 0.17)	0.07 (-0.03 to 0.17)	0.131
Model 2	**0 (Ref.)**	0.07 (-0.02 to 0.16)	0.12 (-0.03 to 0.21)	0.011
Full- adjusted model	**0 (Ref.)**	0.06 (-0.03 to 0.15)	0.10 (0.01 to 0.19)	0.034
Waist circumference changes (cm)	(n=1,967)	(n=1,966)	(n=1,966)	
Model 1	**0 (Ref.)**	0.31 (-0.02 to 0.64)	0.39 (0.07 to 0.72)	0.014
Model 2	**0 (Ref.)**	0.35 (0.05 to 0.66)	0.51 (0.21 to 0.82)	0.001
Full-adjusted model	**0 (Ref.)**	0.39 (0.09 to 0.70)	0.51 (0.20 to 0.82)	0.001
	Odds ratios (95%CI)			
Obesity	(n= 538)	(n= 538)	(n= 537)	
Incidence, % (n)	6.8 (38)	8.59 (48)	8.08 (40)	
Model 1	**1 (Ref.)**	0.84 (0.52 to 1.34)	1.21 (0.78 to 1.85)	0.589
Model 2	**1 (Ref.)**	0.79 (0.42 to 1.27)	1.22 (0.77 to 1.93)	0.643
Full-adjusted model	**1 (Ref.)**	0.80 (0.49 to 1.31)	1.26 (0.79 to 2.02)	0.567
Abdominal obesity	(n=137)	(n=136)	(n=136)	
Incidence, % (n)	15.1 (24)	18.2 (27)	20.59 (21)	
Model 1	**1 (Ref.)**	0.91 (0.49 to 1.69)	0.80 (0.41 to 1.66)	0.648
Model 2	**1 (Ref.)**	0.83 (0.43 to 1.61)	0.94 (0.48 to 1.83)	0.727
Full-adjusted model	**1 (Ref.)**	0.84 (0.43 to 1.63)	0.99 (0.49 to 1.97)	0.841

Abbreviations: PCCD/F: Polychlorinated dibenzo-p-dioxins or dibenzofurans; TEQ: Toxic Equivalency; CI: confidence interval; BMI: body mass index; SD: Standard Deviation.

Linear regression models and logistic regression models were fitted to assess the β-coefficients or odd ratio, and its 95%CI for adiposity parameters and obesity/overweight incidence, respectively across energy-adjusted total dietary PCDD/F intake. Model 1: adjusted for age, sex. Model 2: Model 1 additionally adjusted for physical activity (MET min/week), marital status (single, married, widowed), smoking status (current, former, never), education level (up to primary, secondary, university) and the size of the recruitment centres (<250, 250 to <300, 300 to <400, ≥400). Fully adjusted: Model 2 additionally adjusted to adherence to Mediterranean diet MEDAS score (0-14 points) and the prevalence of having and type 2 diabetes (yes/no), hypertension (yes/no), and hypercholesterolemia (yes/no), or prevalence or taking medications for their control, and hormonal treatment, intervention group and outcome variable at baseline. The robust estimate of variance was used in all analyses to account for the intra-cluster correlation.

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Table S7. Multivariable-adjusted β-coefficients or prevalence ratios and its 95% confidence interval for adiposity parameters and obesity prevalence, respectively, across baseline energy-adjusted total PCDD/F dietary intake tertiles from fish and seafood.

		Energy-adjusted total PCDD/F dietary intake tertiles (TEQ pg/week)-Fish and seafood			
		Tertile 1 n=1,970	**Tertile 2** n=1,964	**Tertile 3** n=1,965	***P- trend***
		β-coefficients (95%CI)			
BMI (kg/m²)					
	Model 1	**0 (Ref.)**	-0.16 (-0.38 to 0.06)	-0.25 (-0.46 to -0.04)	0.021
	Model 2	**0 (Ref.)**	-0.13 (-0.35 to 0.08)	-0.19 (-0.39 to 0.22)	0.088
	Full-adjusted model	**0 (Ref.)**	-0.09 (-0.31 to 0.12)	-0.09 (-0.31 to 0.45)	0.422
Waist circumference (cm)					
	Model 1	**0 (Ref.)**	-0.24 (-0.81 to 0.32)	-0.23 (-0.079 to 0.33)	0.453
	Model 2	**0 (Ref.)**	-0.12 (-0.68 to 0.44)	0.02 (-0.53 to 0.57)	0.902
	Full-adjusted model	**0 (Ref.)**	-0.05 (-0.61 to 0.51)	0.22 (-0.34 to 0.77)	0.417
		Prevalence ratios (95%CI)			
Obesity					
Prevalence, % (n)		71.6 (1,411)	71.5 (1,405)	74.8 (1,470)	
	Model 1	**1 (Ref.)**	0.96 (0.92 to 0.99)	0.98 (0.97 to 1.01)	0.287
	Model 2	**1 (Ref.)**	0.96 (0.92 to 0.99)	0.98 (0.95 to 1.02)	0.461
	Full-adjusted model	**1 (Ref.)**	0.96 (0.93 to 1.00)	0.99 (0.96 to 1.03)	0.949
Abdominal obesity					
Prevalence, % (n)		92.0 (1,812)	92.4 (1,815)	94.8 (1,863)	
	Model 1	**1 (Ref.)**	0.99 (0.97 to 1.01)	1.01 (0.99 to 1.02)	0.299
	Model 2	**1 (Ref.)**	0.99 (0.97 to 1.01)	1.01 (0.99 to 1.03)	0.139
	Full-adjusted model	**1 (Ref.)**	0.99 (0.98 to 1.01)	1.01 (0.99 to 1.03)	0.114

Abbreviations: PCCD/F: Polychlorinated dibenzo-p-dioxins or dibenzofurans; TEQ: Toxic Equivalency; CI: confidence interval; BMI: body mass index. SD: Standard deviation.

Linear and cox regression models were fitted to assess the β-coefficients or prevalence ratio, and its 95%CI for adiposity parameters and obesity/overweight prevalence, respectively, across energy-adjusted total dietary PCDD/F intake. Model 1: adjusted for age and sex. Model 2: Model 1 additionally adjusted for physical activity (MET min/week), marital status (single, married, widowed), smoking status (current, former, never), education level (up to primary, secondary, university) and the size of the recruitment center (<250, 250 to <300, 300 to <400, ≥400). Fully adjusted: Model 2 additionally adjusted for adherence to Mediterranean diet MEDAS score (0-14 points) and type 2 diabetes (yes/no), hypertension (yes/no), and hypercholesterolemia (yes/no), or prevalence or taking medications for their control, and hormonal treatment. The robust estimate of variance was used in all analyses to account for the intra-cluster correlation.

Table S8. Multivariable-adjusted β-coefficients or Odds ratios and its 95% confidence intervals for adiposity changes or obesity incidence, respectively, after 1-year of follow-up across baseline energy-adjusted total dietary PCDD/F intake from fish and seafood.

		Energy-adjusted total PCDD/F dietary intake tertiles (TEQ pg/week) Fish and seafood			
		Tertile 1	**Tertile 2**	**Tertile 3**	***P- trend***
		β-coefficients (95%CI)			
BMI changes (kg/m^2)		(n=1,970)	(n=1,964)	(n=1,965)	
	Model 1	**0 (Ref.)**	-0.04 (-0.14 to 0.06)	0.06 (-0.04 to 0.15)	0.200
	Model 2	**0 (Ref.)**	-0.04 (-0.13 to 0.05)	0.05 (-0.04 to 0.14)	0.243
	Full- adjusted model	**0 (Ref.)**	-0.05 (-0.14 to 0.04)	0.03 (-0.07 to 0.12)	0.484
Waist circumference changes (cm)		(n=1,967)	(n=1,966)	(n=1,966)	
	Model 1	**0 (Ref.)**	-0.07 (-0.39 to 0.25)	0.09 (-0.24 to 0.42)	0.540
	Model 2	**0 (Ref.)**	-0.09 (-0.39 to 0.20)	0.07 (-0.24 to 0.37)	0.586
	Full-adjusted model	**0 (Ref.)**	-0.09 (-0.39 to 0.21)	0.10 (-0.21 to 0.42)	0.466
		Odds ratios (95%CI)			
Obesity		(n= 538)	(n= 538)	(n= 537)	
Incidence, % (n)		6.8 (38)	8.59 (48)	8.08 (40)	
	Model 1	**1 (Ref.)**	0.77 (0.49 to 1.19)	0.72 (0.46 to 1.13)	0.172
	Model 2	**1 (Ref.)**	0.76 (0.48 to 1.19)	0.69 (0.44 to 1.10)	0.144
	Full-adjusted model	**1 (Ref.)**	0.80 (0.49 to 1.31)	1.26 (0.79 to 2.02)	0.166
Abdominal obesity		(n=137)	(n=136)	(n=136)	
Incidence, % (n)		15.1 (24)	18.2 (27)	20.59 (21)	
	Model 1	**1 (Ref.)**	0.81 (0.45 to 1.46)	0.59 (0.29 to 1.19)	0.141
	Model 2	**1 (Ref.)**	0.73 (0.39 to 1.38)	0.44 (0.22 to 0.91)	0.026
	Full-adjusted model	**1 (Ref.)**	0.84 (0.43 to 1.63)	0.99 (0.49 to 1.97)	0.048

Abbreviations: PCCD/F: Polychlorinated dibenzo-p-dioxins or dibenzofurans; TEQ: Toxic Equivalency; CI: confidence interval; BMI: body mass index; SD: Standard Deviation.

Linear regression models and logistic regression models were fitted to assess the β-coefficients or odd ratio, and its 95%CI for adiposity parameters and obesity/overweight incidence, respectively across energy-adjusted total dietary PCDD/F intake. Model 1: adjusted for age, sex. Model 2: Model 1 additionally adjusted for physical activity (MET min/week), marital status (single, married, widowed), smoking status (current, former, never), education level (up to primary, secondary, university) and the size of the recruitment centres (<250, 250 to <300, 300 to <400, ≥400). Fully adjusted: Model 2 additionally adjusted to adherence to Mediterranean diet MEDAS score (0-14 points) and the prevalence of having and type 2 diabetes (yes/no), hypertension (yes/no), and hypercholesterolemia (yes/no), or prevalence or taking medications for their control, and hormonal treatment, intervention group and outcome variable at baseline. The robust estimate of variance was used in all analyses to account for the intra-cluster correlation.

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Table S9. Multivariable-adjusted β-coefficients or prevalence ratios and its 95% confidence interval for adiposity parameters and obesity prevalence, respectively, across baseline energy-adjusted total PCDD/F dietary intake tertiles from fruits and vegetables.

	Energy-adjusted total PCDD/F dietary intake tertiles (TEQ pg/week)-Fruits and vegetables			
	Tertile 1 n=1,970	**Tertile 2** n=1,964	**Tertile 3** n=1,965	***P-trend***
	β-coefficients (95%CI)			
BMI (kg/m²)				
Model 1	**0 (Ref.)**	0.12 (-0.09 to 0.34)	0.15 (-0.06 to 0.37)	0.213
Model 2	**0 (Ref.)**	0.04 (-0.17 to 0.26)	0.06 (-0.15 to 0.27)	0.682
Full-adjusted model	**0 (Ref.)**	0.20 (-0.01 to 0.41)	0.02 (0.02 to 0.44)	0.069
Waist circumference (cm)				
Model 1	**0 (Ref.)**	-0.13 (-0.69 to 0.42)	0.08 (-0.48 to 0.64)	0.642
Model 2	**0 (Ref.)**	-0.08 (-0.64 to 0.48)	0.21 (-0.35 to 0.76)	0.789
Full-adjusted model	**0 (Ref.)**	0.11 (-0.45 to 0.67)	0.24 (-0.31 to 0.80)	0.396
	Prevalence ratios (95%CI)			
Obesity				
Prevalence, % (n)	71.6 (1,411)	71.5 (1,405)	74.8 (1,470)	
Model 1	**1 (Ref.)**	1.01 (0.97 to 1.05)	1.00 (0.96 to 1.04)	0.881
Model 2	**1 (Ref.)**	1.00 (0.96 to 0.04)	1.00 (0.96 to 1.04)	0.966
Full-adjusted model	**1 (Ref.)**	1.01 (0.97 to 1.05)	1.01 (0.97 to 1.05)	0.583
Abdominal obesity				
Prevalence, % (n)	92.0 (1,812)	92.4 (1,815)	94.8 (1,863)	
Model 1	**1 (Ref.)**	0.97 (0.95 to 0.98)	0.98 (0.97 to 1.00)	0.341
Model 2	**1 (Ref.)**	0.97 (0.95 to 0.98)	0.99 (0.97 to 1.00)	0.533
Full-adjusted model	**1 (Ref.)**	0.97 (0.95 to 0.98)	0.99 (0.97 to 1.00)	0.530

Abbreviations: PCCD/F: Polychlorinated dibenzo-p-dioxins or dibenzofurans; TEQ: Toxic Equivalency; CI: confidence interval; BMI: body mass index. SD: Standard deviation.

Linear and cox regression models were fitted to assess the β-coefficients or prevalence ratio, and its 95%CI for adiposity parameters and obesity/overweight prevalence, respectively, across energy-adjusted total dietary PCDD/F intake. Model 1: adjusted for age and sex. Model 2: Model 1 additionally adjusted for physical activity (MET min/week), marital status (single, married, widowed), smoking status (current, former, never), education level (up to primary, secondary, university) and the size of the recruitment center (<250, 250 to <300, 300 to <400, ≥400). Fully adjusted: Model 2 additionally adjusted for adherence to Mediterranean diet MEDAS score (0-14 points) and type 2 diabetes (yes/no), hypertension (yes/no), and hypercholesterolemia (yes/no), or prevalence or taking medications for their control, and hormonal treatment. The robust estimate of variance was used in all analyses to account for the intra-cluster correlation.

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Table S10. Multivariable-adjusted β-coefficients or Odds ratios and its 95% confidence intervals for adiposity changes or obesity incidence, respectively, after 1-year of follow-up across baseline energy-adjusted total dietary PCDD/F intake from fruits and vegetables.

		Energy-adjusted total PCDD/F dietary intake tertiles (TEQ pg/week) Fruits and vegetables			
		Tertile 1	**Tertile 2**	**Tertile 3**	***P- trend***
		β-coefficients (95%CI)			
BMI changes (kg/m^2)		(n=1,970)	(n=1,964)	(n=1,965)	
	Model 1	**0 (Ref.)**	0.04 (-0.06 to 1.34)	-0.02 (-0.12 to 0.08)	0.548
	Model 2	**0 (Ref.)**	0.04 (-0.05 to 0.13)	-0.01 (-0.10 to 0.08)	0.598
	Full- adjusted model	**0 (Ref.)**	0.02 (-0.07 to 0.11)	-0.03 (-0.12 to 0.06)	0.423
Waist circumference changes (cm)		(n=1,967)	(n=1,966)	(n=1,966)	
	Model 1	**0 (Ref.)**	0.10 (-0.22 to 0.43)	0.05 (-0.27 to 0.38)	0.851
	Model 2	**0 (Ref.)**	0.11 (-0.19 to 0.42)	0.07 (-0.24 to 0.38)	0.792
	Full-adjusted model	**0 (Ref.)**	0.14 (-0.17 to 0.44)	0.09 (-0.22 to 0.41)	0.682
		Odds ratios (95%CI)			
Obesity		(n= 538)	(n= 538)	(n= 537)	
Incidence, % (n)		6.8 (38)	8.59 (48)	8.08 (40)	
	Model 1	**1 (Ref.)**	1.13 (0.72 to 1.76)	1.05 (0.67 to 1.66)	0.915
	Model 2	**1 (Ref.)**	1.06 (0.67 to 1.66)	0.97 (0.61 to 1.55)	0.825
	Full-adjusted model	**1 (Ref.)**	1.11 (0.69 to 1.77)	1.01 (0.63 to 1.64)	0.938
Abdominal obesity		(n=137)	(n=136)	(n=136)	
Incidence, % (n)		15.1 (24)	18.2 (27)	20.59 (21)	
	Model 1	**1 (Ref.)**	1.00 (0.54 to 1.88)	1.13 (0.59 to 2.18)	0.680
	Model 2	**1 (Ref.)**	0.93 (0.48 to 1.88)	0.89 (0.42 to 1.89)	0.789
	Full-adjusted model	**1 (Ref.)**	0.94 (0.48 to 1.81)	0.83 (0.38 to 1.82)	0.649

Abbreviations: PCCD/F: Polychlorinated dibenzo-p-dioxins or dibenzofurans; TEQ: Toxic Equivalency; CI: confidence interval; BMI: body mass index; SD: Standard Deviation.

Linear regression models and logistic regression models were fitted to assess the β-coefficients or odd ratio, and its 95%CI for adiposity parameters and obesity/overweight incidence, respectively across energy-adjusted total dietary PCDD/F intake. Model 1: adjusted for age, sex. Model 2: Model 1 additionally adjusted for physical activity (MET min/week), marital status (single, married, widowed), smoking status (current, former, never), education level (up to primary, secondary, university) and the size of the recruitment centres (<250, 250 to <300, 300 to <400, ≥400). Fully adjusted: Model 2 additionally adjusted to adherence to Mediterranean diet MEDAS score (0-14 points) and the prevalence of having and type 2 diabetes (yes/no), hypertension (yes/no), and hypercholesterolemia (yes/no), or prevalence or taking medications for their control, and hormonal treatment, intervention group and outcome variable at baseline. The robust estimate of variance was used in all analyses to account for the intra-cluster correlation.

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Table S11. Multivariable-adjusted β-coefficients or prevalence ratios and its 95% confidence interval for adiposity parameters and obesity prevalence, respectively, across baseline energy-adjusted total PCDD/F dietary intake tertiles from oils and fats.

		Energy-adjusted total PCDD/F dietary intake tertiles (TEQ pg/week)-Oils and fats			
		Tertile 1 n=1,970	**Tertile 2** n=1,964	**Tertile 3** n=1,965	***P-trend***
		β-coefficients (95%CI)			
BMI (kg/m²)					
	Model 1	**0 (Ref.)**	0.08 (-0.13 to 0.29)	0.21 (-0.01 to 0.42)	0.064
	Model 2	**0 (Ref.)**	0.13 (-0.08 to 0.34)	0.27 (0.05 to 0.49)	0.017
	Full-adjusted model	**0 (Ref.)**	0.25 (0.04 to 0.46)	0.43 (0.21 to 0.66)	<0.001
Waist circumference (cm)					
	Model 1	**0 (Ref.)**	0.23 (-0.32 to 0.79)	0.46 (-0.10 to 1.03)	0.111
	Model 2	**0 (Ref.)**	0.45 (-0.11 to 1.01)	0.86 (0.28 to 1.43)	0.003
	Full-adjusted model	**0 (Ref.)**	0.81 (0.25 to 1.37)	1.31 (0.73 to 1.90)	<0.001
		Prevalence ratios (95%CI)			
Obesity					
Prevalence, % (n)		71.6 (1,411)	71.5 (1,405)	74.8 (1,470)	
	Model 1	**1 (Ref.)**	1.03 (0.99 to 1.07)	1.04 (0.99 to 1.08)	0.062
	Model 2	**1 (Ref.)**	1.04 (0.99 to 1.08)	1.04 (0.99 to 1.08)	0.027
	Full-adjusted model	**1 (Ref.)**	1.06 (1.01 to 1.09)	1.07 (1.02 to 1.11)	0.001
Abdominal obesity					
Prevalence, % (n)		92.0 (1,812)	92.4 (1,815)	94.8 (1,863)	
	Model 1	**1 (Ref.)**	1.01 (0.99 to 1.03)	1.02 (1.00 to 1.04)	0.023
	Model 2	**1 (Ref.)**	1.04 (1.00 to 1.08)	1.04 (1.00 to 1.08)	0.004
	Full-adjusted model	**1 (Ref.)**	1.01 (0.99 to 1.03)	1.03 (1.01 to 1.04)	0.003

Abbreviations: PCCD/F: Polychlorinated dibenzo-p-dioxins or dibenzofurans; TEQ: Toxic Equivalency; CI: confidence interval; BMI: body mass index. SD: Standard deviation.

Linear and cox regression models were fitted to assess the β-coefficients or prevalence ratio, and its 95%CI for adiposity parameters and obesity/overweight prevalence, respectively, across energy-adjusted total dietary PCDD/F intake. Model 1: adjusted for age and sex. Model 2: Model 1 additionally adjusted for physical activity (MET min/week), marital status (single, married, widowed), smoking status (current, former, never), education level (up to primary, secondary, university) and the size of the recruitment center (<250, 250 to <300, 300 to <400, ≥400). Fully adjusted: Model 2 additionally adjusted for adherence to Mediterranean diet MEDAS score (0-14 points) and type 2 diabetes (yes/no), hypertension (yes/no), and hypercholesterolemia (yes/no), or prevalence or taking medications for their control, and hormonal treatment. The robust estimate of variance was used in all analyses to account for the intra-cluster correlation.

