REPLACING RED MEAT AND PROCESSED RED MEAT FOR WHITE MEAT, FISH, LEGUMES OR EGGS IS ASSOCIATED WITH LOWER RISK OF INCIDENCE OF METABOLIC SYNDROME

Nerea Becerra-Tomás,[1, 2] Nancy Babio,[1, 2] Miguel Ángel Martínez-González,[2,3] Dolores Corella,[2,4] Ramon Estruch,[2,5] Emilio Ros,[2,6] Montserrat Fitó,[2,7] Lluís Serra-Majem,[2,8] Itziar Salaverria,[9] Rosa M. Lamuela-Raventós,[2,10] José Lapetra,[2,11] Enrique Gómez-Gracia,[12] Miguel Fiol,[2,13] Estefanía Toledo,[2,3] José V. Sorlí,[2,4] Maria Roser Pedret-Llaberia[14] and Jordi Salas-Salvadó[1,2]

[1]Human Nutrition Unit, Faculty of Medicine and Health Sciences, Biochemistry & Biotechnology Department, UniversitatRoviraiVirgili, and Hospital Universitari de Sant Joan de Reus, IISPV, Reus, Spain.

[2]Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III (ISCIII), Madrid, Spain.

[3]Department of Preventive Medicine and Public Health, University of Navarra, Pamplona, Spain.

[4]Department of Preventive Medicine, University of Valencia, València, Spain.

[5]Department of Internal Medicine, Institutd'InvestigacionsBiomèdiques August Pi Sunyer (IDIBAPS), Hospital Clínic, University of Barcelona, Barcelona, Spain.

[6]Lipid Clinic, Endocrinology and Nutrition Service, IDIBAPS, Hospital Clinic, University of Barcelona, Barcelona, Spain.

[7]Cardiovascular Risk and Nutrition Research Group, Institut Hospital del Mar d'InvestigacionsMèdiques (IMIM), Barcelona Biomedical Research Park, Barcelona, Spain.

[8]Research Institute of Biomedical and Health Sciences, University of Las Palmas de Gran Canaria, Las Palmas, Spain.

[9]Department of Cardiology, University Hospital Araba, Vitoria, Spain

[10]Nutrition and Food Science Department. School of Pharmacy, INSA, University of Barcelona, XARTA Barcelona, Spain

[11]Department of Family Medicine, Research Unit, Distrito SanitarioAtenciónPrimariaSevilla, Sevilla, Spain

[12]Department of Preventive Medicine, University of Malaga, Malaga, Spain

[13]Institute of Health Sciences, University of Balearic Islands and Son Espases Hospital, Palma de Mallorca, Spain

[14]Primary Care Division, Catalan Institute of Health, Institutd'InvestigacióenAtencióPrimària Jordi Gol, Tarragona-Reus

Corresponding authors/Request for reprints:

Jordi Salas-Salvadó, MD, PhD, and Nancy Babio, BSc, PhD. Human Nutrition Unit, Faculty of Medicine and Health Sciences, UniversitatRoviraiVirgili. C/SantLlorenç 21, 43201 Reus (Spain). Telephone number: +34 977759312; Fax number: +34 977759322; e-mail address: jordi.salas@urv.catand nancy.babio@urv.cat

Footnotes:

Abbreviations used: CVD: cardiovascular disease; ICC: intra-class correlation coefficient; FFQ: food frequency questionnaire; HR: hazards ratios; MedDiet: Mediterranean diet; MetS: metabolic syndrome; PRM: processed red meat; RM: red meat; RM&PRM: red meat and processed red meat; SFAs: saturated fatty acids

ABSTRACT

Background & Aims: Few studies have assessed the association between consumption of red meat (RM) and processed red meats (PRM) and the incidence of metabolic syndrome (MetS) and results have been inconsistent. We investigated associations between total consumption of meat and its subtypes and incident MetS and estimated the effect of substituting RM or PRM for alternative protein-rich foods.