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Table S12. Multivariable-adjusted β-coefficients or Odds ratios and its 95% confidence intervals for adiposity changes or obesity incidence, respectively, after 1-year of follow-up across baseline energy-adjusted total dietary PCDD/F intake from Oils and fats.

	Energy-adjusted total PCDD/F dietary intake tertiles (TEQ pg/week) Oils and fats			
	Tertile 1	**Tertile 2**	**Tertile 3**	***P- trend***
	β-coefficients (95%CI)			
BMI changes (kg/m^2)	(n=1,970)	(n=1,964)	(n=1,965)	
Model 1	**0 (Ref.)**	-0.12 (-0.22 to -0.2)	-0.06 (-0.16 to 0.04)	0.175
Model 2	**0 (Ref.)**	-0.13 (-0.21 to -0.03)	-0.07 (-0.16 to 0.02)	0.091
Full- adjusted model	**0 (Ref.)**	-0.14 (-0.23 to -0.05)	-0.09 (-0.19 to -0.02)	0.029
Waist circumference changes (cm)	(n=1,967)	(n=1,966)	(n=1,966)	
Model 1	**0 (Ref.)**	-0.39 (-0.07 to -0.07)	-0.25 (-0.58 to 0.07)	0.088
Model 2	**0 (Ref.)**	-0.50 (-0.08 to -0.19)	-0.41 (-0.72 to -0.09)	0.005
Full-adjusted model	**0 (Ref.)**	-0.45 (-0.76 to -0.13)	-0.34 (-0.66 to -0.03)	0.022
	Odds ratios (95%CI)			
Obesity	(n= 538)	(n= 538)	(n= 537)	
Incidence, % (n)	6.8 (38)	8.59 (48)	8.08 (40)	
Model 1	**1 (Ref.)**	1.07 (0.69 to 1.67)	0.95 (0.61 to 1.49)	0.897
Model 2	**1 (Ref.)**	1.06 (0.68 to 1.65)	0.96 (0.60 to 1.54)	0.927
Full-adjusted model	**1 (Ref.)**	1.07 (0.68 to 1.69)	0.98 (0.60 to 1.59)	0.973
Abdominal obesity	(n=137)	(n=136)	(n=136)	
Incidence, % (n)	15.1 (24)	18.2 (27)	20.59 (21)	
Model 1	**1 (Ref.)**	1.14 (0.62 to 2.09)	1.02 (0.53 to 1.97)	0.871
Model 2	**1 (Ref.)**	1.04 (0.55 to 1.99)	0.92 (0.45 to 1.87)	0.859
Full-adjusted model	**1 (Ref.)**	1.16 (0.59 to 2.28)	1.06 (0.49 to 2.29)	0.822

Abbreviations: PCCD/F: Polychlorinated dibenzo-p-dioxins or dibenzofurans; TEQ: Toxic Equivalency; CI: confidence interval; BMI: body mass index; SD: Standard Deviation.

Linear regression models and logistic regression models were fitted to assess the β-coefficients or odd ratio, and its 95%CI for adiposity parameters and obesity/overweight incidence, respectively across energy-adjusted total dietary PCDD/F intake. Model 1: adjusted for age, sex. Model 2: Model 1 additionally adjusted for physical activity (MET min/week), marital status (single, married, widowed), smoking status (current, former, never), education level (up to primary, secondary, university) and the size of the recruitment centres (<250, 250 to <300, 300 to <400, ≥400). Fully adjusted: Model 2 additionally adjusted to adherence to Mediterranean diet MEDAS score (0-14 points) and the prevalence of having and type 2 diabetes (yes/no), hypertension (yes/no), and hypercholesterolemia (yes/no), or prevalence or taking medications for their control, and hormonal treatment, intervention group and outcome variable at baseline. The robust estimate of variance was used in all analyses to account for the intra-cluster correlation.

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e. Article 5:

Title: Dietary intake of Perfluorooctanesulfonic acid (PFOS) and glucose homeostasis parameters in a non-diabetic senior population

Nadine Khoury, María Ángeles Martínez, Stephanie K Nishi, Miguel Ángel Martínez-González, Dolores Corella, Olga Castañer, J Alfredo Martínez, Ángel M Alonso-Gómez, Julia Wärnberg, Jesús Vioque, Dora Romaguera, José López-Miranda, Ramon Estruch, Francisco J Tinahones, José Manuel Santos-Lozano, Lluís Serra-Majem, Aurora Bueno-Cavanillas, Josep A Tur, Sergio Cinza Sanjurjo, Xavier Pintó, José Juan Gaforio, Pilar Matía-Martí, Josep Vidal, Clotilde Vázquez, Lidia Daimiel, Emilio Ros, Carmen Sayon-Orea, Jose V Sorli, Karla-Alejandra Pérez-Vega, Antonio Garcia-Rios, Francisco Ortiz-Díaz, Enrique Gómez-Gracia, M A Zulet, Alice Chaplin, Rosa Casas, Inmaculada Salcedo-Bellido, Lucas Tojal-Sierra, Maria-Rosa Bernal-Lopez, Zenaida Vazquez-Ruiz, Eva M Asensio, Albert Goday, Patricia J Peña-Orihuela, Antonio J Signes-Pastor, Ana Garcia-Arellano, Montse Fitó, Nancy Babio, Jordi Salas-Salvadó

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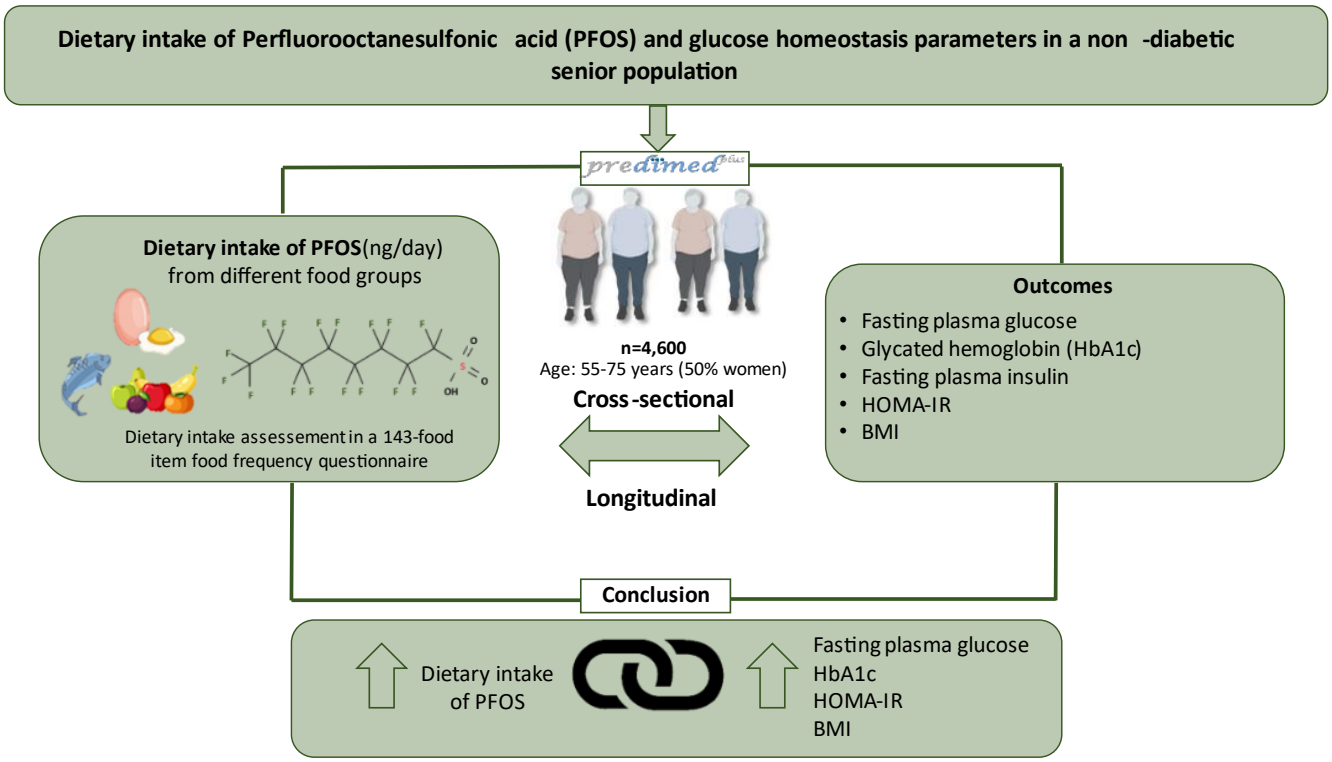
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Summary:

Compared to those in the lowest tertile, participants in the highest tertile of baseline PFOS dietary intake in LB and UB showed higher levels of HbA1c [β-coefficient (CI)] [0.01 % (0.002 to 0.026), and [0.06 mg/dL (0.026 to 0.087), both p-trend ≤ 0.001], and fasting plasma glucose in the LB PFOS dietary intake [1.05 mg/dL (0.050 to 2.046), p-trend = 0.022]. Prospectively, a positive association between LB of PFOS dietary intake and BMI [0.06 kg/m2(0.014 to 0.106) per 1-SD increment of energy-adjusted PFOS dietary intake was shown. Participants in the top tertile showed an increase in HOMA-IR [0.06(0.016 to 0.097), p-trend = 0.005] compared to participants in the reference tertile after 1-year of follow-up.

Discussion: This is the first study to explore the association between DI of PFOS and glucose homeostasis. In this study, a high baseline dietary intake of PFOS was associated with a higher levels of fasting plasma glucose and HbA1c and with an increase in HOMA-IR and BMI after 1-year of follow-up.

RESULTS


Dietary intake of Perfluorooctanesulfonic acid (PFOS) and glucose homeostasis parameters in a non -diabetic senior population
predimed
Dietary intake of PFOS(ng/day) from different food groups
Dietary intake assessement in a 143-food item food frequency questionnaire
n=4,600
Age: 55-75 years (50% women)
Cross-sectional
Longitudinal
Outcomes
• Fasting plasma glucose
• Glycated hemoglobin (HbA1c)
• Fasting plasma insulin
• HOMA-IR
• BMI
Conclusion
Dietary intake of PFOS
Fasting plasma glucose
HbA1c
HOMA-IR
BMI

RESULTS

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Full length article

Dietary intake of Perfluorooctanesulfonic acid (PFOS) and glucose homeostasis parameters in a non-diabetic senior population

Nadine Khoury [a,b,c,1], María Ángeles Martínez [a,b,c,1,*], Stephanie K. Nishi [a,b,c], Miguel Ángel Martínez-González [a,d,e], Dolores Corella [a,f], Olga Castañer [g,l], J. Alfredo Martínez [a,h,i], Ángel M. Alonso-Gómez [a,j,k,l], Julia Wärnberg [a,m], Jesús Vioque [n,o], Dora Romaguera [a,p], José López-Miranda [a,q], Ramon Estruch [a,r], Francisco J Tinahones [a,s], José Manuel Santos-Lozano [a,t], Lluís Serra-Majem [a,u], Aurora Bueno-Cavanillas [n,v], Josep A. Tur [a,w], Sergio Cinza Sanjurjo [x,y,z], Xavier Pintó [a,aa], José Juan Gaforio [l,ab], Pilar Matía-Martín [ac], Josep Vidal [ad,ae], Clotilde Vázquez [a,af], Lidia Daimiel [a,ag], Emilio Ros [a,ah], Carmen Sayon-Orea [a,d], Jose V Sorli [a,f], Karla-Alejandra Pérez-Vega [a,g], Antonio Garcia-Rios [a,q], Francisco Ortiz-Díaz [ai], Enrique Gómez-Gracia [a,m], MA Zulet [a,h,i], Alice Chaplin [a,p], Rosa Casas [a,r], Inmaculada Salcedo-Bellido [n,v,ak], Lucas Tojal-Sierra [a,j,k,l], Maria-Rosa Bernal-Lopez [a,aj], Zenaida Vazquez-Ruiz [a,d], Eva M. Asensio [a,f], Albert Goday [a,g], Patricia J. Peña-Orihuela [a,q], Antonio J. Signes-Pastor [n,o], Ana Garcia-Arellano [a,d], Montse Fitó [a,g], Nancy Babio [a,b,c], Jordi Salas-Salvadó [a,b,c,*]

[a] Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y la Nutrición (CIBEROBN), Institute of Health Carlos III, Madrid, Spain
[b] Universitat Rovira i Virgili, Departament de Bioquímica i Biotecnologia, Alimentació, Nutrició, Desenvolupament i Salut Mental ANUT-DSM, Reus, Spain
[c] Institut d'Investigació Sanitària Pere Virgili (IISPV), Reus, Spain
[d] University of Navarra, Department of Preventive Medicine and Public Health, IDISNA, Pamplona, Spain
[e] Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA, USA
[f] Department of Preventive Medicine, University of Valencia, Valencia, Spain
[g] IMIM (Hospital del Mar Medical Research Institute), Barcelona, Spain
[h] Department of Nutrition, Food Sciences, and Physiology, Center for Nutrition Research, University of Navarra, Pamplona, Spain
[i] Precision Nutrition and Cardiometabolic Health Program, IMDEA Food, CEI UAM + CSIC, Madrid, Spain
[j] Bioaraba Health Research Institute, Cardiovascular, Respiratory and Metabolic Area, Spain
[k] Osakidetza Basque Health Service, Araba University Hospital, Spain
[l] University of the Basque Country UPV/EHU, Vitoria-Gasteiz, Spain
[m] EpiPHAAN Research Group, School of Health Sciences, University of Málaga - Instituto de Investigación Biomédica en Málaga (IBIMA), Málaga, Spain
[n] CIBER de Epidemiología y Salud Pública (CIBERESP), Instituto de Salud Carlos III, Madrid, Spain
[o] Instituto de Investigación Sanitaria y Biomédica de Alicante, Universidad Miguel Hernández (ISABIAL-UMH), Alicante, Spain
[p] Health Research Institute of the Balearic Islands (IdISBa), Palma de Mallorca, Spain
[q] Department of Internal Medicine, Maimonides Biomedical Research Institute of Cordoba (IMIBIC), Reina Sofia University Hospital, University of Cordoba, Cordoba, Spain
[r] Department of Internal Medicine, Hospital Clínic, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Institut de Recerca en Nutrició i Seguretat Alimentària (INSA), University of Barcelona, Barcelona, Spain
[s] Virgen de la Victoria Hospital, Department of Endocrinology, Instituto de Investigación Biomédica de Málaga (IBIMA), University of Málaga, Málaga, Spain
[t] Department of Family Medicine, Research Unit, Distrito Sanitario Atención Primaria Sevilla, Sevilla, Spain
[u] Research Institute of Biomedical and Health Sciences (IUIBS), University of Las Palmas de Gran Canaria & Centro Hospitalario Universitario Insular Materno Infantil (CHUIMI), Canarian Health Service, Las Palmas de Gran Canaria, Spain
[v] Department of Preventive Medicine and Public Health, University of Granada, Granada, Spain
[w] Research Group on Community Nutrition & Oxidative Stress, University of Balearic Islands, Palma de Mallorca, Spain

Abbreviations: BMI, Body mass index; CVD, Cardiovascular disease; Eds, Endocrine disruptors; er-MedDiet, Energy-reduced Mediterranean diet; EFSA, European Food Safety Authority; FFQs, Food frequency questionnaires; HbA1c, Glycated hemoglobin; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; LOD, Limit of detection; LB, lower bound; PFAS, Per- and polyfluoroalkyl substances; PFOS, Perfluorooctane sulfonate; SD, Standard deviation; TWI, Tolerable weekly intake; UB, Upper bound.

* Corresponding authors.
E-mail addresses: mariaangeles.martinez@iispv.cat (M. Ángeles Martínez), jordi.salas@urv.cat (J. Salas-Salvadó).

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RESULTS

[x] CS Milladoiro, Área Sanitaria de Santiago de Compostela, Spain
[y] Instituto de Investigación de Santiago de Compostela (IDIS), Spain
[z] Centro de Investigación Biomédica en Red-Enfermedades Cardiovasculares (CIBERCV), Spain
[aa] Lipids and Vascular Risk Unit, Internal Medicine, Hospital Universitario de Bellvitge-IDIBELL, Hospitalet de Llobregat, Barcelona, Spain
[ab] Departamento de Ciencias de la Salud, Instituto Universitario de Investigación en Olivar y Aceites de Oliva, Universidad de Jaén, Jaén, Spain
[ac] Department of Endocrinology and Nutrition, Instituto de Investigación Sanitaria Hospital Clínico San Carlos (IdISSC), Madrid, Spain
[ad] CIBER Diabetes y Enfermedades Metabólicas (CIBERDEM), Instituto de Salud Carlos III (ISCIII), Madrid, Spain
[ae] Department of Endocrinology, Institut d'Investigacions Biomèdiques August Pi Sunyer (IDIBAPS), Hospital Clínic, University of Barcelona, Barcelona, Spain
[af] Department of Endocrinology and Nutrition, Hospital Fundación Jiménez Díaz. Instituto de Investigaciones Biomédicas IISFJD, University Autonoma, Madrid, Spain
[ag] Nutritional Control of the Epigenome Group. Precision Nutrition and Obesity Program. IMDEA Food, CEI UAM + CSIC, Madrid, Spain
[ah] Lipid Clinic, Department of Endocrinology and Nutrition, Institut d'Investigacions Biomèdiques August Pi Sunyer (IDIBAPS), Hospital Clínic, Barcelona, Spain
[ai] Centro Salud San Vicente del Raspeig, Alicante, Spain
[aj] Internal Medicine Clinical Management Unit, Hospital Regional Universitario de Málaga, Instituto de Investigación Biomédica de Málaga (IBIMA-Plataforma BIONAND), Universidad de Málaga, Spain
[ak] Instituto de Investigación Biosanitaria ibs. GRANADA, 18014 Granada, Spain

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ABSTRACT

Background: Endocrine disruptors (EDs) have emerged as potential contributors to the development of type-2 diabetes. Perfluorooctane sulfonate (PFOS), is one of these EDs linked with chronic diseases and gathered attention due to its widespread in food.
Objective: To assess at baseline and after 1-year of follow-up associations between estimated dietary intake (DI) of PFOS, and glucose homeostasis parameters and body-mass-index (BMI) in a senior population of 4600 non-diabetic participants from the PREDIMED-plus study.
Methods: Multivariable linear regression models were conducted to assess associations between baseline PFOS-DI at lower bound (LB) and upper bound (UB) established by the EFSA, glucose homeostasis parameters and BMI.
Results: Compared to those in the lowest tertile, participants in the highest tertile of baseline PFOS-DI in LB and UB showed higher levels of HbA1c [β-coefficient(CI)] [0.01 %(0.002 to 0.026), and [0.06 mg/dL(0.026 to 0.087), both p-trend ≤ 0.001], and fasting plasma glucose in the LB PFOS-DI [1.05 mg/dL(0.050 to 2.046),p-trend 0.022]. Prospectively, a positive association between LB of PFOS-DI and BMI [0.06 kg/m^2(0.014 to 0.106) per 1-SD increment of energy-adjusted PFOS-DI was shown. Participants in the top tertile showed an increase in HOMA-IR [0.06(0.016 to 0.097), p-trend 0.005] compared to participants in the reference tertile after 1-year of follow-up.
Discussion: This is the first study to explore the association between DI of PFOS and glucose homeostasis. In this study, a high baseline DI of PFOS was associated with a higher levels of fasting plasma glucose and HbA1c and with an increase in HOMA-IR and BMI after 1-year of follow-up.

1. Introduction

Type 2 diabetes mellitus (T2D) is a chronic metabolic disorder posing a significant global health challenge. In 2021, the International Diabetes Federation estimated that 1 in 10 adults were living with T2D and is predicted to rise to 1 in 9 adults by 2030 (IDF Diabetes Atlas 10th edition, 2023). The consequences of uncontrolled T2D are far-reaching and can lead to serious complications affecting various organ systems. Chronic hyperglycemia contributes, among others, to the development of microvascular and macrovascular complications, including diabetic retinopathy, stroke, and other cardiovascular diseases (Deshpande et al., 2008). To understand the multifactorial etiology of the T2D is crucial for establishing effective prevention and management strategies (Zimmet et al., 2001; Egede and Dagogo-Jack, 2005). Nowadays, it is known that there are multiple risk factors that lead to the development of T2D including genetic predisposition, age, sex, obesity status, smoking, physical inactivity, and unhealthy diet (Zimmet et al., 2001; Egede and Dagogo-Jack, 2005). However, in recent years, environmental and anthropogenic contaminants have emerged as potential contributors to the development and progression of this disease, with particular attention given to the role of endocrine disruptors (EDs) (Diamanti-Kandarakis et al., 2009; Gore et al., 2015; Wang et al., 2019).

Per- and polyfluoroalkyl substances (PFAS] are a diverse group of almost 15,000 anthropogenic chemicals (eBioMedicine, 2023). Since the 1940 s, these compounds have been extensively utilized due to their unique hydrophobic and lipophobic properties and molecular stability, making them highly stable and effective as water, grease, and stain resistants (Glüge et al., 2020; Paul et al., 2009). Despite their serious consequences on health, PFAS continue to be in use in various countries, contributing to the production of surfactants, lubricants, Teflon products, textiles, furniture, carpets, papers, and firefighting foams (Lau et al., 2004).

Perfluorooctane sulfonate (PFOS) is included in the PFAS family, and stands out as one of the most studied compounds (EFSA, 2020), it is an ED that has gathered considerable attention due to its widespread distribution and presence in foods specifically animal-origin products (Costopoulou et al., 2022; EFSA, 2020), and for its potential negative health effects. Due to its structural similarities to natural fatty acids, PFOS can bind to certain proteins and hormones, resulting in its long elimination half-life and bio-accumulation in the human body (Lindstrom et al., 2011; Heuvel et al., 1991). Furthermore, human exposure to PFAS is reported to start during gestation, potentially overpassing the placenta and persist through breastfeeding, leading to an important accumulation in body (Fábelová et al., 2023; Rovira et al., 2019). One of the key characteristics of PFOS is its notable persistence, characterized by high half-lives, which is estimated to range from 3.4 to 5.7 years (Rosato et al., 2024). PFOS is the most widely spread PFAS, and it has been used in various industrial and consumer applications. Consequently, it is accumulated in the food chain, being diet the main human exposure to this chemical (Olsen et al., 2007; EFSA, 2020). The chronic exposure and the long half-life of PFOS contribute to its accumulation in the body over time.

There is an increase amount of evidence examining the relationships between exposure to PFOS and the risk of obesity (Averina et al., 2021), anemia (Jain, 2021), dyslipidemia and mortality (Wen and Xu, 2022). In addition, previous studies have assessed the associations between PFAS exposure and increased risk of diabetes and glucose parameters (Xu

[1] These authors have contributed equally to this work.

et al., 2022; Brosset and Ngueta, 2023). However, other authors did not find consistent results (Han et al., 2021). In addition, the epidemiological evidence in senior population regarding dietary intake (DI) of PFOS, glycemic parameters and BMI is limited (Margolis and Sant, 2021; Qi et al., 2020) and despite the recognized susceptibility of specific groups to the adverse health effect of PFOS exposure, such as infants and pregnant women, the elderly populationis also a vulnerable group that is overlooked and needs to receive more attention. The elderly often experience a longer lifetime exposure to various contaminants, including PFOS, which may result in higher cumulative effects over time (Geller and Zenick, 2005). Additionally, age-related changes in metabolism, diminished organ function, and potential alterations in immune response can influence the way PFOS is absorbed, distributed, and eliminated (Palmer and Jensen, 2022).

Given the continuous increase in T2D and the significant anthropological and environmental exposures to PFOS, it is essential to evaluate in the context of the whole exposome, these chemicals and its impact on human health. In this way, we will be able to expand the understanding of the mechanism of this disease and to develop strategies for minimizing exposure (Lindstrom et al., 2011; Heuvel et al., 1991).

We hypothesized that high DI of PFOS has a detrimental impact on glycemic parameters and body mass index (BMI). Consequently, the overall objective of this study was to assess the associations between the estimated DI of PFOS and glucose homeostasis parameters and BMI in a cohort population from PREDIMED-Plus without diagnosed diabetes, cross-sectionally and longitudinally.

2. Materials and methods

2.1. Study design and population

The data of the PREDIMED-Plus (PREvención con DIeta MEDiterránea) cohort was used for the present cross-sectional and longitudinal study. PREDIMED-Plus is a currently ongoing randomized controlled trial taking place in 23 Spanish centers. The primary outcome of this trial is to evaluate the effect of an intensive weight loss intervention founded on a Mediterranean diet with reduced energy intake (er-MedDiet), promotion of physical activity, and behavioral support (intervention group), to and ad libitum Mediterranean diet following standard care advice (control group). The PREDIMED-Plus study aims to evaluate the incidence of cardiovascular disease (CVD) and mortality. Additional information about the PREDIMED-Plus study can be accessed at https://www.predimedplus.com, and has been described in prior publications (Sayón-Orea et al., 2019; Salas-Salvadó et al., 2019; Martínez-Gonzá lez et al., 2019). Participants were recruited in Spain (October 2013 - December 2016, including a total of 6,874) participants were randomly assigned to either the control group or the intervention group in a 1:1 ratio. Eligible participants were men (55–75 years) and women (60–75 years) with overweight or obesity (BMI = 27–40 kg/m2) and without a documented history of cardiovascular diseases (CVD) and met at least 3 criteria of metabolic syndrome (Alberti et al., 2005). In this present analysis, a total of 4,600 participants without diabetes were included in the cross-sectional assessment. They had complete baseline data from food frequency questionnaires (FFQs). Participants were excluded if their energy intake appeared implausible (Willet, 2012). Additionally, individuals with type 2 diabetes (T2D) were excluded from this analysis. For the prospective analysis, out of the initial 4,600 participants, 504 were further excluded due to insufficient FFQ

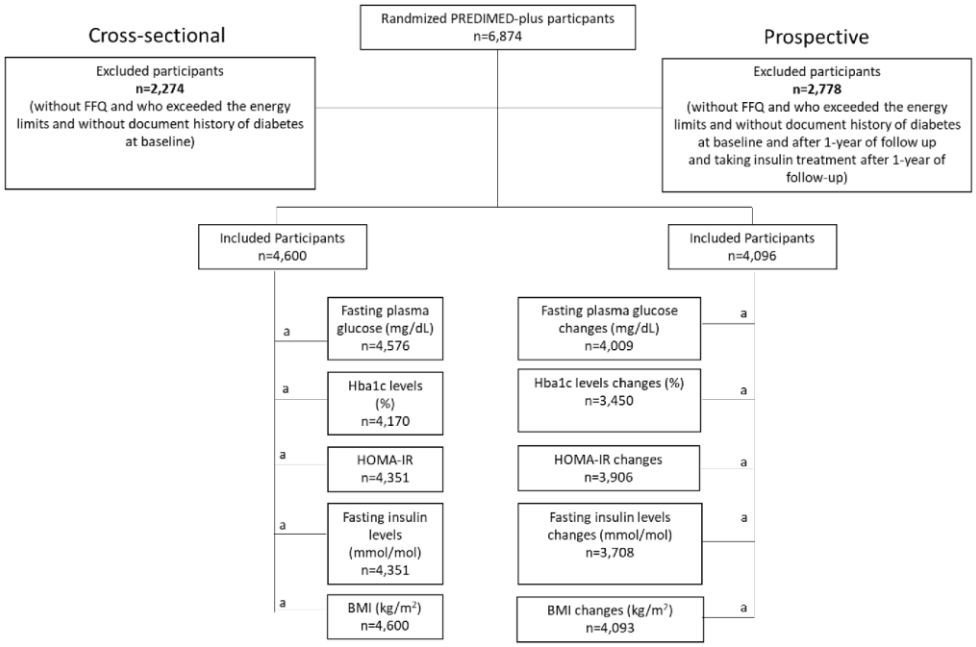


Fig. 1. Flow chart. a Included participants for each outcome after excluding those with respective non-available measurements.

information, implausible energy intake, or because they had started insulin treatment during the one-year follow-up period (Fig. 1).

2.2. PFOS dietary intake assessment

To assess PFOS dietary intake, the study population underwent a validated face-to-face baseline food frequency questionnaire (FFQ) administered by trained staff members (De La Fuente-Arrillaga et al., 2010). Participants were asked to report their food consumption frequencies and quantity by this validated questionnaire (De La Fuente-Arrillaga et al., 2010). For the water intake assessment, a fluid-specific questionnaire that has been already validated as a tool for assessing intake of water and other types of beverages, was used in our population (Ferreira-Pêgo et al., 2016) (Supplementary Material, Figure S1).

We considered the most recent data on PFOS levels in food (measured in ng/g) (EFSA, 2020). These food items were grouped into appropriate food groups based on guidelines provided by the European Food Safety Authority (EFSA) (Supplementary Material, Table S2) (EFSA, 2020). To calculate the PFOS-DI, we also considered the previously mentioned consumption frequency. As a result, the dietary PFOS intake was expressed in ng/day.

The PFOS levels for each food group were obtained from earlier studies conducted by the EFSA, which assessed PFOS concentrations in a wide range of food samples from various European regions between 2012 and 2020. We primarily relied on data from 2020 as it represented the most up-to-date information (EFSA, 2020). In the DI estimation, we used the mean of the lower bound (LB) and upper bound (UB) levels within food categories defined by the EFSA.

It's important to note that the terms "lower bound" and "upper bound" refer to the range in which PFOS is detected. The LB represents the lowest value within that range, while the UB represents the highest estimated range for PFOS levels.

The mean LB is characterized by being the least conservative and also called "best case scenario" because when a non-detect is reported for a component, the concentration in the food is zero and the mean UB is characterized by being highly conservative and also called "worst case scenario" because when a non-detect is reported for a component, the concentration in food items is at the limit of detection (LOD). According to EFSA, when analyzing data on the presence of PFAS, results that fall below the LOD are considered left-censored. The recommended method for handling such data, involves a substitution approach. This method is particularly suitable for chemicals likely to be found in food such as PFOS, assigns a value of zero (minimum possible value) to results reported as < LOD LB. Simultaneously, an UB is determined by assigning the numerical value of LOD. The choice of LOD depends on the laboratory's reporting method. This approach ensures a cautious and realistic estimation of PFOS levels (EFSA, 2020). Consequently, in this study, both LB and UB were taken into consideration to estimate the lower and upper limits of dietary intake and exposure to PFOS in our study population (EFSA, 2020).

Therefore, the estimated DI of PFOS UB and LB (expressed in ng/day) was obtained from the following equation:

$$DI\ PFOS_{LB/UB}(ng/day) = \sum C_i \times A_i$$

Where DI represents the downward (LB) / upward (UB) Dietary intake of PFOS in ng/day; C_i represents the PFOS concentration of a specific food group (in ng/g); Ai signifies the quantity of a particular food item consumed, measured in grams per day (g/day).

2.3. Estimation of dietary exposure to PFOS

According to the EFSA, dietary exposure to PFOS was estimated in this cohort considering the LB (being this approach more realistic) (EFSA, 2020), following this equation:

$$Dietary\ exposure\ to\ PFOS(ng/kg\ of\ body\ weight/week) = \left(\left(\sum C_i \times A_i\right)/BW_i\right) \times 7$$

"Ci" refers to the PFOS concentration of a particular food group, measured in nanograms per gram (ng/g). "Ai" stands for the amount of that specific food consumed, expressed in grams per day (g/day). "BWi" represents the body weight of each participant, measured in kilograms (kg). To determine the weekly dietary exposure, the calculated result is multiplied by 7.

2.4. Outcomes

Samples for fasting plasma glucose and glycated hemoglobin (HbA1c) were obtained from our study cohort population and analyzed by a routine laboratory method. The fasting plasma insulin levels were measured centrally using an Elecsys immunoanalyzer (Roche Diagnostics, Meylan, France) with the assistance of an electrochemiluminescence immunoassay. To estimate insulin resistance, the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) index was used following this formula (Matthews et al., 1985):

$$HOMA - IR = Insulin_{(mU/mL)} \times glucose_{(mg/dL)}/405$$

Trained PREDIMED-Plus staff conducted assessments of various anthropometric variables at baseline and after 1-year of follow-up. The Body weight of our cohort population was measured two different times wearing light clothes. This was assessed using precise electronic calibrated scales. The average of the two measurements was used. Height was measured using a stadiometer mounted on a wall. BMI was obtained dividing the weight of each participant (in kilograms) by the square of height of each subject (in meters).

2.5. Assessment of covariates

Additional covariates were evaluated using general face-to-face questionnaires that collect information on socio-demographics (sex, marital status, age, and level of education), lifestyle (smoking habits and physical activity), history of type 2 diabetes and family history of cardiovascular diseases. Physical activity was assessed using a validated Minnesota-REGICOR Short Physical Activity questionnaire (Molina et al., 2017). Moreover, a validated 17-item questionnaire was used to assess the adherence to the Mediterranean diet (Schröder et al., 2011). Waist circumference was obtained for each participant measuring at the midpoint between the lowest rib and the iliac crest using a specialized tape.

2.6. Statistical analyses

The most updated database of the PREDIMED-PLUS study (from December 2020) was considered. The population was divided in tertiles of energy-adjusted PFOS intake, expressed as mean ± standard deviation (SD) for continuous variables and percentages (number) for categorical variables. Tertiles of PFOS dietary intake were adjusted for total energy intake (in ng/day), using the residual-regression method (Willett and Stampfer, 1986), where the first tertile corresponds to lower DI of PFOS, second tertile corresponds to medium DI of PFOS and third tertile corresponds to higher DI of PFOS.

To compare the baseline characteristics across these tertiles, the Chi-square test was used for categorical variables, and one-way ANOVA was applied for continuous variables.

Multivariable linear regression models were fitted to cross-sectionally and longitudinally assess the associations [β-coefficient (95 % confidence interval (CI)] between the baseline energy-adjusted DI of PFOS, glucose-related parameters (fasting plasma glucose, HbA1c, fasting plasma insulin and HOMA-IR) and BMI. These associations were explored considering both, tertiles of energy-adjusted DI of PFOS, and

RESULTS

Table 1
Main baseline characteristics of the included participants overall and across energy-adjusted total PFOS lower bound dietary intake tertiles.

	All	Energy-adjusted total PFOS lower bound dietary intake – (ng/d)			
		T1 n = 1,534 17.63 ± 3.50	**T2** n = 1,533 25.52 ± 1.94	**T3** n = 1,533 35.22 ± 5.93	**P-value**
Sex					
Women	50.3 (2,316)	42.9 (658)	50.7 (778)	57.4 (880)	< 0.001
Age (years) (n = 4,600)	64.9 ± 4.90	64.4 ± 5.07	65.3 ± 4.91	65.0 ± 4.69	< 0.001
Educational level (n = 4,600)					0.020
Up to primary	48.0 (2,209)	49.1 (754)	50.4 (772)	44.5 (683)	
Secondary	29.1 (1,339)	28.4 (436)	27.6 (423)	31.3 (480)	
University	22.9 (1,052)	22.4 (344)	22.0 (338)	24.1 (370)	
Marital status (n = 4,600)					0.211
Single	13.3 (613)	15.2 (233)	12.6 (193)	12.2 (187)	
Married	76.3 (3,508)	75.2 (1,154)	76.3 (1,170)	77.2 (1,184)	
Widowed	10.4 (479)	9.58 (147)	11.1 (170)	10.6 (162)	
Smoking status (n = 4,600)					0.001
Never	45.4 (2,088)	41.6 (638)	46.2 (708)	48.4 (742)	
Former	42.5 (1,953)	44.3 (679)	41.7 (639)	41.4 (635)	
Current	12.1 (559)	14.1 (217)	12.1 (186)	10.2 (156)	
Physical activity (MET min/week) (n = 4,600)	2499 ± 2318	2422 ± 2492	2403 ± 2124	2673 ± 2314	0.001
Waist circumference (cm) (n = 4,600)					
Men	111 ± 8.73	110 ± 8.76	111 ± 8.75	110 ± 8.65	0.114
Women	103 ± 9.03	103 ± 9.15	103 ± 9.15	103 ± 8.84	0.605
BMI (kg/m^2) (n = 4,600)	32.4 ± 3.42	32.4 ± 3.35	32.5 ± 3.45	32.4 ± 3.46	0.855
Adherence to Mediterranean diet MEDAS score (0–17 points) (n = 4,600)	8.44 ± 2.70	7.57 ± 2.61	8.49 ± 2.56	8.25 ± 2.67	< 0.001
Dietary assessment (n = 4,600)					
Fruits (g/d)	359 ± 208	321 ± 191	367 ± 199	389 ± 227	< 0.001
Vegetables (g/d)	327 ± 140	284 ± 127	325 ± 134	372 ± 144	< 0.001
Legumes (g/d)	20.5 ± 11.2	19.5 ± 11.4	20.7 ± 10.5	21.4 ± 11.5	< 0.001
Total cereals (g/d)	151 ± 78.3	162 ± 84.6	149 ± 77.5	141 ± 70.9	< 0.001
Biscuits (g/d)	27.7 ± 30.1	31.5 ± 33.9	28.7 ± 30.1	22.8 ± 24.9	< 0.001
Dairy products (g/d)	339 ± 198	346 ± 204	337 ± 198	336 ± 192	0.335
Red meat and derivatives (g/d)	80.9 ± 44.4	79.9 ± 45.8	81.4 ± 43.4	81.6 ± 43.9	0.548
White meat (g/d)	61.6 ± 33.8	55.8 ± 33.8	61.3 ± 31.5	67.7 ± 34.9	< 0.001
Fish and shellfish (g/d)	101 ± 47.3	57.5 ± 21.9	96.8 ± 21.5	150 ± 37.9	< 0.001
Nuts (g/d)	14.9 ± 16.9	14.4 ± 17.5	14.5 ± 16.2	16.0 ± 16.9	0.014
Total olive oil (g/d)	40.3 ± 16.7	39.9 ± 17.3	40.7 ± 16.2	40.4 ± 16.6	0.445
Virgin olive oil (g/d)	32.3 ± 20.7	31.2 ± 21.3	32.7 ± 20.8	32.9 ± 19.9	0.039
Total energy (Kcal/d)	2382 ± 554	2396 ± 585	2369 ± 552	2381 ± 524	0.405
Proteins (g/d)	97.2 ± 21.8	89.4 ± 21.0	96.3 ± 20.5	106 ± 20.8	< 0.001
Saturated fatty acids (g/d)	26.2 ± 8.49	26.5 ± 9.16	26.2 ± 8.31	26.0 ± 7.95	0.294
Polyunsaturated fatty acids (g/d)	18.0 ± 6.59	17.7 ± 6.89	17.9 ± 6.36	18.4 ± 6.48	0.021
Monounsaturated fatty acids (g/d)	53.9 ± 16.0	53.6 ± 16.7	53.9 ± 15.6	54.1 ± 15.8	0.644
Carbohydrates (g/d)	244 ± 73.2	255 ± 76.9	243 ± 73.3	235 ± 67.8	< 0.001
Dietary fiber (g/d)	26.2 ± 8.82	24.7 ± 8.66	26.3 ± 8.79	27.6 ± 8.76	< 0.001
Total sugar (g/d)	8.58 ± 13.2	10.2 ± 14.1	8.64 ± 13.2	6.90 ± 11.8	< 0.001
Water (mL/day)	1139 ± 267	1133 ± 269	1137 ± 263	1148 ± 268	0.305
Alcohol (g/day)	11.2 ± 14.9	12.5 ± 16.6	10.7 ± 14.4	10.3 ± 13.7	0.001
Fasting plasma insulin levels (mmol/mol) (n = 4,351)	18.4 ± 10.0	18.4 ± 10.4	18.6 ± 9.92	18.1 ± 9.84	0.394
Fasting plasma glucose levels (mg/dL) (n = 4,576)	102 ± 13.5	102 ± 14.2	103 ± 13.2	102 ± 12.9	0.152
HbA1c levels (%) (n = 4,170)	5.75 ± 0.39	5.73 ± 0.38	5.76 ± 0.41	5.77 ± 0.39	0.011

Abbreviations PFOS: Perfluorooctanesulfonic acid; CI: confidence interval; HbA1c: hemoglobin A1c; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; BMI: body mass index.
Data are expressed as means ± SD and percentages (number) for continuous and categorical variables, respectively. P-values for comparisons were tested by one-way ANOVA or Chi-square test, as appropriate across tertiles.

energy-adjusted DI of PFOS with one SD increment, continuous. For the prospective analysis, the outcome considered were the changes in aforementioned glucose-related parameters and BMI after 1-year of follow-up.

Three multivariable models were conducted considering several confounders: i) Model 1: sex and age-adjusted ii) Model 2: model 1 further adjusted for marital status (married, widowed, single or divorced or separated, or religious), physical activity (METs min/week), education level (primary or lower, secondary or academic, or graduate), the size of the recruitment center representing the total field center workload (<250, 250 to < 300, 300 to < 400, ≥400 randomized participants) and smoking status (never, former, or current) and BMI (kg/m^2); iii) Model 3: further adjusted for adherence to Mediterranean diet using the 17-item MEDAS score and family history of cardiovascular disease. For prospective analyses, model 3 was further adjusted for the intervention group, diabetes medications used and each respective baseline glucose-related parameter (Supplementary Material, Figure S2).