Methods: We analyzed 1868 participants (55-80 years-old) recruited into the PREDIMED study who had no MetS at baseline and were followed for a median of 3.2 years. MetS was defined using updated harmonized criteria. Anthropometric variables, dietary habits, and blood biochemistry were determined at baseline and yearly thereafter. Multivariable-adjusted hazard ratios (HRs) of MetS were estimated for the two upper tertiles (versus the lowest one) of mean consumption of meat and its subtypes during the follow-up as exposure.

Results: Comparing the highest vs the lowest tertile of consumption, we observed an increased risk of MetS incidence, with HRs of 1.23 (95% confidence interval [CI]: 1.03-1.45) and 1.46 (CI: 1.22-1.74) for total meat and pooled RM and PRM, respectively. Compared with participants in the lowest tertile, those in the highest tertile of poultry and rabbit consumption had a lower risk of MetS incidence. The risk of MetS was lower when one-serving/day of RM or PRM was replaced by legumes, poultry and rabbit, fish or eggs.

Conclusion: RM and PRM consumption was associated with higher risk of MetS. Replacing RM or PRM with other protein-rich foods related to a lower risk of MetS and should, therefore, be encouraged.

This trial was registered at controlled-trials.com as ISRCTN35739639.

Key words: Total meat, red meat, processed red meat, metabolic syndrome, PREDIMED-study

INTRODUCTION

Metabolic syndrome (MetS) is a cluster of metabolic disorders associated with abdominal obesity that is associated with an increased risk of cardiovascular disease (CVD) and diabetes[1]. It has been suggested that adherence to the Mediterranean diet (MedDiet) and a healthy lifestyle are cornerstones in the prevention and treatment of MetS[2].On the other hand, a Western dietary pattern, characterized by a high consumption of red meat, processed meat, butter and margarine and refined grain has been associated with an increased prevalence and incidence[3]of MetS.

Some studies have reported a positive association between meat consumption– mainly red meat and processed meat–and hypertension[4], abdominal obesity[5], and type 2 diabetes[6,7], all of which are MetS components. Cross-sectional[8–12] and prospective studies[3,9,13] have examined the association between red meat consumption and MetS, with controversial results. To our knowledge only three prospective studies have analyzed the association between red meat consumption and MetS[3,9,13]. In the Atherosclerosis Risk in Communities study, a direct association was observed between meat consumption (hamburger, hot dogs, processed meats, bacon, meat sandwiches or mixed dishes, meat as a main dish) and MetS incidence in middle-aged women and men[3]. Along the same lines, in a study limited to one of the centers of the PREDIMED trial we found an increased risk of MetS development in those individuals in the highest baseline quartile of red meat and processed red meat consumption compared to those in the first quartile after one year of follow-up[9]. Finally, in a cohort of Japanese ancestry a 4.7-fold increased risk of developing MetS was observed in those individuals in the top tertile of red meat consumption compared to those in the lower tertile, although the relationship was lost after adjustment for saturated fatty acid intake[13]. As far as we know, only two previous studies related exposure to poultry consumption with MetS prevalence[12] or incidence[13] and reported no associations. In the present analysis we provide the results obtained in the full cohort of the PREDIMED study, a nutritional intervention trial for the primary prevention of cardiovascular disease[14] for the associations between total meat and specific types of meat consumption (especially red meat and processed red meat) and the incidence of MetS during the total study follow-up. We also estimated the effects on MetS incidence of replacing red meat and processed red meat with alternative protein-rich foods.