Moreover, we conducted sensitivity analyses considering the linear regression model 3 to assess the association between baseline energy-adjusted DI of PFOS in continuous (with 1 SD increment) and fasting plasma glucose or HbA1c depending on age (<65 years old ≥ 65 years old), sex (men or women) and weight status (overweight or obesity). In addition, sensitivity analysis was conducted after 1-year of follow-up using the linear regression model 3 to assess the association between baseline energy-adjusted DI of PFOS in continuous (with 1 SD increment) and changes in HOMA-IR depending on age (<65 years old or equal or ≥ 65 years old), sex (men or women), weight status (overweight or obesity) and allocation group (intervention or control group). No

significant interaction was observed for all cases (P-value > 0.05) using the likelihood ratio test.

P-value < 0.05 was considered as significant. All analyses were conducted with robust estimates of the variance to correct for intra-cluster (members of the same household) correlation and using the Stata 14 software program (StataCorp).

3. Results

A total of 4,600 participants with no diabetes from the PREDIMED-Plus cohort population (49.7 % men and 50.3 % women, with a mean (SD) age of 65 ± 4.90 years) were included in this analysis. In Table 1, the baseline characteristics of the participants overall and according to tertiles of total energy-adjusted dietary PFOS intake (ng/day) were presented.

Compared to participants in the lower DI tertile of PFOS, those participants the highest PFOS intake were more frequently women, were less frequently smokers, do more leisure-time physically activity, and showed lower adherence to the Mediterranean diet. They consume higher amounts of nuts, fruits and vegetables, fish and shellfish, legumes, white meat, dietary fiber, virgin olive oil, protein, and poly-unsaturated fatty acids. On the other hand, they consumed lower amounts of biscuits, cereals, sugar and alcohol.

In the present study the major food groups contributors to total DI of PFOS were fish and seafood (62 %), followed by eggs (25 %), and fruits and vegetables (8 %) (Fig. 2).

In our cross-sectional analysis (Table 2), positive associations were observed between the LB of energy-adjusted DI of PFOS and fasting plasma glucose and HbA1c levels, being the magnitude of these associations increased through the adjustment (β-coefficient [95 %CI]) (1.05 mg/dL [0.050 to 2.046] and 0.05 % [0.024 to 0.082], P for trend 0.022 and 0.001 for fasting plasma glucose and HbA1c, respectively, in the full adjusted model). In addition, we found a positive association between the UB of energy-adjusted DI of PFOS and HbA1c levels in the fully-adjusted model (β-coefficient [95 %CI]) (0.06 % [0.026 to 0.087], P for trend < 0.001. No other significant associations were found for the rest of glucose-related parameters in the LB nor the UB analysis. However, the direction of these associations goes in line with what was expected (higher fasting plasma insulin levels, higher HOMA-IR and higher BMI in the LB and UB of the highest tertile of PFOS DI).

In the prospective analysis (Table 3), a positive association between baseline energy-adjusted DI of PFOS in the LB (ng/day) and an increase in BMI after 1-year of follow-up (β coefficient [95 %CI] 0.06 kg/m^2 [0.014 to 0.106]) was shown. In addition, participants in the top tertile of baseline energy-adjusted DI of PFOS in the UB (ng/day) showed an increase in HOMA-IR (β-coefficient [95 %CI]; 0.06 [0.016 to 0.097]; P for trend = 0.005) compared to those in the first tertile after one year of follow-up. No other significant associations were observed for changes in fasting plasma glucose, HbA1c, fasting plasma insulin and BMI. In the sensitivity analysis energy-adjusted DI PFOS and fasting plasma glucose in the LB was positively associated only in overweight participants. Moreover, a higher baseline DI of PFOS was positively associated with higher levels of HbA1c in the LB as well as in the UB in participants < 65 years old. In addition, DI of PFOS was cross-sectionally associated with higher HbA1c in men and overweight participants in the UB. After 1-year of follow-up, baseline energy-adjusted PFOS intake (with 1 SD increment) was positively associated with an increase in HOMA-IR in participants < 65 years old (Supplementary Material, figures S3a-d and figures S4a-b).

4. Discussion

To the best of our knowledge, this is the first study to explore the association between DI of PFOS and glucose homeostasis. We found that in an adult Mediterranean population at high cardiovascular risk, high baseline DI of PFOS was associated with a higher levels of fasting plasma glucose and HbA1c and with an increase in HOMA-IR and BMI after 1-year of follow-up.

In our study, we found that fish and seafood, followed by eggs, and fruits and vegetables were the major food contributors to the total dietary intake of PFOS. These results are in line with other published articles (Fábelová et al., 2023; Rovira et al., 2019; Sznajder-Katarzyńska et al., 2018). A recent study conducted in Greece found that eggs, fish and seafood seem to be responsible for about 50 % of PFOS dietary exposure (Costopoulou et al., 2022). In addition, EFSA published a scientific opinion in 2020 stating that fish and seafood, fruits and vegetables contribute the most to the total PFOS DI (EFSA, 2020) for all ages. The high abundance of PFOS in animal-origin foods could be explained due the lipophilic properties of this chemical, which can lead to their accumulation and persistence in the food chain, being animal and animal-based products, especially seafood and eggs, the main sources to total PFOS dietary intake exposure. In addition, its presence in fruits and

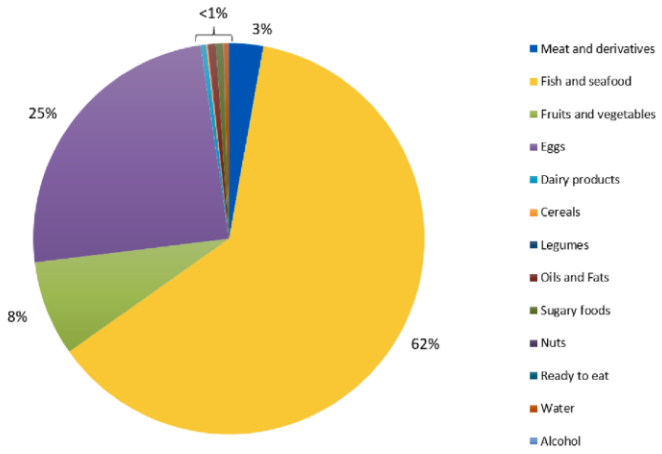


Fig. 2. Food groups contribution to the total dietary intake of PFOS.

Table 2
Cross-sectional baseline association between energy-adjusted dietary intake of PFOS and glucose-related parameters and body-mass-index.

	Energy-Adjusted dietary intake of PFOS across tertiles and in continuous with 1 SD increment in ng/d β coefficient (95 % CI)									
	LB					UB				
	T1	T2	T3	P-trend	Continuous with 1 SD increment	T1	T2	T3	P-trend	Continuous with 1 SD increment
Mean ± SD (ng/d)	17.62 ± 3.49	25.51 ± 1.94	35.21 ± 5.94			1104 ± 251	1738 ± 170	2552 ± 475		
Fasting plasma glucose (mg/dL) (n = 4,576)										
Model 1	**0 (ref)**	**1.15 (0.165 to 2.128)**	0.76 (-0.215 to 1.729)	0.079	0.18 (-0.206 to 0.576)	**0 (ref)**	0.94 (-0.037 to 1.924)	0.37 (-0.608 to 1.350)	0.531	0.12 (-0.276 to 0.520)
Model 2	**0 (ref)**	**1.20 (0.228 to 2.179)**	0.90 (-0.072 to 1.881)	**0.041**	0.25 (-0.146 to 0.639)	**0 (ref)**	**1.04 (0.065 to 2.02)**	0.53 (-0.453 to 1.516)	0.347	0.19 (-0.206 to 0.594)
Fully-adjusted	**0 (ref)**	**1.30 (0.316 to 2.289)**	**1.05 (0.050 to 2.046)**	**0.022**	0.29 (-0.107 to 0.693)	**0 (ref)**	**1.13 (0.143 to 2.128)**	0.67 (-0.341 to 1.685)	0.245	0.24 (-0.164 to 0.655)
Mean ± SD (ng/d)	17.53 ± 3.45	25.35 ± 1.91	35.01 ± 5.95			1096 ± 248	1721 ± 166	2531 ± 466		
HbA1c level (%) (n = 4,170)										
Model 1	**0 (ref)**	0.02 (-0.011 to 0.048)	0.03 (-0.000 to 0.057)	0.053	0.00 (-0.007 to 0.016)	**0 (ref)**	0.00 (-0.025 to 0.334)	**0.03 (0.000 to 0.059)**	**0.044**	**0.01 (-0.005 to 0.018)**
Model 2	**0 (ref)**	0.02 (-0.011 to 0.048)	**0.03 (0.005 to 0.063)**	**0.026**	0.01 (-0.004 to 0.019)	**0 (ref)**	0.00 (-0.025 to 0.033)	**0.04 (0.007 to 0.066)**	**0.013**	**0.01 (-0.002 to 0.022)**
Fully-adjusted	**0 (ref)**	0.03 (-0.001 to 0.058)	**0.05 (0.024 to 0.082)**	**0.001**	**0.01 (0.002 to 0.026)**	**0 (ref)**	0.01 (-0.013 to 0.045)	**0.06 (0.026 to 0.087)**	< **0.001**	**0.02 (0.006 to 0.030)**
Mean ± SD (ng/d)	17.59 ± 3.49	25.52 ± 1.94	35.26 ± 5.99			1102 ± 251	1737 ± 169	2553 ± 478		
Fasting plasma insulin (mmol/mol) (n = 4,351)										
Model 1	**0 (ref)**	0.49 (-0.243 to 1.219)	0.01 (-0.725 to 0.743)	0.948	−0.25 (-0.531 to 0.032)	**0 (ref)**	**0.97 (0.232 to 1.707)**	−0.01 (-0.724 to 0.702)	0.815	−0.22 (-0.498 to 0.049)
Model 2	**0 (ref)**	0.43 (-0.261 to 1.312)	0.12 (-0.573 to 0.824)	0.776	−0.23 (-0.501 to 0.039)	**0 (ref)**	0.93 (0.214 to 1.641)	0.14 (-0.546 to 0.829)	0.827	−0.19 (-0.456 to 0.077)
Fully-adjusted	**0 (ref)**	0.62 (-0.068 to 1.315)	0.48 (-0.222 to 1.186)	0.208	−0.02 (-0.099 to 0.058)	**0 (ref)**	1.15 (0.431 to 1.876)	0.52 (-0.182 to 1.217)	0.222	−0.05 (-0.324 to 0.216)

(continued on next page)

Table 2 (*continued*)

	Energy-Adjusted dietary intake of PFOS across tertiles and in continuous with 1 SD increment in ng/d β coefficient (95 % CI)									
	LB					UB				
	T1	T2	T3	P-trend	Continuous with 1 SD increment	T1	T2	T3	P-trend	Continuous with 1 SD increment
Mean ± SD (ng/d)	17.59 ± 3.49	25.52 ± 1.94	35.26 ± 5.99			1102 ± 251	1737 ± 169	2553 ± 478		
HOMA IR (n = 4,351)										
Model 1	**0 (ref)**	0.17 (-0.054 to 0.395)	0.02 (-0.205 to 0.238)	0.965	−0.07 (-0.149 to 0.017)	**0 (ref)**	**0.30 (0.073 to 0.529)**	−0.00 (-0.213 to 0.205)	0.798	0.06 (-0.139 to 0.022)
Model 2	**0 (ref)**	0.16 (-0.049 to 0.378)	0.07 (-0.139 to 0.274)	0.578	−0.05 (-0.131 to 0.027)	**0 (ref)**	0.30 (0.076 to 0.527)	0.05 (-0.150 to 0.258)	0.713	−0.04 (-0.117 to 0.040)
Fully-adjusted	**0 (ref)**	**0.21 (0.003 to 0.423)**	0.16 (-0.045 to 0.365)	0.166	−0.10 (-0.373 to 0.170)	**0 (ref)**	0.04 (0.130 to 0.591)	0.15 (-0.054 to 0.359)	0.229	−0.01 (-0.086 to 0.074)
Mean ± SD (ng/d)	17.63 ± 3.50	25.52 ± 1.94	35.22 ± 5.93			1105 ± 252	1739 ± 170	2553 ± 474		
BMI (kg/m²) * (n = 4,600)										
Model 1	**0 (ref)**	0.05 (-0.194 to 0.288)	−0.07 (-0.316 to 0.169)	0.522	0.00 (-0.099 to 0.101)	**0 (ref)**	−0.02 (0.265 to 0.219)	−0.13 (-0.375 to 0.111)	0.275	−0.03 (-0.132 to 0.070)
Model 2	**0 (ref)**	0.07 (-0.172 to 0.305)	0.01 (-0.232 to 0.252)	0.960	0.04 (-0.058 to 0.143)	**0 (ref)**	0.00 (-0.239 to 0.244)	−0.04 (-0.287 to 0.199)	0.714	0.01 (-0.089 to 0.114)
Fully-adjusted	**0 (ref)**	0.13 (-0.111 to 0.370)	0.12 (-0.131 to 0.364)	0.381	0.08 (-0.017 to 0.188)	**0 (ref)**	0.07 (-0.175 to 0.313)	0.06 (-0.184 to 0.313)	0.631	0.06 (-0.044 to 0.163)

Abbreviations: PFOS: Perfluorooctanesulfonic acid; CI: confidence interval; HbA1c: hemoglobin A1c; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; LB: lower bound of energy-Adjusted dietary intake of PFOS; UB: upper bound of energy-Adjusted dietary intake of PFOS; BMI: body mass index; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance.
Linear regression models were fitted to assess the β-coefficients and its 95 % CI for glycemic index parameters across energy-adjusted total dietary PFOS intake and with 1 SD increment in ng/d. Model 1 adjusted for age and sex. Model 2: model 1 additionally adjusted for physical activity (MET min/week), marital status (single, married, widowed), smoking status (current, former, never), education level (up to primary, secondary, university) and the size of the recruitment center (<250, 250 to < 300, 300 to < 400, ≥400 and BMI (kg/m²). Model 3: model 2 additionally adjusted for adherence to Mediterranean diet MEDAS score (0–17 points) and family history of cardiovascular diseases. All analyses were conducted with robust estimates of the variance to correct for intra-cluster correlation.

* Excluding BMI (kg/m²) as a confounder in the models.

Table 3
Prospective association between baseline energy-adjusted dietary intake of PFOS and changes in glucose-related parameters and body-mass-index changes.

	Energy-Adjusted dietary intake of PFOS across tertiles and in continuous with 1 SD increment in ng/d β coefficient (95 % CI)									
	LB					UB				
	T1	T2	T3	P-trend	Continuous with 1 SD increment	T1	T2	T3	P-trend	Continuous with 1 SD increment
Mean ± SD (ng/d)	17.7 ± 3.45	25.6 ± 1.95	35.1 ± 5.82			1109 ± 248	1741 ± 170	2546 ± 471		
Fasting plasma glucose changes (mg/dL) (n = 4,009)										
Model 1	**0 (ref)**	−0.27 (-1.209 to 0.669)	−0.54 (-1.480 to 0.391)	0.254	−0.17 (-0.551 to 0.200)	**0 (ref)**	−0.24 (-1.181 to 0.700)	−0.12 (-1.063 to 0.814)	0.815	−0.12 (-0.494 to 0.249)
Model 2	**0 (ref)**	−0.32 (-1.269 to 0.620)	−0.30 (-1.543 to 0.336)	0.209	−0.19 (-0.564 to 0.189)	**0 (ref)**	−0.32 (-1.263 to 0.625)	0.20 (-1.145 to 0.734)	0.692	−0.15 (-0.522 to 0.222)
Fully-adjusted	**0 (ref)**	0.02 (-0.841 to 0.881)	−0.21 (-1.075 to 0.662)	0.627	−0.08 (-0.431 to 0.260)	**0 (ref)**	−0.07 (-0.925 to 0.792)	0.05 (-0.812 to 0.915)	0.893	−0.07 (-0.412 to 0.268)
Mean ± SD (ng/d)	17.6 ± 3.39	25.4 ± 1.91	34.8 ± 5.82			**1101** ± 246	1724 ± 165	2517 ± 457		
HbA1c level changes (%) (n = 3,450)										
Model 1	**0 (ref)**	−0.01 (-0.032 to 0.018)	−0.02 (-0.045 to 0.006)	0.128	−0.00 (-0.015 to 0.007)	**0 (ref)**	−0.01 (-0.033 to 0.017)	−0.02 (-0.048 to 0.004)	0.095	−0.01 (-0.018 to 0.004)
Model 2	**0 (ref)**	−0.00 (-0.031 to 0.019)	−0.02 (-0.047 to 0.005)	0.113	−0.00 (-0.016 to 0.007)	**0 (ref)**	−0.01 (-0.032 to 0.018)	−0.02 (-0.049 to 0.003)	0.076	−0.01 (-0.019 to 0.004)
Fully-adjusted	**0 (ref)**	−0.00 (-0.027 to 0.019)	−0.01 (-0.039 to 0.010)	0.243	−0.00 (-0.015 to 0.007)	**0 (ref)**	−0.01 (-0.033 to 0.013)	−0.02 (-0.042 to 0.009)	0.200	−0.01 (-0.018 to 0.004)
Mean ± SD (ng/d)	17.7 ± 3.44	25.6 ± 1.95	35.1 ± 5.84			1109 ± 248	1744 ± 169	2547 ± 472		
Fasting plasma insulin levels changes (mmol/mol) (n = 3,708)										
Model 1	**0 (ref)**	−0.27 (-0.943 to 0.402)	0.33 (-0.559 to 1.218)	0.439	0.18 (-0.119 to 0.489)	**0 (ref)**	−0.55 (-1.217 to 0.119)	0.58 (-0.312 to 1.474)	0.168	0.27 (-0.037 to 0.583)
Model 2	**0 (ref)**	−0.26 (-0.944 to 0.414)	0.32 (-0.560 to 1.209)	0.445	0.18 (-0.118 to 0.489)	**0 (ref)**	−0.54 (-1.216 to 0.139)	0.60 (-0.315 to 1.518)	0.166	0.28 (-0.034 to 0.596)
Fully-adjusted	**0 (ref)**	−0.18 (-0.782 to 0.422)	0.37 (-0.419 to 1.152)	0.335	0.08 (-0.194 to 0.361)	**0 (ref)**	−0.29 (-0.926 to 0.346)	0.67 (-0.137 to 1.478)	0.086	0.19 (-0.105 to 0.479)

(*continued on next page*)

Table 3 (*continued*)

	Energy-Adjusted dietary intake of PFOS across tertiles and in continuous with 1 SD increment in ng/d β coefficient (95 % CI)									
	LB					**UB**				
	T1	**T2**	**T3**	**P-trend**	**Continuous with 1 SD increment**	**T1**	**T2**	**T3**	**P-trend**	**Continuous with 1 SD increment**
Mean ± SD (ng/d)	17.7 ± 3.43	25.6 ± 1.95	35.1 ± 5.82			1109 ± 246	1741 ± 169	2547 ± 470		
HOMA-IR changes (n = 3,906)										
Model 1	**0 (ref)**	−0.00 (-0.041 to 0.037)	0.03 (-0.009 to 0.065)	0.102	0.01 (-0.008 to 0.023)	**0 (ref)**	−0.55 (-1.217 to 0.119)	0.58 (-0.312 to 1.474)	**0.029**	0.01 (-0.005 to 0.025)
Model 2	**0 (ref)**	0.00 (-0.038 to 0.041)	0.02 (-0.013 to 0.062)	0.182	0.00 (-0.011 to 0.020)	**0 (ref)**	0.02 (-0.014 to 0.060)	0.04 (-0.001 to 0.080)	0.058	0.01 (-0.009 to 0.022)
Fully-adjusted	**0 (ref)**	0.00 (-0.039 to 0.040)	0.03 (-0.007 to 0.069)	0.101	0.01 (-0.007 to 0.024)	**0 (ref)**	0.03 (-0.010 to 0.069)	**0.06 (0.016 to 0.097)**	**0.005**	0.01 (-0.001 to 0.030)
Mean ± SD (ng/d)	17.7 ± 3.43	25.6 ± 1.95	35.1 ± 5.78			1116 ± 247	1748 ± 171	2552 ± 468		
BMI changes (kg/m^2) * (n = 4,093)										
Model 1	**0 (ref)**	0.01 (-0.106 to 0.136)	**0.13 (0.008 to 0.249)**	**0.033**	**0.07 (0.026 to 0.123)**	**0 (ref)**	−0.02 (-0.146 to 0.098)	0.08 (-0.035 to 0.205)	0.877	0.06 (0.010 to 0.111)
Model 2	**0 (ref)**	0.03 (-0.089 to 0.152)	**0.14 (0.021 to 0.262)**	**0.019**	**0.08 (0.030 to 0.127)**	**0 (ref)**	0.00 (-0.119 to 0.124)	0.10 (-0.019 to 0.220)	0.802	**0.06 (0.015 to 0.115)**
Fully-adjusted	**0 (ref)**	−0.05 (-0.162 to 0.061)	0.09 (-0.029 to 0.202)	0.117	**0.06 (0.014 to 0.106)**	**0 (ref)**	−0.08 (-0.193 to 0.033)	0.06 (-0.057 to 0.174)	0.230	0.05 (-0.001 to 0.094)

Abbreviations: PFOS: Perfluorooctanesulfonic acid; CI: confidence interval; HbA1c: hemoglobin A1c; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; LB: lower bound of energy-Adjusted dietary intake of PFOS; UB: upper bound of energy-Adjusted dietary intake of PFOS; BMI: body mass index; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance.
Linear regression models were fitted to assess the β-coefficients and its 95 % CI for glycemic index parameters across energy-adjusted total dietary PFOS intake and with 1 SD increment in ng/d. Model 1 adjusted for age and sex. Model 2: model 1 additionally adjusted for physical activity (MET min/week), marital status (single, married, widowed), smoking status (current, former, never), education level (up to primary, secondary, university) and the size of the recruitment center (<250, 250 to < 300, 300 to < 400, ≥400 and BMI (kg/m^2). Model 3: model 2 additionally adjusted for adherence to Mediterranean diet MEDAS score (0–17 points), family history of cardiovascular diseases, intervention group, each outcome at baseline and oral diabetes medications. All analyses were conducted with robust estimates of the variance to correct for intra-cluster correlation.

vegetables is due to the widespread contamination of soil and surface water which reach and can be stored in these food items (Bizkarguenaga et al., 2016; Domingo et al., 2012). Nevertheless, it is important to note that the significant contributors identified in this study may vary in other cohorts due to differences in lifestyle and dietary patterns.