MATERIAL AND METHODS

Study design and participants

This study is a secondary analysis of a previously published randomized clinical trial, the PREDIMED (PREvención con DIeta MEDiterránea, www.predimed.es) study. Briefly, PREDIMED is a randomized, multicentre, parallel-group field trial that was conducted in Spain between October 2003 and December 2010 to assess the effectiveness of the MedDiet on the primary prevention of CVD. The protocol and design have been described elsewhere[14]. The trial was registered at http://www.controlledtrials.com/ISRCTN35739639 and included 7444 men and women (aged 55–80 and 60–80 years, respectively), without previously documented cardiovascular disease. Participants were eligible if they had either type 2 diabetes or at least three of the following cardiovascular risk factors: hypertension (systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or on antihypertensive medication), high plasma LDL-cholesterol (≥ 160 mg/dL), low plasma HDL-cholesterol (< 40mg/dL in men; <50mg/dL in women), overweight or obesity (BMI ≥ 25kg/m^2), current smoking, or a family history of premature coronary heart disease. Participants were randomized to one of three intervention groups: a MedDiet supplemented with 1 liter/week of extra-virgin olive oil, a MedDiet supplemented with 30 g/day of mixed nuts, or a control diet (adviceto follow a low fat-diet).The main results in relation to cardiovascular events have been published[15].

In the present report, data were analyzed considering the PREDIMED study as a observational cohort. We selected participants from all the PREDIMED recruiting centers with biochemical determinations available for at least 2 years of follow-up (n=5081).

Because our main aim was to explore the associations between different types of meat consumption and the risk of MetS development, we excluded participants with MetS at baseline (n=3707). We also excluded participants who had not completed a baseline food frequency questionnaire (FFQ) and those who reported total energy intake values outside the pre-specified limits (500-3500 kcal/d in women and 800-4000 kcal/d in men). Finally, 2094 individuals were available for evaluation. The protocol was approved by the institutional review boards of each recruitment center and all participants provided written informed consent.

Dietary assessment

Dietary intake was evaluated at baseline and yearly during follow-up using a previously validated FFQ[16]. The reproducibility of the FFQ used in the PREDIMED study for food groups, and energy and nutrient intake, explored by the Pearson correlation coefficient (r), ranged from 0.50 to 0.82, and the intra-class correlation coefficient (ICC) ranged from 0.63 to 0.90. The validity indices of the FFQ in relation to the dietary records for food groups, nutrient and energy intake ranged (r) from 0.24 to 0.72, while the ICC ranged from 0.40 to 0.84. The ICC was 0.75 for total meat/meat products, 0.59 for fish or seafood, 0.40 for legumes, and 0.58 for eggs. Information about meat consumption was assessed using 13 items included in the FFQ. Energy and nutrient intake were estimated using Spanish food composition tables[15].

Trained dieticians asked the participants about the frequency with which they consumed red meat, poultry or rabbit, processed meat products, fish, eggs and legumes: 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 times per day or more than six times per day. The responses were transformed to grams per day and then categorized into red meat (RM) including pork, veal, beef and lamb; processed red meat

(PRM) including offal , ham, sausages, pâté, hamburgers and bacon. Red meat and processed red meat were merged into one category (RM&PRM) and poultry and rabbit, into another category, including chicken, turkey and rabbit, while total meat included all of the above categories. All dietary variables at baseline and yearly during the follow-up were adjusted for total energy intake using the residuals method[17].

Ascertainment of Metabolic Syndrome

The primary end point of the PREDIMED trial was a composite of major cardiovascular clinical events (non-fatal myocardial infarction, non-fatal stroke or cardiovascular death). For the present study, we considered MetS incidence and its components to be the outcome. The definition of MetS we used was in accordance with the updated harmonized criteria of the International Diabetes Federation and the American Heart Association/National Heart, Lung, and Blood Institute[1]. Individuals were diagnosed with MetS if they had three or more of the following components: elevated waist circumference for European individuals (≥ 88cm in women and ≥102cm in men), hypertriglyceridemia (>150mg/dl) or drug treatment for elevated triglycerides, low concentrations of HDL-cholesterol (<50mg/dl and <40mg/dL in women and men, respectively) or drug treatment for low HDL-cholesterol, elevated blood pressure (systolic ≥130 mm Hg and/or diastolic ≥ 85 mm Hg) or taking antihypertensive medication; and high fasting plasma glucose (≥100 mg/dl) or drug treatment for hyperglycemia.