Our results are in line with previous published papers, which assessed these chemicals in biological samples (serum and plasma). In Chinese adults an increase in serum PFOS was recently correlated with an increase in fasting plasma glucose, fasting plasma insulin and HOMA-IR (Zeeshan et al., 2021). In individuals at high risk of diabetes, cross-sectional positive associations were reported between plasma concentration of PFOS and different glucose-related measurements such as fasting glucose and insulin, HbA1C and HOMA-IR (Cardenas et al., 2017). Finally, a study conducted in adolescents and adults showed that PFOS concentrations levels increased HOMA-IR and beta-cell function (Lin et al., 2009).

The mechanisms explaining these associations are largely unknown. Limited studies suggest a potential effect of these EDs on pancreatic cells. For example, Qin et al., 2022 using *in vivo* and *in vitro* models found that PFOS exposure may disrupt calcium homeostasis and signaling within beta cells leading to an inability to secrete insulin in response to increased blood glucose levels (Qin et al., 2022). Therefore, based on the evidence of these seminal results more prospective and mechanistic studies to assess the specific effect of these chemicals on human health are needed.

As well as in other studies, in the current study an association between DI of PFOS and BMI was also found after 1-year of follow-up. In young adults, a positive association between serum PFOS and overweight was reported in individuals aged between 9 and 21 years old (Domazet et al., 2016). Furthermore, in a study involving 1612 Chinese adults, a positive association between blood PFOS concentration and a higher rate of overweight in women (Tian et al., 2019) was reported. Nelson et al., 2010 also found in men aged 60–80 years a positive association between PFOS exposure and BMI (Nelson et al., 2010).

The most updated tolerable weekly intake (TWI) of PFAS established by the EFSA in 2020 is 4.4 ng/kg of body weight/week (EFSA, 2020). In our study, the mean LB dietary exposure of the cohort was 2.17 ng/kg body weight/week, being this mean below the reference value. This result is in concordance with other Spanish and European populations such as from France, Belgium and Italy (Rovira et al., 2019; Cornelis et al., 2012; Yamada et al., 2014; Klenow et al., 2013). Even though PFOS is the largest contributor to the total dietary PFAS exposure (Barbo et al., 2023; Olsen et al., 2007; EFSA, 2020), our dietary exposure results could be slightly underestimated because other PFAS were not taken into consideration to perform the current the dietary exposure. Nevertheless, even with this current estimation obtained here, we saw a significant positive association between baseline higher DI of PFOS and fasting plasma glucose, HbA1c levels, HOMA-IR and BMI after 1-year of follow-up. Considering our results, the TWI of these perfluoralkyl compounds may have to be re-evaluated by the EFSA to get a more updated approach than the one from 2020.

Our study has several limitations that need to be mentioned. These findings cannot be applied to other subjects because the participants included in this analysis were senior individuals from the Mediterranean region who had overweight/obesity and metabolic syndrome. It's important to acknowledge that the use of a FFQ to evaluate food intake has certain limitations due to the possibility of measurement errors and it was not specifically designed and validated to assess to dietary exposure to PFOS. Nonetheless, despite this drawback, FFQs have been extensively employed as an epidemiological tool since the 1990 s (Shim et al., 2014). Furthermore, while PFOS constitutes the primary contributor to overall dietary PFAS exposure, it is worth noting that our assessment of dietary intake might be slightly underestimated. This is attributed to the fact that the current dietary intake assessment did not include other PFAS compounds in its calculations. In addition, it is important to note that this study exclusively examined exposure to PFOS through dietary sources and the concentrations were taken from available data and did not take into account other exposures such as inhalation or dermal contact or biological samples. However, it is worth mentioning that as before commented the major source of exposure to these chemicals is primarily through the diet and water.

To conclude, this is the first study to our knowledge that explores the association between DI of PFOS and glucose homeostasis. We found that in an adult Mediterranean population at high cardiovascular risk, high baseline DI of PFOS was associated with a higher levels of fasting plasma glucose and HbA1c and with an increase in HOMA-IR and BMI after 1-year of follow-up. These findings support the growing body of evidence linking the potential effect of PFOS intake on glucose homeostasis and insulin regulation. However, recognizing the broader PFAS categories and all different congeners is crucial for comprehensive evaluations of the overall impact of PFAS on human health, for this reason, the association remains complex and further large prospective studies using different population are necessary to warrant our results.

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CRediT authorship contribution statement

Nadine Khoury: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **María Ángeles Martínez:** Writing – review & editing, Methodology, Formal analysis, Data curation, Conceptualization. **Stephanie K. Nishi:** Conceptualization. **Miguel Ángel Martínez-González:** Funding acquisition, Conceptualization. **Dolores Corella:** Conceptualization. **Olga Castañer:** Writing – review & editing, Conceptualization. **J. Alfredo Martínez:** Data

curation. **Ángel M. Alonso-Gómez:** Data curation. **Julia Wärnberg:** Data curation. **Jesús Vioque:** Data curation. **Dora Romaguera:** Data curation. **José López-Miranda:** Data curation. **Ramon Estruch:** Data curation. **Francisco J Tinahones:** Data curation. **José Manuel Santos-Lozano:** Data curation. **Lluís Serra-Majem:** Data curation. **Aurora Bueno-Caunivanillas:** Data curation. **Josep A. Tur:** Writing – review & editing. **Sergio Cinza Sanjurjo:** Writing – review & editing. **Xavier Pintó:** Writing – review & editing. **José Juan Gaforio:** Data curation. **Pilar Matía-Martín:** Data curation. **Josep Vidal:** Writing – review & editing. **Clotilde Vázquez:** Conceptualization. **Lidia Daimiel:** Data curation. **Emilio Ros:** Data curation. **Carmen Sayon-Orea:** Writing – review & editing. **Jose V Sorli:** Data curation. **Karla-Alejandra Pérez-Vega:** Writing – review & editing. **Antonio Garcia-Rios:** Writing – review & editing. **Francisco Ortiz-Díaz:** Data curation. **Enrique Gómez-Gracia:** Data curation. **MA Zulet:** Writing – review & editing. **Alice Chaplin:** Writing – review & editing. **Rosa Casas:** Writing – review & editing. **Inmaculada Salcedo-Bellido:** Writing – review & editing. **Lucas Tojal-Sierra:** Writing – review & editing. **Maria-Rosa Bernal-Lopez:** Writing – review & editing. **Zenaida Vazquez:** Writing – review & editing. **Eva M. Asensio:** Writing – review & editing. **Albert Goday:** Writing – review & editing. **Patricia J. Peña-Orihuela:** Writing – review & editing. **Antonio J. Signes-Pastor:** Writing – review & editing. **Ana Garcia-Arellano:** Writing – review & editing. **Montse Fitó:** Data curation. **Nancy Babio:** Writing – original draft, Visualization, Methodology, Formal analysis, Conceptualization. **Jordi Salas-Salvadó:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2024.108565.

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Supplementary Material

Dietary intake of Perfluorooctanesulfonic acid (PFOS) and glucose homeostasis parameters in a non-diabetic senior population

Nadine Khoury[1,2,3,a], María Ángeles Martínez[1,2,3,a*], Stephanie K. Nishi[1,2,3], Miguel Ángel Martínez-González[1,4,5] , Dolores Corella[1,6], Olga Castañer[12,7], J. Alfredo Martínez[1,8,9], Ángel M. Alonso-Gómez[1,10], Julia Wärnberg[1,11], Jesús Vioque[12,13], Dora Romaguera[1,14], José López-Miranda[1,15], Ramon Estruch[1,16], Francisco J Tinahones[1,17], José Manuel Santos-Lozano [1,18], Lluís Serra-Majem[1,19], Aurora Bueno-Cavanillas[12,20], Josep A. Tur[1,21], Sergio Cinza Sanjurjo[22,23,24], Xavier Pintó[1,25], José Juan Gaforio[12,26], Pilar Matía-Martín[27], Josep Vidal[28,29], Clotilde Vázquez[1,30], Lidia Daimiel[1,31], Emili Ros[1,32], Carmen Sayon-Orea[1,4], Jose V Sorli[1,6], Karla-Alejandra Pérez-Vega[1,7]; Antonio Garcia-Rios[1,15], Francisco Ortiz-Díaz [33]; Enrique Gómez-Gracia[1,11], MA Zulet[1,8,9], Alice Chaplin[1,14], Rosa Casas[1,16], Inmaculada Salcedo-Bellido[12,20], Lucas Tojal-Sierra[1,10], Maria-Rosa Bernal-Lopez[1,34], Zenaida Vazquez-Ruiz[1,4], Eva M. Asensio[1,6], Albert Goday[1,7], Patricia J. Peña-Orihuela[1,15], Antonio J. Signes-Pastor[12,13], Ana Garcia-Arellano[1,4], Montse Fitó[1,7], Nancy Babio[1,2,3], Jordi Salas-Salvadó[1,2,3*]

1. Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y la Nutrición (CIBEROBN), Institute of Health Carlos III, Madrid, Spain
2. Universitat Rovira i Virgili, Departament de Bioquímica i Biotecnologia, Alimentaciò, Nutrició, Desenvolupament i Salut Mental ANUT-DSM, Reus, Spain
3. Institut D'Investigació Sanitària Pere Virgili (IISPV), Reus, SpainInstitut d'Investigació Sanitària Pere Virgili (IISPV), Reus, Spain
4. University of Navarra, Department of Preventive Medicine and Public Health, IDISNA, Pamplona, Spain
5. Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA, USA
6. Department of Preventive Medicine, University of Valencia, Valencia, Spain
7. IMIM (Hospital del Mar Medical Research Institute), Barcelona, Spain.
8. Department of Nutrition, Food Sciences, and Physiology, Center for Nutrition Research, University of Navarra, Pamplona, Spain
9. Precision Nutrition and Cardiometabolic Health Program. IMDEA Food, CEI UAM + CSIC, Madrid, Spain
10. Bioaraba Health Research Institute, Cardiovascular, Respiratory and Metabolic Area; Osakidetza Basque Health Service, Araba University Hospital; University of the Basque Country UPV/EHU, Vitoria-Gasteiz, Spain.
11. EpiPHAAN research group, School of Health Sciences, University of Málaga - Instituto de Investigación Biomédica en Málaga (IBIMA), Málaga, Spain
12. CIBER de Epidemiología y Salud Pública (CIBERESP), Instituto de Salud Carlos III, Madrid, Spain
13. Instituto de Investigación Sanitaria y Biomédica de Alicante. Universidad Miguel Hernández (ISABIAL-UMH). Alicante, Spain
14. Health Research Institute of the Balearic Islands (IdISBa), Palma de Mallorca, Spain.
15. Department of Internal Medicine, Maimonides Biomedical Research Institute of Cordoba (IMIBIC), Reina Sofia University Hospital, University of Cordoba, Cordoba, Spain
16. Department of Internal Medicine, Hospital Clinic, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Institut de Recerca en Nutrició i Seguretat Alimentària (INSA), University of Barcelona, Barcelona, Spain
17. Virgen de la Victoria Hospital, Department of Endocrinology, Instituto de Investigación Biomédica de Málaga (IBIMA). University of Málaga, Málaga, Spain.

18. Department of Family Medicine, Research Unit, Distrito Sanitario Atención Primaria Sevilla, Sevilla, Spain
19. Research Institute of Biomedical and Health Sciences (IUIBS), University of Las Palmas de Gran Canaria & Centro Hospitalario Universitario Insular Materno Infantil (CHUIMI), Canarian Health Service, Las Palmas de Gran Canaria, Spain.
20. Department of Preventive Medicine and Public Health, University of Granada, Granada, Spain
21. Research Group on Community Nutrition & Oxidative Stress, University of Balearic Islands, Palma de Mallorca, Spain
22. CS Milladoiro, Área Sanitaria de Santiago de Compostela
23. Instituto de Investigación de Santiago de Compostela (IDIS)
24. Centro de Investigación Biomédica en Red-Enfermedades Cardiovasculares (CIBERCV)
25. Lipids and Vascular Risk Unit, Internal Medicine, Hospital Universitario de Bellvitge-IDIBELL, Hospitalet de Llobregat, Barcelona Spain
26. Departamento de Ciencias de la Salud, Instituto Universitario de Investigación en Olivar y Aceites de Oliva, Universidad de Jaén, Jaén, Spain
27. Department of Endocrinology and Nutrition, Instituto de Investigación Sanitaria Hospital Clínico San Carlos (IdISSC), Madrid, Spain
28. CIBER Diabetes y Enfermedades Metabólicas (CIBERDEM), Instituto de Salud Carlos III (ISCIII), Madrid, Spain
29. Department of Endocrinology, Institut d'Investigacions Biomédiques August Pi Sunyer (IDIBAPS), Hospital Clinic, University of Barcelona, Barcelona, Spain.
30. Department of Endocrinology and Nutrition, Hospital Fundación Jimenez Díaz. Instituto de Investigaciones Biomédicas IISFJD. University Autonoma, Madrid, Spain
31. Nutritional Control of the Epigenome Group. Precision Nutrition and Obesity Program. IMDEA Food, CEI UAM + CSIC, Madrid, Spain
32. Lipid Clinic, Department of Endocrinology and Nutrition, Institut d'Investigacions Biomèdiques August Pi Sunyer (IDIBAPS), Hospital Clínic, Barcelona, Spain
33. Centro Salud San Vicente del Raspeig, Alicante, Spain
34. Internal Medicine Clinical Management Unit, Hospital Regional Universitario de Málaga, Instituto de Investigación Biomédica de Málaga (IBIMA-Plataforma BIONAND). Universidad de Málaga

***Correspondence:** María Ángeles Martínez: mariaangeles.martinez@iispv.cat; Jordi Salas-Salvadó: jordi.salas@urv.cat

[a] These authors have contributed equally to this work.

Table S1. Food included in our analysis based on the food frequency questionnaire.

Food groups	*Food items*
Fish and seafood	White fish (g/day) Blue fish: sardines, tuna, bonito, salmon (g/day) Salted fish (cod) (g/day) Oysters, clams, octopus, baby squid, cuttlefish (g/day) Calamari, octopus, cuttlefish (g/day) Prawns (g/day) Canned fish and shellfish without oil (sardines, anchovies, bonito, tuna) (g/day) Canned fish and shellfish with oil (sardines, anchovies, bonito, tuna) (g/day)
Fruits and vegetables	Chard, spinach, cauliflower (g/day) Cabbage (g/day) Lettuce, endives (g/day) Raw tomato (g/day) Carrot, pumpkin (g/day) Green beans (g/day) Onion (g/day) Andalusian gazpacho (g/day) Eggplant, zucchini (g/day) Cucumbers (g/day) Peppers (g/day) Asparagus (g/day) Other vegetables (artichoke, leek, celery) (g/day) Garlic (g/day) Parsley, thyme, bay leaf, oregano, etc. (g/day) Baked potatoes (g/day) Homemade fries (g/day) Mushrooms (g/day) Orange, grapefruit, tangerines (g/day) Orange (g/day) Banana (g/day) Apple or pear (g/day) Strawberries, cherries (g/day) Plums, peach, apricot, nectarine (g/day) Watermelon (g/day) Cantaloupe (g/day) Kiwi (g/day) Grapes (g/day) Fruits in syrup or in its juice (g/day) Dates, dried figs, raisins, prunes (g/day)
Meat and meat products	Pork meat (g/day) Cow or beef meat (g/day) Lamb meat (g/day) Liver meat (g/day) Organ meat (g/day) Ham Serrano (g/day) Cooked ham (g/day) Charcuterie (g/day) Patés- foie-gras (g/day) Hamburger, meatballs (g/day) Bacon (g/day) Chicken or turkey with/without skin (g/day)

	Rabbit or hare (g/day)
Eggs	Chicken eggs (g/day)
Milk and Dairy Products	Whole Milk (g/day) Semi-skimmed milk (g/day) Skimmed milk (g/day) Condensed milk (g/day) Heavy cream (g/day) Milk shakes (g/day) Ice creams (g/day) Yogurt (g/day) Nonfat yogurt (g/day) Petit suisse (g/day) Custard (g/day) Flan, pudding (g/day) Cottage or curd cheese (g/day) White cheese (g/day) Cream cheese (g/day) Other cheeses: cured, semi-cured (Manchego, Bola, Emmental...) (g/day)
Cereals	White bread (g/day) Whole wheat bread (g/day) Breakfast cereals (g/day) Whole grains: muesli, oat flakes, all-bran (g/day) White rice (g/day) Whole grain rice (g/day) Pasta (g/day) Who le grain pasta (g/day)
Legumes	Lentils (g/day) Beans (g/day) Peas (g/day) Chickpeas (g/day)
Oils and Fats	Olive oil (g/day) Virgin olive oil (g/day) Aceite de oliva de orujo (g/day) Corn oil (g/day) Sunflower oil (g/day) Soy oil (g/day) Mix of the above (g/day) Margarine (g/day) Butter (g/day)
Sugar and confectionary	Maria cookies (g/day) Whole grain or fiber crackers (g/day) Chocolate chip cookies (g/day) Homemade cakes and pastries (g/day) Croissant (g/day) Donuts (g/day) Muffin (g/day) Cakes (g/day) Churros (g/day) Chocolates and sweets (g/day) Cocoa powder-soluble cocoa (g/day) Nougat (g/day) Mantecados, marzipan (g/day) Jam (g/day) Sugar (g/day) Honey (g/day)

Nuts	Almonds (g/day) Pistachios (g/day) Walnuts (g/day) Other types of nuts (g/day)
Ready-to-eat	Potato chips (g/day) Pizza (g/day) Croquettes (g/day) Soups and sachet creams (g/day) Mustard (g/day) Mayonnaise (g/day) Fried tomato sauce, ketchup (g/day) Spicy: tabasco, bell pepper, paprika (g/day) Snacks (g/day)
Drinking Water	Tap water (g/day) Bottled water (g/day)
Alcoholic beverages	Rosé wine (g/day) Muscatel wine (g/day) Young red wine (g/day) Old red wine (g/day) White wine (g/day) Catalonian wine (g/day) Beer (g/day) Liqueurs, anise (g/day) Spirits: whiskey, vodka, gin, cognac (g/day)

RESULTS

predimed plus

SPANISH BEVERAGE INTAKE ASSESSMENT QUESTIONNAIRE

Centre | Participant | Visit | Date

Instructions:
Please indicate your answer for your consumption last month.
For each type of beverage consumed, indicate the number of times per day or per week, and with an "X" the moment of the day that you consumed it.
For example, if you drank 2 glasses of wine per week with lunch, mark "lunch" in the "moment of the day" column and put a 2 in "per week" column. If a drink is consumed every day, for example water, indicate how many times "per day" you consumed it. For example: 6 times a day.
Do not take into account the liquids used in the kitchen or in other culinary preparations, such as sauce or homemade dessert.
If you drink coffee with milk, mark it in the category "coffee with milk" and not in the dairy categories.