Assessment of covariates

At baseline and yearly during follow-up, participants completed a 47-item questionnaire about lifestyle variables, medical history and medication use; a validated Spanish version of the Minnesota Leisure Time Physical Activity Questionnaire[18]; a 14-item validated questionnaire designed to assess adherence to the MedDiet[19]; and a validated semi-quantitative FFQ with 137 items [16].

Trained personnel measured height in centimeters, weight in kilograms, and waist circumference by standard methods and blood pressure in triplicate with a 5-min interval between each measurement by using a validated oscillometer (Omron HEM705CP, Hoofddorp, the Netherlands) BMI was calculated by dividing weight in kilograms by the square of height in meters.

Fasting blood samples were collected from all participants. Total cholesterol, triglycerides and glucose concentrations were measured using standard methods. HDL-cholesterol was determined after precipitation with phosphotungstic acid and magnesium chloride. The laboratory technicians were blinded to the intervention group.

Statistical analyses

To take advantage of the yearly dietary assessments, we averaged the meat consumption from baseline to the end of the follow-up or from baseline to the last follow-up FFQ before the occurrence of MetS (if it ever occurred) as the relevant exposure. Because participants who developed MetS during follow-up might have changed their dietary habits after the diagnosis of MetS, their average consumption was calculated from baseline to the year before MetS diagnosis. Then, participants were categorized into tertiles of average daily consumption of total meat and its different subtypes during follow-up. The baseline characteristics of the study population are expressed as percentages and numbers for categorical variables and mean ± SD or median (IQR) for continuous variables. The Chi-square and one-way ANOVA tests were used to appraise differences in the baseline characteristics according to tertiles of the average energy-adjusted daily consumption of total meat. Multivariable Cox regression models were fitted to assess the hazards ratios (HR) of incident MetS and its components during follow-up for tertiles of total meat, RM, RM & PRM, PRM, and poultry and rabbit. The Cox regression models were adjusted for several potential confounders. Model 1 was adjusted for intervention group, sex, age, leisure time physical activity (METs/min-day), BMI (kg/m^2), smoking (current , former or never) at baseline; model 2 was additionally adjusted for quintiles of daily average consumption (g/d) during follow-up of vegetables, fruit, legumes, cereals, fish, dairy products, biscuits, olive oil , nuts and alcohol(continuous and adding the quadratic term); and model 3 was additionally adjusted for the prevalence of MetS components at baseline: abdominal obesity (yes/no), hypertriglyceridemia (yes/no), low HDL-cholesterol (yes/no), high blood pressure (yes/no), and high fasting plasma glucose (yes/no). The first tertile was used as the reference category in all models. The time variable was calculated as the difference between the date of death or end of follow-up (the date of the last visit or the last recorded clinical event [MetS incidence] of participants who were still alive) and the date of recruitment.

Statistical interaction between tertiles of total meat or its different subtypes and potential confounding variables such as sex, diabetes status and BMI were checked including product terms in the multivariable model. Because no significant interactions were observed with sex, age or BMI, the product terms were removed.

To assess the linear trend, the median value of each tertile of total meat and different subtypes of meat consumption was included in the Cox regression models as a continuous variable. We conducted subsequent multivariable analyses to examine the HRs for MetS of substituting RM and PRM with one portion/day of other protein-rich foods such as fish, poultry and rabbit, legumes and eggs. These dietary variables were included in the same fully adjusted model as continuous variables, and the differences in their β-coefficients, variances and covariance were used to calculate the β-coefficient

±SE for the substitution effect. Thereafter, these parameters were used to estimate the HR and 95% CI. The level of significance for all statistical tests was set at $P < 0.05$ for bilateral contrast. All analyses were performed with the SPSS software (version 22.0).

RESULTS

A total of 1868 individuals free of MetS at baseline and without extreme total energy values in FFQ were included in the final longitudinal analyses after 226 individuals had been excluded because data on some of the MetS components during follow-up were missing. The mean daily consumption of total meat was 124 g, for which RM & PRM were the major contributors (55%).

After a median follow-up of 3.2 years (interquartile range 1.9-5.8), 980 participants without MetS at baseline (53.8% women) developed new-onset MetS. **Table 1** depicts the baseline characteristics of the study subjects by tertiles of average daily consumption of total meat. Participants, in the top tertile were more likely than those in the bottom tertile to have abdominal obesity and use oral antidiabetic agents or insulin; they also consumed less fruit, legumes, dairy products, nuts, and olive oil.

The risk of MetS development across tertiles of total meat consumption and its different subtypes is presented in **Table 2**. Participants in the top tertile of total meat and RM & PRM consumption had a greater risk of incident MetS than those in the bottom tertile, with HRs of 1.23 (95%CI: 1.03-1.45) for total meat and 1.46 (95%CI: 1.22-1.74) for RM & PRM. When RM and PRM were analyzed separately, similar direct associations were observed, with HRs of 1.27 (95%CI: 1.06-1.52) and 1.37 (95%CI; 1.15-1.62), respectively. On the other hand, the consumption of poultry and rabbit was inversely associated with the risk of MetS [HR: 0.83 (95% CI: 0.70-0.99) for the upper tertile compared to the lowest tertile].

Table 3 shows HR and 95% CI of the MetS components for the daily average tertiles of energy-adjusted total meat consumption and its different subtypes. An increased intake of total meat was associated with an increased risk in the incidence of all MetS components, except high blood pressure. Results were similar when RM and PRM were merged and when PRM was analyzed alone.

Individuals in the top tertile of RM consumption showed a 40%, 25% and 36% higher risk of abdominal obesity, hypertriglyceridemia and low HDL-cholesterol, respectively, compared to those in the bottom tertile. Conversely, compared with participants in the bottom tertile of poultry and rabbit consumption, those in the top tertile had a lower risk of all MetS components, except for abdominal obesity.

The risk of MetS was lower when one serving/day of legumes (150 g boiled), poultry and rabbit (150 g), fish (150 g) or eggs (60 g) were substituted for RM (150 g).The corresponding HR and 95%CI were 0.32 (0.09-0.60), 0.34 (0.20-0.66), 0.40 (0.24-0.87), 0.37 (0.19-0.76), respectively. Results were similar when one-serving/day of PRM (150g) was replaced (**Figure1**). The replacement of one serving/day of RM for one serving/day of PRM was non-significantly associated with a lower risk of MetS development [HR: 0.72(95%CI; 0.34-2.92)].

DISCUSSION

To the best of our knowledge, this is the first epidemiologic study that has evaluated the association between total meat and different subtypes of meat and the risk of MetS development in older individuals at high cardiovascular risk. The results showed that a high consumption of total meat (around more than one serving/day), especially RM & PRM, was associated with increased risk of MetS after adjusting for several potential confounders. In contrast, poultry and rabbit consumption was associated with a reduced risk of MetS and all its components except abdominal obesity. The consumption of total meat, RM & PRM and PRM was also associated with components of the MetS such as abdominal obesity, hypertriglyceridemia, low HDL-cholesterol and high fasting glucose. In addition, the substitution of one serving/day of poultry and rabbit, legumes, fish or eggs for one serving/day of RM or PRM was associated with a significant lower risk of developing MetS.

Our results regarding RM, PRM and RM & PRM are in line with most of the previous cross-sectional[8–10,12] and prospective studies[3,9].Although Damião and co-workers showed that individuals with a higher red meat consumption in a Japanese–Brazilian population had an increased risk of developing MetS, this association disappeared after adjustment for saturated fatty acid (SFA) intake[13]. This discrepancy may be due to over-adjustment, because SFA may be mediators of the association rather than confounders.

Contrary to our results, two previous studies found no association between consumption of poultry and the risk of MetS[12,13]. This discrepancy may be due to differences in the meat subtypes included in the poultry category of these studies. Cocate et al., grouped poultry and fish in the same category[12], while Damião et al. did not mention which meats were included in their definition of poultry[13]. In our study, chicken, turkey and rabbit were included in the same category.