TYPE OF BEVERAGE		FREQUENCY OF CONSUMPTION										
		TIMES			MOMENT OF THE DAY							
		RARELY OR NEVER	PER WEEK	PER DAY	BEFORE BREAKFAST	BREAKFAST	BETWEEN BREAKFAST AND LUNCH	LUNCH	BTEWEEN LUNCH AND DINNER	DINNER	AFTER DINNER	DURING NIGHT
Tap water	200 cc											
Bottled water (sparkling/ still)	200 cc											
Natural fruit juices	200 cc											
Bottled fruit juices	200 cc											
Natural vegetable juices (*gazpacho*, tomato, etc.)	200 cc											
Bottled vegetable juices (*gazpacho*, tomato, etc.)	200 cc											
Whole milk	200 cc											
Semi-skimmed milk	200 cc											
Skimmed milk	200 cc											
Drinking yogurt	100 cc											
	200 cc											
Milkshakes	200 cc											
Vegetable drinks (soy, oat, almond, etc.)	200 cc											
Soups	200 cc											
Jellies and sorbets	120 cc											
Sugar sweetened beverages	200 cc											
	330 cc											
Artificially sweetened beverages	330 cc											
	200 cc											
Espresso (sweetened)	30-50 cc											
Espresso (unsweetened or sugar-free)	30-50 cc											
White coffee (sweetened)	125 cc											
White coffee (unsweetened or sugar-free)	125 cc											
Tea (sweetened)	200 cc											
Tea (unsweetened or sugar-free)	200 cc											
Other infusions (sweetened)	200 cc											
Other infusions (unsweetened or sugar-free)	200 cc											
Beer	200 cc											
	330 cc											
Non-alcoholic beer	330 cc											
	200 cc											
Wine, champagne	120 cc											
High alcoholic content beverages (whisky, rum, vodka, gin)	50 cc											
Mixed alcoholic drinks (cocktails, gin tonic, *piña colada*, etc.)	200 cc											
Energy drinks (Red Bull, Burn, etc.)	200 cc											
Sport/ Isotonic drinks	200 cc											
	330 cc											
Meal replacement shakes	200 cc											
Other drinks:												

Figure S1. Spanish beverage intake assessment used in our analysis.

Table S2. Mean levels (µg /kg) of PFOS in selected food groups according to EFSA.

Food category	PFOS Mean (µg/kg)			
	N	Lower bound	Upper bound	EFSA reference
Vegetables and vegetable products	477	0.003	0.15	2020
Fruit and fruit products	143	0.027	0.25	2020
Livestock meat	461	0.028	0.17	2020
Poultry	169	0.009	0.13	2020
Game mammals (meat)	574	0.94	1.59	2020
Fish and fish offal	2205	0.16	14.21	2020
Milk and dairy products	13	0.001	0.12	2020
Liquid milk	235	0.001	0.14	2020
Eggs and egg products	174	0.27	0.35	2020
Animal and vegetable fats and oils	38	0.004	0.11	2020
Alcoholic beverages	6	0.000	0.002	2020
Drinking water	451	0.0001	0.003	2020
Legumes, nuts and oilseeds	20	0.0001	0.11	2012
Sugar and confectionary	45	0.0035	0.053	2012

N: number of food samples

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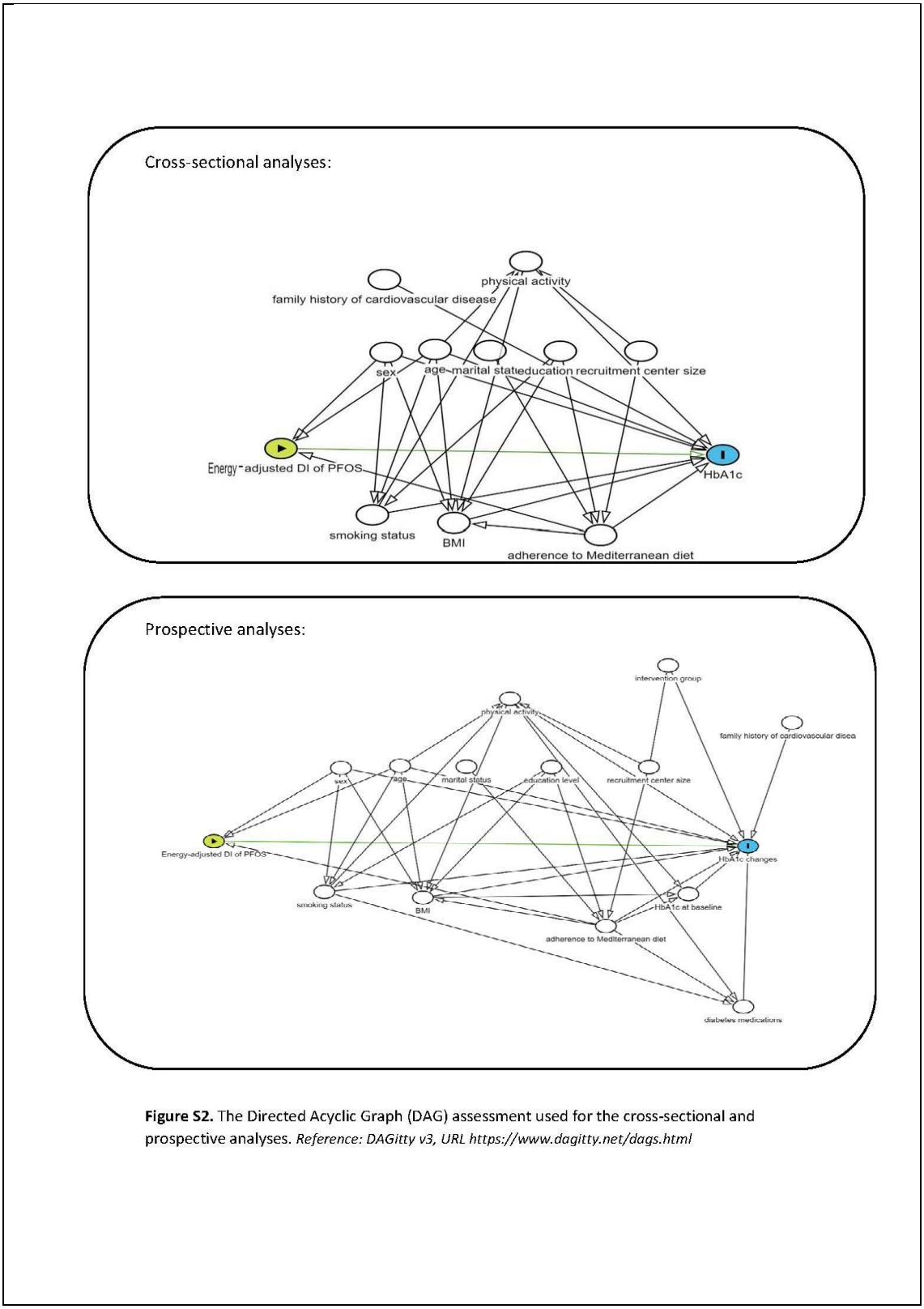


Figure S2. The Directed Acyclic Graph (DAG) assessment used for the cross-sectional and prospective analyses. *Reference: DAGitty v3, URL https://www.dagitty.net/dags.html*

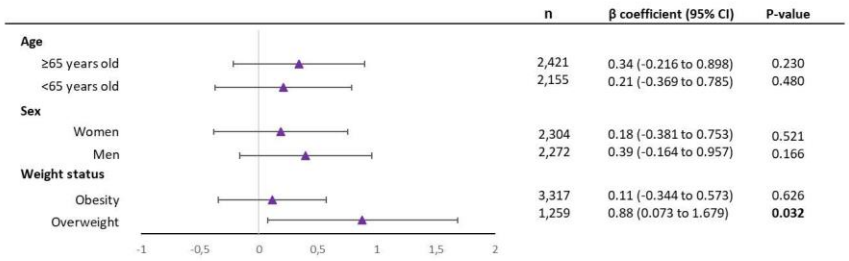


Figure S3.a. Association between 1 SD increment in energy-adjusted PFOS intake and fasting plasma glucose (mg/dL) (EFSA lower bound).

Linear regression models were fitted to assess the β-coefficients and its 95% CI of fasting plasma glucose across baseline energy-adjusted total dietary PFOS intake. Adjusted for age and sex, physical activity (MET min/week), marital status (single, married, widowed), smoking status (current, former, never), education level (up to primary, secondary, university), the size of the recruitment center (<250, 250 to <300, 300 to <400, ≥400 and BMI (kg/m²), adherence to Mediterranean diet MEDAS score (0-17 points) and family history of cardiovascular disease. All analyses were conducted with robust estimates of the variance to correct for intra-cluster correlation. No significant interaction was observed for all cases (P-value >0.05)

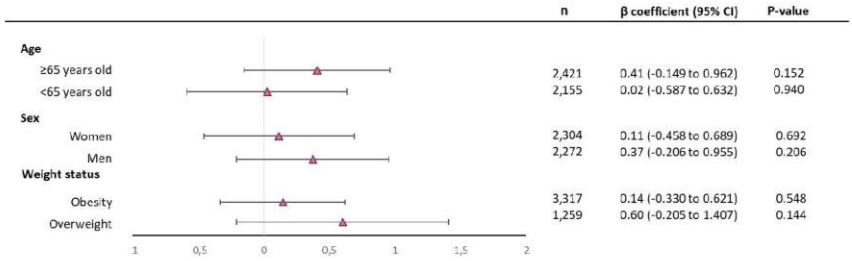


Figure S3.b. Association between 1 SD increment in energy-adjusted PFOS intake and fasting plasma glucose (mg/dL) (EFSA upper bound).

Linear regression models were fitted to assess the β-coefficients and its 95% CI of fasting plasma glucose across baseline energy-adjusted total dietary PFOS intake. Adjusted for age and sex, physical activity (MET min/week), marital status (single, married, widowed), smoking status (current, former, never), education level (up to primary, secondary, university), the size of the recruitment center (<250, 250 to <300, 300 to <400, ≥400 and BMI (kg/m²), adherence to Mediterranean diet MEDAS score (0-17 points) and family history of cardiovascular disease. All analyses were conducted with robust estimates of the variance to correct for intra-cluster correlation. No significant interaction was observed for all cases (P-value >0.05)

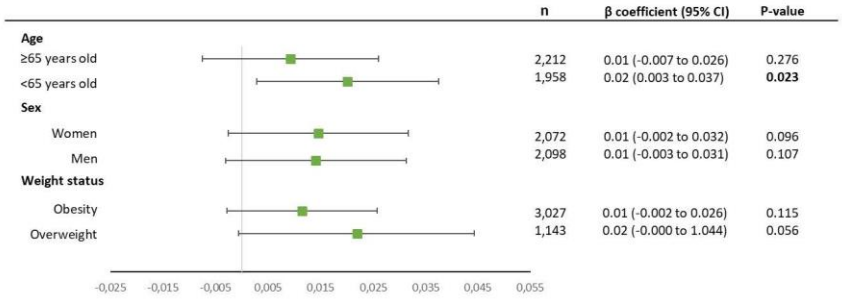


Figure S3.c. Association between 1 SD increment in energy-adjusted PFOS intake and HbA1c levels (%) (EFSA lower bound).

Linear regression models were fitted to assess the β-coefficients and its 95% CI of fasting plasma glucose across baseline energy-adjusted total dietary PFOS intake. Adjusted for age and sex, physical activity (MET min/week), marital status (single, married, widowed), smoking status (current, former, never), education level (up to primary, secondary, university), the size of the recruitment center (<250, 250 to <300, 300 to <400, ≥400 and BMI (kg/m²), adherence to Mediterranean diet MEDAS score (0-17 points) and family history of cardiovascular disease. All analyses were conducted with robust estimates of the variance to correct for intra-cluster correlation. No significant interaction was observed for all cases (P-value >0.05)

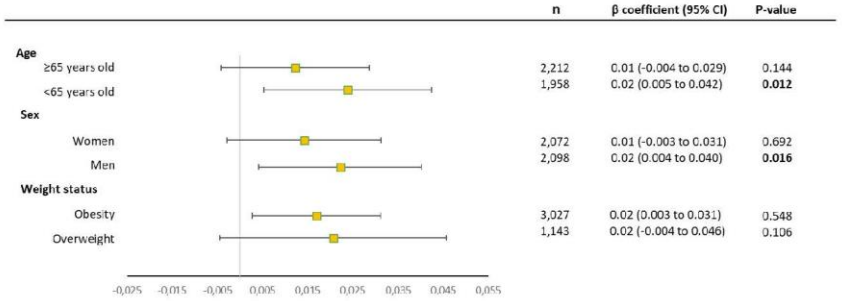


Figure S3.d. Association between 1 SD increment in energy-adjusted PFOS intake and HbA1c levels (%) (EFSA upper bound).

Linear regression models were fitted to assess the β-coefficients and its 95% CI of fasting plasma glucose across baseline energy-adjusted total dietary PFOS intake. Adjusted for age and sex, physical activity (MET min/week), marital status (single, married, widowed), smoking status (current, former, never), education level (up to primary, secondary, university), the size of the recruitment center (<250, 250 to <300, 300 to <400, ≥400 and BMI (kg/m²), adherence to Mediterranean diet MEDAS score (0-17 points) and family history of cardiovascular disease. All analyses were conducted with robust estimates of the variance to correct for intra-cluster correlation. No significant interaction was observed for all cases (P-value >0.05)

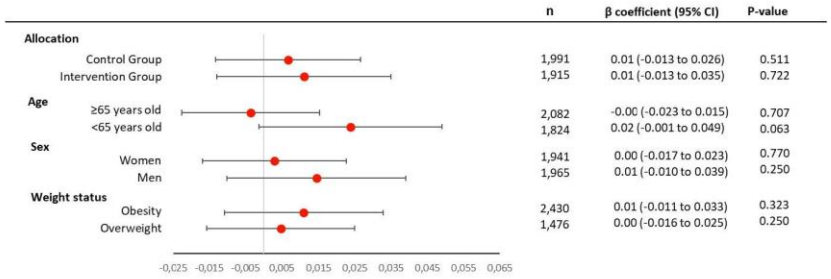


Figure S4.a. Association between baseline 1 SD increment of baseline energy-adjusted PFOS intake and HOMA changes (EFSA lower bound).

Linear regression models were fitted to assess the β-coefficients and its 95% CI for glucose-related parameters across energy-adjusted total dietary PFOS intake. Model 3: adjusted for age and sex, physical activity (MET min/week), marital status (single, married, widowed), smoking status (current, former, never), education level (up to primary, secondary, university), the size of the recruitment center (<250, 250 to <300, 300 to <400, ≥400 and BMI (kg/m²), adherence to Mediterranean diet MEDAS score (0-17 points), family history of cardiovascular disease, intervention group, each outcome at baseline and oral diabetes medications. All analyses were conducted with robust estimates of the variance to correct for intra-cluster correlation. No significant interaction was observed for all cases (P-value >0.05)

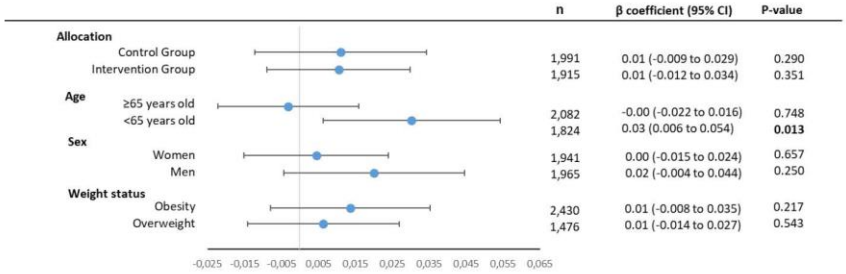


Figure S4.b. Association between 1 SD increment of baseline energy-adjusted PFOS intake and HOMA changes (EFSA Upper bound).

Linear regression models were fitted to assess the β-coefficients and its 95% CI for glucose-related parameters across energy-adjusted total dietary PFOS intake. Model 3: adjusted for age and sex, physical activity (MET min/week), marital status (single, married, widowed), smoking status (current, former, never), education level (up to primary, secondary, university), the size of the recruitment center (<250, 250 to <300, 300 to <400, ≥400 and BMI (kg/m2), adherence to Mediterranean diet MEDAS score (0-17 points), family history of cardiovascular disease, intervention group, each outcome at baseline and oral diabetes medications. All analyses were conducted with robust estimates of the variance to correct for intra-cluster correlation. No significant interaction was observed for all cases (P-value >0.05)

VI. DISCUSSION

UNIVERSITAT ROVIRA I VIRGILI
A MULTI-DIMENSIONAL APPROACH OF FOOD CLASSIFICATIONS AND ITS ASSOCIATION WITH CARDIOMETABOLIC RISK FACTORS
Nadine Alkhoury

1. General discussion

To the best of our knowledge, our results in chapter 1 were the first to evaluate the prospective associations between the nutritional dimension of diet assessed using the FSAm-NPS DI (or 2015 NS-NPM) and several cardiometabolic risk factors. In addition, our second publication in chapter 1 was the first study that showed the discriminatory power of the updated Nutri-Score dietary index (2023 NS-NPM DI) in a population of children and adolescents.

The results from the first publication in chapter 1 showed that the consumption of foods with higher scores of FSAm-NPS (foods with a less favorable rating on the Nutri-Score scale, indicating lower nutritional quality) was associated with unfavorable changes in cardiometabolic risk factors after 1 year of follow-up, specifically with an increase in adiposity (BMI and waist circumference) and an increased levels of fasting plasma glucose, triglycerides, and diastolic blood pressure in elderly Mediterranean individuals at high CVD risk. In line with our study, a recent systematic review and meta-analysis conducted by Barrett et al., 2024[67], one of the objectives assessed by the authors was to examine and compare the associations between diet quality as measured by nutrient profiling systems and diet-related health outcomes. When comparing participants in the highest and lowest quantiles of diet quality as assessed by nutrient profiling systems, they found a reduced risk for most diet-related outcomes examined, including a 25% to 32% lower risk of cardiovascular disease, total cancer, all-cause mortality, and BMI change over time. Moreover, they reported that the associations between high-quality diets, as defined by these nutrient profiling systems, and better health outcomes were relatively substantial and comparable to the benefits assessed with healthy dietary patterns like the Mediterranean diet[67]. In our study, a higher 1-year increase in the FSAm-NPS, reflecting a decrease in the nutritional quality of diet, was also positively associated with increases in adiposity measured by BMI and waist circumference. The results are in line with other studies; in the SU.VI.MAX French cohort, a positive association between the FSAm-NPS DI and body weight and BMI gain[189] was found in both men and women. In addition, an increased risk of obesity was observed only in men after 13 years of follow-up. A study using data from the French NutriNet-Santé

cohort has analyzed the prospective associations between the FSAm-NPS DI and adiposity markers and overweight/obesity risk, the results showed that participants with a higher dietary index (lower diet quality) were more likely to have a significant increase in BMI over time and an increased risk of overweight and obesity[190]. Furthermore, in a prospective study conducted in the context of the SU.VI.MAX cohort, the authors found an association between the FSAm-NPS DI and the risk of metabolic syndrome and its components. Our results are partially in line with the SU.VI.MAX study where the authors assessed the prospective association between the FSAm-NPS DI and metabolic syndrome and its components[191]. Even though these authors found significant and positive association between the FSAm-NPS DI and the incidence of metabolic syndrome, when the components were assessed individually, only systolic and diastolic blood pressure showed a significant association. In our study, we also found a positive association with diastolic, but not for systolic blood pressure. These results may be explained because the FSAm-NPS DI considers the salt content of food that has been proved to be related to hypertension[192]. However, we cannot discard that some other factors of the score, as well as their synergistic effect, may also explain these results on blood pressure. In contrast to the SU.VI.MAX study, we have demonstrated significant prospective associations with triglycerides and fasting glucose. These results in relation to triglycerides and glucose may be explained because the participants in our study were elderly Mediterranean individuals (aged between 55 and 75 years) with overweight/obesity and metabolic syndrome. Therefore, as they were already at the risk of CVD, the results of our study could be influenced by their pre-existing risk and conditions. In contrast, in the SU.VI.MAX study, the participants were younger and healthier (women aged between 35 and 60 years and men aged between 45 and 60 years). In addition, few studies have studied the association between FSAm-NPS DI and elevated glucose levels however, in a recent study conducted by Deng et al., 2024, the researchers aimed to explore the association between dietary patterns assessed with FSAm-NPS and prediabetes in 1,460 participants from the cross-sectional study KORA FF4 and they showed that a diet with a higher FSAm-NPS dietary index was positively associated with prediabetes[193].

It is important to note that the FSAm-NPS focuses on the nutritional dimension and serves as the underlying system of some front-of-pack labels to allow consumers to easily compare foods belonging to the same category. Importantly, FSAm-NPS has the advantage of considering a large number of elements that are associated with cardiometabolic health from a nutritional point of view, in particular the content, per 100 g of food of fruits and vegetables (proxy of the amount of antioxidants, vitamins, and minerals), legumes, nuts, proteins (e.g., proxy of the amount of calcium and iron), olive oil, rapeseed oil, and walnut oils.

Furthermore, the second article of this chapter is one of the first studies assessing the relevance of the 2023 updated Nutri-Score algorithm dietary index on evaluating its discriminatory power and compare it to the 2015 version of the algorithm in French children and adolescents. Our results showed that the nutritional quality of the diet is in generally better for participants in the lowest quartile of NS-NPM DI compared to those in the highest quartile, implying that both Nutri-Score algorithms are able to characterize the quality of children and adolescent's diet. In addition, our results suggest that compared with the previous algorithm, with the updated algorithm higher discriminative differences between quartiles were observed for fish and seafood, poultry, processed meat and vitamin D, all in the expected direction, indicating that this updated version of the algorithm of Nutri-Score represents an improvement to characterize diet quality. Participants with more favorable DI (lower NS-NPM DI) corresponding to participants consuming foods with more favorable rating in the Nutri-Score scale, consumed higher amounts of fruits, vegetables, whole grain cereals, milk, poultry, fish and seafood, vegetable oils, proteins, carbohydrates, added sugars, fibers and vitamins and minerals. Additionally, they consumed lower amounts of, processed meat, sweetened foods, lipids, saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA) and simple sugars.

Previous studies have evaluated the validity and the discriminatory power of the original Nutri-Score and found that overall, the updated FSA NPS demonstrates strong consistency in characterizing individual diets within the French context and different other countries[56,57,194–196]. In a recent Canadian study conducted by Lee et al., with 13,495 adult participants, it was reported that the NS-NPM DI based on 2015 Nutri-Score

algorithm, showed better ability to discriminate diet quality than the Dietary Approach to Stop Hypertension (DASH) dietary index with a moderate agreement with the dietary index scoring system based on Canada's Food Guide[195]. In addition, Paper et al., assessed the cross-sectional association between dietary indexes that underpin 3 front-of-pack labels: the Nutri-Score, the proposed Canadian 'High In' Symbol and the Diabetes Canada Clinical Practice Guidelines in 1,836 adults from the Esteban study and found that the dietary index underpinning the Nutri-Score algorithm discriminated adequately individuals according to the nutritional quality of their diets through food consumption and nutrient intakes [197]. These results reinforce our findings and show the consistency of algorithm to be applied in populations of different life stages. Some of the results observed in our study can be related to the elements considered in the Nutri-Score algorithm and its ability to assess the overall nutritional quality of foods and beverages[198]. The NS-NPM, takes into account various nutrients and food components and assigns scores that reflect the overall nutritional value of a product. Because energy, saturated fat, simple sugars, salt, fibers, and protein are all included in the calculation of Nutri-Score[59], it is anticipated that a discrimination would be observed at the individual level. In addition, while vitamins and minerals are linked to the presence of proteins, fruits, and vegetables (elements considered in the score calculation), they are not explicitly included in the algorithm, which strengthens the discriminatory ability of the NS-NPM to characterize the overall nutritional quality beyond the elements taken directly into consideration in the NS-NPM algorithm.

Furthermore, both DIs showed an adequate ability to discriminate the intake of added sugars. While the two algorithms (2015 and 2023 NS-NMP) only take into account total sugars, the results showed that the classification is consistent and that the main type of sugar discriminated is indeed added sugars not the sugars naturally present in fruit or dairy products, which is of importance for public health purposes for addressing obesity, particularly in children who have a high intake of added/free sugars.

Overall, the distribution of the various food groups within the updated Nutri-Score was aligned with the previous one. The recent update has resulted in improved ratings for certain food groups, specifically fish and seafood, vegetable oils, nuts, poultry and processed meat. These enhancements can be attributed to the adjustment in the

maximum points allocated for protein and fiber content and for nuts and vegetable oils. This adjustment helps offset the higher caloric density of these foods, which are rich in essential micronutrients, such as vitamin D in fish. Additionally, the modifications in the attribution of protein points in the updated algorithm improved the initial classification and ensured a good discrimination between red meat (including processed meat) and poultry. Consequently, while poultry received relatively favorable ratings in both versions of the Nutri-Score algorithm, unprocessed meat classification – except for very lean cuts – was shifted towards less favorable ratings in the updated version. These modifications may explain the higher discrimination in the consumption of poultry and red meat in the 2023 NS-NPM DI compared to the 2015 NS-NPM DI.

The 2023 NS-NPM modified the overall classification for breakfast cereals, with a shift towards less favorable rating. This shift tended to group these products within similar ratings and somewhat reduced the algorithm´s discriminant capacity within this specific food group, which may explain the relatively lower discriminatory capacity of the 2023 NS-NPM DI in this group compared to the 2015 version. Furthermore, in our analysis, when using the 2015 NS-NPM DI, we observed an unexpected direction in bread and breakfast cereals consumption among participants. We found that participants in quartile 4 consumed less bread/breakfast cereals compared to those in quartile 1 (better nutritional quality). Typically, we might expect higher consumption in quartile 4, as those products tend to have lower nutritional quality overall (high sugar content and/or low fiber content) However, it is important to note that while the relative difference in bread consumption between the quartiles was statistically significant for the 2015 NS-NPM, it was no longer the case for the 2023 NS-NPM. Although bread and breakfast cereals consumption trends between the highest and lowest quartiles did not align with initial expectations for the 2015 NS-NPM, the lack of statistical significance in the 2023 NS-NPM DI suggests an enhancement in the reliability and validity of the updated dietary index.

Front-of-pack nutrition labels take into consideration only the nutrients that a food product includes (such as sugar, fiber, and saturated fat) and discriminate the healthfulness of foods and beverages uniquely on the basis of their nutritional composition while excluding other aspects of the diet which are not related to nutrients,

including the degree of food processing [74,88]. However, the extent and purpose of food processing was found to play a role in human health[199] beyond the nutritional composition, through a variety of mechanisms possibly triggered by non-nutritional components, such as cosmetic additives, food contact materials, neo-formed compounds, and the degradation of the food matrix[200]. In the large Moli-sani study cohort in Italy [201], the researchers have examined two food dimensions, assessed with the FSAm-NPS, used to derive the Nutri-Score front-of-pack label, and the NOVA classification for UPF consumption in relation to mortality. The authors found that a significant portion of the higher mortality risk was explained by a high degree of food processing, but not vice versa [201]. Similarly, a study conducted by Gomez-Donoso et al. in the SUN cohort[58], showed that the association between FSAm-NPS DI and all-cause mortality was stronger among participants with high UPF consumption compared to those with lower intake, however no association was found for cardiovascular mortality[58]. Additionally, the study by Bullon-Vela et al. suggested that the combination of nutrient-poor diets and high UPF consumption presents the highest risk of premature mortality however not with cardiovascular mortality[202].

In a recent meta-analysis conducted by Montericcio et al.[68], the authors concluded that the observed weak and nonsignificant associations between the DIs underpinning front-of-pack labels and major health outcomes in their analysis, could be partly explained by an intrinsic limitation common to all nutrient profiling systems: they focus exclusively on nutrient composition while disregarding the nature and purpose of food processing [87,203]. In addition, they stated that front-of-pack nutrition labels should incorporate warnings about the degree of food processing [201,204] and the presence of additives, pesticides, or compounds released from packaging in contact with food, as these could all potentially contribute to cardiometabolic disorders [205,206].

For these reasons, beyond the nutritional dimension of food assessed with NS-NPM DI, it is important to take into consideration other food dimensions when evaluating the impact of diet on cardiometabolic health. In chapter 2, we aimed to investigate the association between UPF consumption and cardiometabolic risk factors in the CORALS cohort. To our knowledge, this study is one of the first to assess the associations between UPF consumption and various cardiometabolic risk factors in young children.

DISCUSSION

In this large cross-sectional study, UPF consumption was positively associated with z scores of BMI, waist circumference, fat mass index, and fasting plasma glucose concentration and inversely associated with HDL cholesterol concentration. Our findings are in line with those of previous studies[207–209]. Consumption of UPFs at age 4 years was associated with increased BMI z scores at age 10 years in the Generation XXI cohort, while no association was found at age 7 years[207]. Another study showed that high UPF consumption in children aged 7 to 13 years was associated with increased BMI growth trajectories[208]. Similarly, lower UPF intake in Spanish children aged 4 to 7 years was associated with lower BMI z scores at age 7 years, though this association became nonsignificant after adjusting for maternal factors[209]. A global study by Neri et al[210] revealed that increased UPF consumption was associated with higher dietary energy density and intake of free sugars, alongside decreased total fiber intake, potentially contributing to childhood obesity[210]. Additionally, findings from the Avon Longitudinal Study of Parents and Children showed that high UPF consumption was associated with unfavorable fat mass index trajectories from age 7 to 24 years[208]. Similarly, in a Brazilian cohort, UPF consumption during preschool years was associated with increases in waist circumference from preschool to school age[211]. Other studies found no significant association between UPF consumption and HDL cholesterol and fasting plasma glucose concentrations[209,212]. Therefore, to our knowledge, our study is the first in children to find significant associations with the aforementioned risk factors and is in line with other studies assessing adult populations[213]. Our results are in line with previous studies showing that the main UPF products consumed are pastries, sweet beverages, cookies, and candies[214–216]. In addition, our results support the findings of other European studies that have shown that children of mothers with lower education or with lower socioeconomic status are more likely to consume UPFs. These findings suggest that educational and socioeconomic factors may contribute to the purchase of low-cost and unhealthy foods, such as UPFs, increasing the risk of health disorders[209,217,218]. Several possible mechanisms could explain our results. First, UPFs contain higher amounts of sodium, energy, fat, and sugar and lower amounts of fiber, which are well recognized as contributors to cardiometabolic risk factors[219]. In addition, some UPFs may be linked to a higher glycemic response, and it has been shown that high consumption of sugar-sweetened beverages may delay the internal satiety signal, leading to excessive calorie

intake and higher glycemic load[220–222]. Moreover, excessive consumption of energy, saturated fat, and sugar contributes to weight gain and a higher risk of obesity, which is an important risk factor in CVD[223]. Furthermore, our study showed that children who consumed high amounts of UPFs tended to have lower intakes of fruits and vegetables, which, along with a healthy dietary pattern, are known to be beneficial for cardiometabolic health[224]. Most of the associations were maintained in our study after further adjusting the models to Mediterranean diet adherence, suggesting that other intrinsic UPF factors may play an important role in determining these associations (eg, additives). Animal and cellular studies revealed potential cardiovascular risks from authorized additives such as sulfites, monosodium glutamate, and emulsifiers[225–230]. Food processing generates contaminants such as acrylamide and acrolein, which have been linked, respectively, to increased odds and risk of cardiovascular disease[99,134]. UPF may contain chemicals such as bisphenols and perfluoroalkyl substances that have been associated with a higher risk of cardiometabolic outcomes in children[205].

Our results provide new insight into the association between UPF consumption and health and the importance of recognizing that early dietary habits in childhood might have future implications on cardiometabolic health. While the magnitude of the associations reported in our study may be considered of limited clinical relevance, it is important to note that our study consisted of young children. Therefore, if such minimal differences can reveal a significant association, they may serve as an early warning of future cardiometabolic conditions.

In Chapter 3, we explored the contamination dimension of food assessed by the dietary intake of endocrine disrupting chemicals and their link to cardiometabolic risk factors in an elderly Mediterranean population with metabolic syndrome. It is well known that there are several risk factors that could contribute to the development of cardiometabolic risk factors, with an unhealthy diet being one of the most significant. Food high in concerning nutrients (such as saturated fat and sugar) and UPFs have been associated with adverse cardiometabolic outcomes[67,71,199,231]. However, in recent years, environmental and anthropogenic contaminants have emerged as potential contributors to the development and progression of these complications, with particular

attention given to the role of EDCs[29,30,151,232]. In the first paper of chapter 3, we aimed to assess cross-sectionally and prospectively the associations between the estimated dietary intake of PCDD/Fs and adiposity or obesity risk in a middle-aged Spanish Mediterranean cohort. We saw in this large cohort of middle-aged subjects at high cardiovascular risk that a higher dietary intake of PCDD/Fs was shown to be cross-sectionally associated with higher measures of adiposity parameters and a higher prevalence of obesity and abdominal obesity. In addition, a higher dietary intake of PCDD/Fs at baseline was positively associated with changes in waist circumference after 1 year of follow-up. No other significant associations were observed for BMI nor for obesity or abdominal obesity incidence, although the direction of these associations was the same as those observed in the cross-sectional analysis. Current results are in line with other epidemiological and mechanistic studies[233,234]. Some studies have also investigated the association between similar chemical substances such as dioxin-like polychlorinated biphenyls (DL-PCBs) and adiposity through the dietary intake[233,234]. For example, in a prospective Spanish study conducted with healthy participants, a positive association between higher dietary intake of DL-PCBs and obesity risk was found[235]. As reviewed by Hassan et al., 2021[236], excessive exposure to PCB in humans was also related to different metabolic disorders including hepatic steatosis, obesity, diabetes mellitus, endocrine metabolism, thyroid metabolism, and dyslipidaemia[236]. It was determined that red meat (28%), fish and seafood (18%) and fruits and vegetables (17%) were the main food groups contributing to the total PCDD/Fs dietary intake in our study. Our results showed a higher dietary intake of PCDD/ Fs in women compared to men ($p < 0.001$), this could be explained because women present a higher consumption of fish, seafood and fruits and vegetables. This is in line with other studies reporting that red meat and seafood were the most contributors to the total intake of PCDD/Fs[233,237,238]. The high dietary intake of PCDD/Fs coming from fruits and vegetables could be explained due to the high consumption of these food items in our study population and not by the high levels of TEQ detected in this food category. According to the literature, it is important to consider the concentration levels of these chemicals and the specific frequency of consumption of each food item from each population profile to estimate the exposure[239,240]. In fact, the major contributors detected here could be different in other cohort study populations depending on their lifestyle and dietary patterns. In our

PREDIMED-Plus study, participants have a high adherence to the Mediterranean diet. Because of that, fruits and vegetables (instead of dairy products) are one of the main contributors to total dioxin exposure along with meat and fish. Our findings are also in line with other studies measuring PCDD/F in blood. Fattore et al., 2006 reported higher serum PCDD/F levels in middle-aged women than in men[234]. Beyond the dietary intake, it is reasonable to expect that women present higher PCDD/Fs serum levels than men since PCDD/Fs are principally stored in adipose tissue, and women tend to have naturally greater percentage of body fat[241]. Moreover, in the cross-sectional analyses of Chang et al., 2016[146], it has been found a positive and significant association between serum PCDD/Fs concentrations and the prevalence of abdominal obesity[146]. They also found that participants with abdominal obesity had significantly higher serum dioxin levels compared to those participants without abdominal obesity[146]. In addition, a study conducted by Lee et al., 2007[242], found a positive relationship between serum concentrations of dioxins and waist circumference and prevalence of metabolic syndrome[242]. Furthermore, Uemura et al., 2009 reported that body levels of dioxins and related compounds, particularly those of DL-PCBs, were cross-sectionally associated with BMI and the risk of metabolic syndrome[148]. Animal studies also suggest that these compounds can have potential effects on adiposity. Hoyeck et al., 2020 demonstrated in animals that low-dose TCDD exposure in female mice impair metabolic adaptability to a high fat diet, indicating that dioxin exposure may be a causative factor of obesity and diabetes pathogenesis in females[243]. Another study also provided new evidence that dioxins, could present obesogenic effects and metabolic disturbances in adult mice[244]. According to the EFSA, the TWI of dioxins and furans in food (established in 2018) is 2.0 pgTEQ/$kg_{body\ weight}$/week[130]. The TWI set by the European Commission's Scientific Committee on Food in 2001 was seven times higher than the current EFSA TWI[130]. It is important to consider that in this current study the 87% of our population exceeded the PCDD/Fs TWI with a mean of 2.99 pg TEQ/$kg_{body\ weight}$/week. The current dietary intake mean is similar to other studies conducted around the world and in accordance, even slightly above, with a previous study carried out in Spain[245] in which the mean dietary exposure also exceeded the EFSA TWI limits. Compared to other countries, the mean level of exposure of our participants is similar to other studies conducted in Netherlands or Ireland[246]. In contrast, studies from Italy[247], France[240] and Ireland[248] reported lower

PCDD/Fs exposure compared to our participants. On the other hand, in countries such as Germany[237], the United Kingdom[249] and Taiwan[250] a higher PCDD/Fs exposure has been detected. It has been observed that the PCDD/Fs dietary exposure in healthy general populations of numerous countries in Europe has been declining in the last years[245,251]. Dioxin emissions have been decreasing as a result of demanding laws governing waste treatment and disposal, which may account for the majority of this decline. According to several studies, food consumption of PCDDs/PCDFs decreased to a greater extent than DL-PCBs[252,253]. The improvements and efforts made in the waste incineration process concerning PCDDs/PCDFs emissions in recent years could be the reason for these decreases[252]. However, the problem is that due to the large discharges produced and the lack of periodic review of the legislation, we still have high levels of dioxins remaining, especially in the trophic chain[251].

In the second paper of chapter 3, we aimed to cross-sectionally and longitudinally assess the associations between the estimated dietary intake of PFOS and glucose homeostasis parameters and BMI in participants without diagnosed diabetes at baseline from the PREDIMED-Plus study cohort. To the best of our knowledge, this is the first study to explore the association between dietary intake of PFOS and glucose homeostasis. We found that in an adult Mediterranean population at high cardiovascular risk, high baseline dietary intake of PFOS was associated with a higher levels of fasting plasma glucose and HbA1c and with an increase in HOMA-IR and BMI after 1- year of follow-up. In our study, we found that fish and seafood, followed by eggs, and fruits and vegetables were the major food contributors to the total dietary intake of PFOS. These results are in line with other published articles[254–256]. A recent study conducted in Greece found that eggs, fish and seafood seem to be responsible for about 50 % of PFOS dietary exposure[139]. In addition, EFSA published a scientific opinion in 2020 stating that fish and seafood, fruits and vegetables contribute the most to the total PFOS dietary intake[140] for all ages. The high abundance of PFOS in animal-origin foods could be explained due the lipophilic properties of this chemical, which can lead to their accumulation and persistence in the food chain, being animal and animal-based products, especially seafood and eggs, the main sources to total PFOS dietary intake exposure. In addition, its presence in fruits and vegetables is due to the widespread contamination of soil and

surface water which reach and can be stored in these food items[257,258]. Nevertheless, it is important to note that the significant contributors identified in this study may vary in other cohorts due to differences in lifestyle and dietary patterns. Our results are in line with previous published papers, which assessed these chemicals in biological samples (serum and plasma). In Chinese adults an increase in serum PFOS was recently correlated with an increase in fasting plasma glucose, fasting plasma insulin and HOMA-IR[259]. In individuals at high risk of diabetes, cross-sectional positive associations were reported between plasma concentration of PFOS and different glucose-related measurements such as fasting glucose and insulin, HbA1C and HOMA-IR[260]. Finally, a study conducted in adolescents and adults showed that PFOS concentrations levels increased HOMA-IR and beta-cell function[261]. The mechanisms explaining these associations are largely unknown. Limited studies suggest a potential effect of these EDCs on pancreatic cells. For example, Qin et al., 2022 using in vivo and in vitro models found that PFOS exposure may disrupt calcium homeostasis and signaling within beta cells leading to an inability to secrete insulin in response to increased blood glucose levels[262]. Therefore, based on the evidence of these seminal results more prospective and mechanistic studies to assess the specific effect of these chemicals on human health are needed. As well as in other studies, in the current study an association between dietary intake of PFOS and BMI was also found after 1-year of follow-up. In young adults, a positive association between serum PFOS and overweight was reported in individuals aged between 9 and 21 years old[263]. Furthermore, in a study involving 1612 Chinese adults, a positive association between blood PFOS concentration and a higher rate of overweight in women[264] was reported. Nelson et al., 2010 also found in men aged 60–80 years a positive association between PFOS exposure and BMI[265]. The most updated TWI of PFAS established by the EFSA in 2020 is 4.4 ng/$kg_{body\ weight}$/week[140]. In our study, the mean LB dietary exposure of the cohort was 2.17 ng/$kg_{body\ weight}$/week, being this mean below the reference value. This result is in concordance with other Spanish and European populations such as from France, Belgium and Italy[123,255,266,267]. Even though PFOS is the largest contributor to the total dietary PFAS exposure[140,268], our dietary exposure results could be slightly underestimated because other PFAS were not taken into consideration to perform the current the dietary exposure. Nevertheless, even with this current estimation obtained here, we saw a significant positive association between

baseline higher DI of PFOS and fasting plasma glucose, HbA1c levels, HOMA-IR and BMI after 1-year of follow-up. Considering our results, the TWI of these perfluoralkyl compounds may have to be re-evaluated by the EFSA to get a more updated approach than the one from 2020.