Various mechanisms can explain the associations observed between meat consumption and MetS incidence. For instance, red meat is a food group rich in compounds harmful for cardiometabolic risk, such as cholesterol, SFA and heme iron There is compelling evidence suggesting that SFA have a lower thermogenic effect and are more prone to oxidation than unsaturated fatty acids from plant sources[20], and this type of fat has been associated with a higher likelihood of weight gain in animals[21]. Indeed, in a recent meta-analysis[5], consumption of RM and PRM has been associated with higher waist circumference and BMI. Moreover, consumption of SFA from RM, but not from white meat, has also been associated with MetS, which suggests that this nutrient has an important role in the pathogenesis of metabolic disorders[12]. Heme iron from red meat, but not from other food sources, has also been associated with MetS[22]. Iron is potentially harmful because it catalyses cellular reactions and produces reactive oxygen species that increasethe oxidative stress.This has a particular effect on pancreatic beta cells, which can lead to insulin resistance[23].

Processed meat products are treated by salting, curing, or smoking, thus having high sodium content, besides harmful additives such as nitrites and nitrates, aromatic polycyclic hydrocarbons, and heterocyclic amines. Nitrites and nitrates can be converted into nitrosamines that have been associated with an increased risk of diabetes in experimental animal models[24]. Moreover, blood nitrites have been associated with endothelial dysfunction and impaired insulin response in adults[25], thus increasing the risk of MetS development. Finally, excessive sodium intake is clearly related to high blood pressure.

The mechanism by which poultry consumption may decrease MetS risk remains unclear. The substitution of poultry for RM and PRM entails a lower intake of SFA, heme iron, glycotoxins and sodium, which may be involved in the development of MetS through the aforementioned mechanisms. In fact, in observational studies the risk of type 2 diabetes was reduced when one serving of poultry/day was substituted for one serving of total red meat/day[6]. Our results also show that substituting a serving of poultry, fish, legumes or eggs for RM and PRM can protect against MetS development. A recent meta-analysis of prospective studies showed an inverse association between fish consumption and the risk of MetS incidence[26].The mechanisms explaining this inverse association may be the high fish content of n-3 fatty acids , which have anti-inflammatory effects and may help reduce insulin resistance in muscle, improve the plasma lipoprotein profile and endothelial function, and control blood pressure[27]. In epidemiologic studies legume consumption has been associated with a reduced risk of MetS components such as increased waist circumference and high blood pressure[28]. Legumes have a high fiber and magnesium content, which has been associated with a better lipid profile and improved glucose and inflammatory responses[29] that may be responsible in part for these beneficial effects. The inverse association found with MetS when substituting eggs for RM and PRM may be explained in part because eggs are a good source of folate, B vitamins, and carotenoids and promote the absorption of other antioxidants present in vegetables[30]. Robust observational evidence suggests that high egg consumption is not associated with an increased risk of coronary heart disease or stroke, with the probable exception of high consumption levels among diabetic persons[31].

Although our study focuses on the risk of MetS attributable to exposure to a specific food group (meat and processed meat), it should be considered that the effect of the overall dietary pattern is likely to have a considerably greater effect than those of individual food groups or nutrients. For example, there is consistent evidence that some dietary patterns, such us the MedDiet, DASH and Nordic diet, have beneficial effects on MetS[32]. Probably, the joint effect of the whole dietary pattern is larger than the sum of itsr parts. Nevertheless, the associations we found remained significant after adjusting for other food groups within the background diet.

Our study has some limitations. First, the results cannot be generalized to other populations because study subjects are older individuals at high cardiovascular risk. Second, MetS was a secondary outcome of the PREDIMED study, hence the results are exploratory in nature. Third, our study has been conducted in the frame of a nutritional field trial with dietary patterns that might have a differential effect on the incidence of MetS or its components. However, this confounding effect was minimized by adjusting analyses for the intervention group. Fourth, as in any prospective study, there can be unknown or unmeasured confounding factors, such as the amounts of nitrates, nitrites and heterocyclic amines consumed, all of which have been related to the occurrence and progress of MetS and its components. This possibility may have introduced some degree of residual confounding.