2. Strengths and limitations

Several limitations in this doctoral thesis are derived from the conducted studies and deserve to be highlighted as follows:

Generalization to other population

- For the publications conducted within the framework of PREDIMED-Plus cohort, the results cannot be generalized to other populations since participants included in the analysis were elderly Mediterranean individuals with overweight/obesity and metabolic syndrome. Therefore, as they were already at the risk of CVD, the results of this study could be influenced by this condition.
- For the publication conducted within the framework of Esteban study, the generalization of the present findings must be approached with caution, as the study participants were mainly healthy children and adolescents from mainland France.
- For the publication conducted within the framework of CORALS cohort, the study involved preschool children from Spain, which means that the generalization of our findings to other populations may not be appropriate.

Potential dietary intake bias

- For the publications conducted within the framework of PREDIMED-Plus cohort The assessment of food intake through a FFQ is prone to possible measurement errors. However, despite this limitation, food-based FFQs have been widely used as a tool in epidemiological studies since the 1990s[269].
- For the publication conducted within the framework of Esteban study, the use of 24-hour records may bias the estimation of the intake of nutrient intakes, which in turn is crucial for the correct estimation of the NS-NPM DI. However,

we employed high-quality dietary data, incorporating repeated 24-hour records obtained through a validated web-based tool[270]. This approach ensures precise estimates of individual intakes.

- For the publication conducted within the framework of CORALS cohort, some grade of misclassification may be present in our study because UPF consumption was estimated from a food and beverage frequency questionnaire that was not specifically developed to assess this type of food, which could lead to either an overestimation or underestimation of consumption within various NOVA categories. In addition, the use of a F&B-FQ may be influenced by social desirability bias which, could also result in inaccurate estimates.

Potential confounding bias

- We cannot exclude the possibility that that associations may be due to residual confounding or that undetected cardiometabolic disorders may be present in our study population in all the publications included in this doctoral thesis.

Potential dietary intake estimation bias

Estimation of dietary intake of PCDD/Fs and PFOS

The present doctoral thesis only assessed exposure to PCDDs/PCDFs through dietary sources, but not from other sources such as inhalation or dermal contact. However, the exposure to these chemicals occurs largely (90%) through the diet. Nowadays, estimation of PCDD/F from food sources is feasible. However, more detailed information in the literature on different foods, including their origin, production methods, content and packaging, would be helpful for more accurate estimations. Additionally, it was challenging to compare current results with other articles based on dietary exposure estimations, due to the limited literature data available on that topic. Moreover, we lacked information about the food preparation or cooking techniques that could change PCDD/Fs levels in the finished food product

In addition, the present doctoral thesis also assessed exposure to PFOSs through dietary sources. While PFOS constitutes the primary contributor to overall dietary PFAS

exposure, it is worth noting that our assessment of dietary intake might be slightly underestimated. This is attributed to the fact that the current dietary intake assessment did not include other PFAS compounds in its calculations. In addition, it is important to note that this study exclusively examined exposure to PFOS through dietary sources and the concentrations were taken from available data and did not take into account other exposures such as inhalation or dermal contact or biological samples. However, it is worth mentioning that the major source of exposure to these chemicals is primarily through the diet and water.

Synergetic effects of different EDCs

In the present thesis, we have not investigated potential synergistic interactions of different EDCs such as the combined assessment of PCDD/Fs and PFOS on the cardiometabolic risk factors studied, including their dual impact and potential interactive mechanisms.

This doctoral thesis also has some strengths that need to be highlighted as follows:

- The prospective design, which reduces the possibility of reverse causation bias.
- The large sample size, and the comprehensive data collected by trained staff.
- Control for several potential confounding factors.
- Translating the nutrient profiles from the food level to individual dietary indices allowed us to account for the complexity of the overall diet. This approach allows a more comprehensive assessment of dietary quality, taking into account the interactive or synergistic effects that individual dietary nutrients may exert within the context of the food matrix[271].
- In this dissertation, we evaluated cardiometabolic risk factors that have not considered in other similar studies[104,272–274].

VII. CONCLUSIONS

UNIVERSITAT ROVIRA I VIRGILI
A MULTI-DIMENSIONAL APPROACH OF FOOD CLASSIFICATIONS AND ITS ASSOCIATION WITH CARDIOMETABOLIC RISK FACTORS
Nadine Alkhoury

CONCLUSIONS

Hypothesis:

(1) The consumption of food with higher FSAm-NPS dietary index (lower nutritional quality) is associated with cardiometabolic risk factors in adults.

The results of this prospective cohort study conducted in a population with overweight/obesity and metabolic syndrome suggest that the consumption of foods with higher FSAm-NPS (reflecting a lower nutritional quality) is associated with an increase in some cardiometabolic risk factors (adiposity parameters, fasting plasma glucose, triglycerides, and diastolic blood pressure). However, no significant associations were identified for critical cardiometabolic risk factors such as HDL-cholesterol, LDL-cholesterol, and systolic blood pressure.

(2) The updated 2023 NS-NPM discriminate food consumption and nutrient intake better than the 2015 NS-NPM in a population of French children and adolescents.

Overall, the distribution of the various food groups within the updated Nutri-Score was aligned with the previous one. The recent update has resulted in improved ratings for certain food groups, specifically fish and seafood, vegetable oils, nuts, poultry and processed meat.

(3) Higher consumption of ultra-processed food is associated with worse cardiometabolic risk factors in children.

The results of this cross-sectional study of 1426 children suggests that higher consumption of UPFs was positively associated with body mass index, waist circumference, fat mass index, and fasting plasma glucose and negatively associated with high-density lipoprotein cholesterol concentrations.

Theoretical substitution of 100 g of UPFs with 100 g of unprocessed or minimally processed foods was associated with lower z scores of BMI, fat mass index and fasting plasma glucose.

(4) Higher dietary intake of endocrine disruptors (PCDD/Fs and PFOS) is associated with cardiometabolic risk factors (adiposity, obesity status and glucose homeostasis parameters).

The results suggest that in an aged population at high cardiovascular risk, the dietary intake of PCDD/Fs was associated with a higher prevalence of obesity and higher adiposity parameters at baseline. In addition, a higher baseline dietary intake of PCDD/Fs was positively associated with changes in waist circumference after 1 year of follow-up. Furthermore, dietary intake of PFOS was associated with glucose homeostasis parameters. We found that high baseline DI of PFOS was associated with a higher levels of fasting plasma glucose and HbA1c and with an increase in HOMA-IR and BMI after 1-year of follow-up.

Overall, the main conclusions and consequences of the present doctoral thesis are:

The associations between each of the three food classifications dimensions and cardiometabolic risk factors are significant, indicating that the three approaches should be considered independently and importantly when assessing the impact of food on cardiometabolic risk factors. These findings support the suitability of the dietary index underlying the Nutri-Score algorithm as a nutritional quality indicator aimed at improving diets and preventing the development of cardiometabolic risk factors. In addition, our results highlight the need for public health initiatives to promote the replacement of UPFs with unprocessed or minimally processed foods for the prevention of cardiometabolic risk factors in children. Furthermore, our findings support the growing epidemiological evidence linking the dietary intake of PCDD/Fs and PFOS with cardiometabolic risk factors (adiposity parameters, glucose homeostasis and insulin regulation). Even though the production of PCDD/Fs and PFOS has stopped in most countries, PCDD/Fs and PFOS seems to continue to pose a serious health risk since the dietary intake of our population and of other countries are still above the levels recommended by the EFSA. It is therefore very important to assess the unintentionally exposed population and to keep establishing awareness and prophylactic measures to reduce these levels. In addition, we need to recognize the broader PFAS categories and all different congeners for comprehensive evaluations of the overall impact of PFAS on

human health. Finally, it is important to note that these associations remain complex and further large prospective studies using different populations and longer periods of follow-up are needed to validate our results.

VIII. FUTURE PERSPECTIVE

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FUTURE PERSPECTIVES

This doctoral thesis contributes new evidence to the growing literature on the relationship between diet and cardiometabolic risk factors. The results offer new insights into the connections between each of the three food dimension classifications and cardiometabolic risk factors, highlighting the need to consider each dimension independently and with equal importance.

Nevertheless, future studies should address the limitations of this thesis, to provide clearer and more comprehensive understanding of the associations between the nutritional quality, processing levels, and contamination of foods and their impact on cardiometabolic risk factors:

- Prospective studies with longer period of follow-up are required in both general and specific populations, as well as in different geographical regions, to validate our findings. This approach would facilitate the comparison of results across various studies and aid in communicating the outcomes to the general population in a clear and comprehensible way.
- To address the limitation of the FFQ used to assess UPF consumption in this thesis, future studies should consider utilizing more objective and precise dietary assessment methods, such as 24-hour dietary recalls, food diaries or biomarkers of nutrition (such as metabolomics or other omics methodologies) which can provide a more accurate reflection of participants' UPF consumption.
- Future studies are needed to include all PFAS compounds in the dietary intake assessments to provide a more accurate estimation of overall dietary intake. Additionally, other exposure routes, such as inhalation and dermal contact, should be considered to validate the associations shown for the dietary intake of PFOS and PCDD/Fs.
- One of the aims in this doctoral thesis was to evaluate the relationship between dietary intake of PFOS and parameters of glucose homeostasis and body mass index. We prioritized a more feasible method for our large sample size, focusing on dietary intake due to its validation and essential role in our study. However, the lack of assessing the concentration of PFOS in biological samples should be considered in the future. Biomonitoring for such a large sample size, aside from

being invasive for participants, can be a costly and expensive process. It could be valuable to select a subsample of our population with high dietary exposure to PFOS for biomonitoring purposes. This approach would assist in future analyses by exploring the correlation between dietary intake of PFOS and its concentration levels in biological samples. This could enhance the understanding of the relationship between PFOS exposure and cardiometabolic risk factors, providing a more comprehensive picture of the health impact of PFOS. Additionally, we will aim to evaluate the exposure to mixtures of different EDCs including a biomonitoring for both PFAS and PCDD/Fs in a subsample. This approach acknowledges the complex reality of human exposure to EDCs, where individuals are simultaneously exposed to various EDCs in everyday environments. By studying these mixtures by conducting a biomonitoring, we could uncover the synergistic impact of EDCs on cardiometabolic health outcomes.

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X. Appendixes

UNIVERSITAT ROVIRA I VIRGILI
A MULTI-DIMENSIONAL APPROACH OF FOOD CLASSIFICATIONS AND ITS ASSOCIATION WITH CARDIOMETABOLIC RISK FACTORS
Nadine Alkhoury

APPENDIXES

1. Publications performed and published during the thesis

Khoury N, Martínez MÁ, Garcidueñas-Fimbres TE, Pastor-Villaescusa B, Leis R, de Las Heras-Delgado S, Miguel-Berges ML, Navas-Carretero S, Portoles O, Pérez-Vega KA, Jurado-Castro JM, Vázquez-Cobela R, Mimbrero G, Andía Horno R, Martínez JA, Flores-Rojas K, Picáns-Leis R, Luque V, Moreno LA, Castro-Collado C, Gil-Campos M, Salas-Salvadó J, Babio N. Ultraprocessed Food Consumption and Cardiometabolic Risk Factors in Children. JAMA Netw Open. 2024 May 1;7(5):e2411852. doi: 10.1001/jamanetworkopen.2024.11852. PMID: 38758555. Journal: JAMA Network Open. First Decile, Quartile 1. Impact Factor: 10.5

Khoury N, Ángeles Martínez M, Nishi SK, Ángel Martínez-González M, Corella D, Castañer O, Alfredo Martínez J, Alonso-Gómez ÁM, Wärnberg J, Vioque J, Romaguera D, López-Miranda J, Estruch R, Tinahones FJ, Manuel Santos-Lozano J, Serra-Majem L, Bueno-Cavanillas A, Tur JA, Cinza Sanjurjo S, Pintó X, Juan Gaforio J, Matía-Martín P, Vidal J, Vázquez C, Daimiel L, Ros E, Sayon-Orea C, V Sorli J, Pérez-Vega KA, Garcia-Rios A, Ortiz-Díaz F, Gómez-Gracia E, Zulet MA, Chaplin A, Casas R, Salcedo-Bellido I, Tojal-Sierra L, Bernal-Lopez MR, Vazquez-Ruiz Z, Asensio EM, Goday A, Peña-Orihuela PJ, Signes-Pastor AJ, Garcia-Arellano A, Fitó M, Babio N, Salas-Salvadó J. Dietary intake of Perfluorooctanesulfonic acid (PFOS) and glucose homeostasis parameters in a non-diabetic senior population. Environ Int. 2024 Apr;186:108565. doi: 10.1016/j.envint.2024.108565. Epub 2024 Mar 15. PMID: 38574403.

Journal: Environment International. First Decile, Quartile 1. Impact Factor: 10.3

Khoury N, Martínez MÁ, Paz-Graniel I, Martínez-González MÁ, Corella D, Castañer O, Martínez JA, Alonso-Gómez ÁM, Wärnberg J, Vioque J, Romaguera D, López-Miranda J, Estruch R, Tinahones FJ, Lapetra J, Serra-Majem JL, Bueno-Cavanillas A, Tur JA, Sanjurjo SC, Pintó X, Gaforio JJ, Matía-Martín P, Vidal J, Vázquez C, Daimiel L, Ros E, Sayon-Orea C, Sorlí JV, Pérez-Vega KA, Garcia-Rios A, Bellvert NG, Gómez-Gracia E, Zulet MA, Chaplin A, Casas R, Salcedo-Bellido I, Tojal-Sierra L, Bernal-Lopez MR, Vázquez-Ruiz Z, Asensio EM, Goday A, Peña-Orihuela PJ, Signes-Pastor AJ, Garcia-Arellano A, Fitó M, Babio N, Salas-Salvadó J. Dietary intake of polychlorinated dibenzo-p-dioxins and furans, adiposity and obesity status. Environ Res. 2023 Jun 15;227:115697. doi: 10.1016/j.envres.2023.115697. Epub 2023 Mar 25. PMID: 36972775.

Journal: Environmental Research. First Decile, Quartile 1. Impact Factor: 7.7

Khoury N, Gómez-Donoso C, Martínez MÁ, Martínez-González MÁ, Corella D, Fitó M, Martínez JA, Alonso-Gómez ÁM, Wärnberg J, Vioque J, Romaguera D, León-Acuña A, Tinahones FJ, Santos-Lozano JM, Serra-Majem L, Massó Guijarro P, Tur JA, Martín Sánchez V, Pintó X, Delgado-Rodríguez M, Matía-Martín P, Vidal J, Vázquez C, Daimiel L, Ros E, Bes-Rastrollo M, Barragan R, Castañer O, Torres-Peña JD, Notario-Barandiaran L, Muñoz-Bravo C, Abete I, Prohens L, Cano-Ibáñez N, Tojal Sierra L, Fernández-García JC, Sayon-Orea C, Pascual M, Sorli JV, Zomeño D, Peña-Orihuela PJ, Signes-Pastor AJ, Basterra-Gortari FJ, Schröeder H, Salas Salvadó J, Babio N. Associations Between the Modified Food Standard Agency Nutrient Profiling System Dietary Index and

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Journal: Frontiers in Nutrition. Quartile 2. Impact Factor: 5.0

Nishi SK, **Khoury N**, Valle Hita C, Zurbau A, Salas-Salvadó J, Babio N. Vegetable and Fruit Intake Variety and Cardiovascular Health and Mortality: A Systematic Review and Meta-Analysis of Observational Studies. Nutrients. 2023 Nov 24;15(23):4913. doi: 10.3390/nu15234913. PMID: 38068771; PMCID: PMC10707746.

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De las Heras-Delgado S; Shyam S, **Khoury N**, Salas-Salvadó J, Babio N. Nutrient Profiles of Spanish Beverages: A Comparative Evaluation using Original and Updated Nutri-Score Algorithm. *Under Review*

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Nishi S. K, Paz Graniel I, Ni J, Valle Hita C, **Khoury N**, Garcia Gavilán JF, Babio N, Salas-Salvadó J. Effect of Tree Nuts and Peanut Consumption on Blood Lipids: An updated Systematic Review and Meta-analysis of Randomized Controlled Trials. *Under Review*

Valle-Hita C, Babio N, Serra-Majem L, Vioque J, Zomeño MD, Corella D, Pintó X, Cano-Ibañez N, Tur JA, Cuadrado E, Martínez J.A, Díaz-López A, Torres-Collado L, Goday A, Fernández-Carrión R, Nissenshon M, Riera-Mestre A, Garrido-Garrido E, Bouzas C, Abete I, Daimiel L, Cornejo-Pareja I, Vázquez-Ruiz Z, **Khoury N**, Pérez-Vega KA, Salas-Salvadó J. Long-
term association between water intake and kidney function in a population at high car diovascular risk. *Under review.*

2. Oral and poster communications as presenting author

Oral presentation:

41th International Symposium on Diabetes and Nutrition, organized by Diabetes and Nutrition Study Group (DNSG), Sweden

Khoury N, et al., “Dietary intake of Perfluorooctanesulfonic acid (PFOS) and glucose homeostasis parameters in a non-diabetic senior population” Environ Int. 2024 Apr;186:108565. doi: 10.1016/j.envint.2024.108565. June 2024

PHD DAY, Institut d'Investigació Sanitària Pere Virgili (IISPV), Reus

Khoury N, et al., “Dietary intake of Perfluorooctanesulfonic acid (PFOS) and glucose homeostasis parameters in a non-diabetic senior population” Environ Int. 2024 Apr;186:108565. doi: 10.1016/j.envint.2024.108565. June 2024

CyTA Junior 2024, Barcelona

Khoury N, et al., "Dietary intake of Perfluorooctanesulfonic acid (PFOS) and glucose homeostasis parameters in a non-diabetic senior population" Environ Int. 2024 Apr;186:108565. doi: 10.1016/j.envint.2024.108565. April 2024

PHD DAY, Institut d'Investigació Sanitària Pere Virgili (IISPV), Reus

Khoury N, et al., "Dietary intake of polychlorinated dibenzo-p-dioxins and furans, adiposity and obesity status." Environmental research vol. 227 (2023): 115697. doi:10.1016/j.envres.2023.115697. June 2023

40th International Symposium on Diabetes and Nutrition, organized by Diabetes and Nutrition Study Group (DNSG), Croatia

Khoury N, et al., "Dietary intake of polychlorinated dibenzo-p-dioxins and furans, adiposity and obesity status." Environmental research vol. 227 (2023): 115697. doi:10.1016/j.envres.2023.115697. June 2023

XIII Symposium Ciber Fisiopatología de la obesidad y nutrición, Barcelona

Khoury N, et al., "Dietary intake of polychlorinated dibenzo-p-dioxins and furans, adiposity and obesity status." Environmental research vol. 227 (2023): 115697. doi:10.1016/j.envres.2023.115697. November 2022

Poster presentation:

XIII Symposium Ciber Fisiopatología de la obesidad y nutrición, Barcelona

Khoury N, et al., "Dietary intake of polychlorinated dibenzo-p-dioxins and furans, adiposity and obesity status." Environmental research vol. 227 (2023): 115697. doi:10.1016/j.envres.2023.115697. November 2022

Workshops:

Fourth edition of the European Researchers' Night, Tarragona. September 2023

3. Research Stay

Length: 3 months (November 2023 – February 2024)

Institution: Nutritional Epidemiology Research Group (EREN) at Sorbonne Paris Nord University (Bobigny, France).

Supervisor: Prof. Chantal Julia

Objective: To investigate the ability of the 2015 and 2023 Nutri-Score Nutrient Profiling Model to discriminate food consumption and nutrient intake in a population of French children and adolescents.

4. Grants and prizes

Agencia de Gestión de Ayudas Universitarias y de Investigación (AGAUR)

Grant for Pre-Doctoral Fellowship

AURORA traineeship

Grant for a 3-month research stay in Nutritional Epidemiology Research Group (EREN) at Sorbonne Paris Nord University (Bobigny, France).

PHD DAY, Institut d'Investigació Sanitària Pere Virgili (IISPV), Reus

Khoury N, et al., "Dietary intake of polychlorinated dibenzo-p-dioxins and furans, adiposity and obesity status." Environmental research vol. 227 (2023): 115697. doi:10.1016/j.envres.2023.115697. June 2023

Prize: Best flash presentation