Our study also has strengths, such as the relatively long follow-up, the control for a large number of potential confounders, the analysis of different meat subtypes and yearly repeated dietary assessments during follow-up, which allows updating the consumption of the foods under consideration and is rarely undertaken in large observational studies.

In conclusion, the present study suggests that total meat (when consumed to a level of around more than one serving/day), RM and PRM promote MetS development. In contrast, poultry consumption is associated with a lower risk of MetS. The substitution of other protein-rich foods for RM or PRM is also associated with a lower risk of MetS. Therefore, replacing RM and PRM by other healthy foods should be recommended to decrease the risk of MetS in individuals at high cardiovascular risk. Further studies are warranted to confirm these findings and elucidate the possible mechanisms involved.

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Authors' responsibilities: MA.M-G, D.C, R.E, E.R, L.S-M, , J.L, E.G-G, M.F, and J.S-S designed the PREDIMED study; N.B-T, N.B, MA.M-G, D.C, R.E, E.R, M.F, L.S-M, I.S, RM.L-R, J.L, E.G-G, M.F, E.T, JV-S, R.P. and J.S-S conducted the research; N.B-T and N.B analyzed data; N.B-T,N.B, and J.S.-S wrote the manuscript; MA.M-G, D.C, R.E, E.R, L.S-M, M.F, J.L, J.S-S were the coordinators of subject recruitment and follow-up at the outpatient clinics; N.B-T, N.B and J.S-S had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors have read and approved the final manuscript.

Conflict of Interest Statement and Funding sources:

The authors disclose no conflict of interest related with the article.

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Table 1.Baseline characteristics of the study population according to tertiles of energy-adjusted average daily consumption of total meat[a]

	Total meat consumption (g/day)			
	T1	**T2**	**T3**	**P-value[b]**
	≤106.92	**106.94-137.80**	**≥137.82**	
	n=622	**n=623**	**n=623**	
Age, years	67.3 ± 6.0	66.9 ± 6.0	66.5 ± 6.2	0.06
Women, % (n)	54.0 (336)	52.5 (327)	50.9 (317)	0.54
Waist circumference, cm	95.5 ± 9.8	94.00 ± 9.5	95.7 ± 9.9	0.05
Women	93.2 ± 10.5	91.0 ± 10.9	92.8 ± 10.2	0.02
Men	98.1 ± 8.1	97.3 ± 6.1	98.6 ± 8.5	0.11
BMI, kg/m^2	28.4 ± 3.4	28.1 ± 3.5	28.5 ± 3.6	0.15
Leisure time physical activity, METs-min/d	272 ± 270	269 ± 244	282 ± 248	0.66
Former smokers, % (n)	24.8 (154)	25.7 (160)	27.1 (169)	0.63
Current smokers, % (n)	17.4 (108)	14.6 (91)	15.1 (94)	0.36
Blood pressure, mmHg				

Systolic	145.9 ± 20.0	147.3 ± 21.0	146.3 ± 20.5	0.35
Diastolic	81.4 ± 10.9	82.3 ± 10.7	82.3 ± 10.7	0.27
Biochemistry, mg/dL				
Fasting blood glucose	101.2 ± 37.1	99.0 ± 34.5	99.1 ± 34.6	0.01
HDL-cholesterol, median [IRQ]	59.0 [51.0-68.0]	58.7 [51.0-68.0]	57.0 [50.0-66.5]	0.41
Triglycerides, median [IRQ]	97.0 [75.0-120.0]	94.0 [76.0-116.0]	96.0 [73.0-121.0]	0.56
Current medication use, % (n)				
Use of hypoglycemic agents	13.2 (82)	12.7 (79)	17.2 (107)	0.04
Use hypolipidemic agents	46.9 (292)	47.2 (294)	44.1 (275)	0.31
Use of antihypertensive agents	65.3 (406)	66.5 (414)	63.9 (398)	0.59
Insulin treatment	2.3 (14)	4.7 (29)	6.1 (38)	<0.01
Metabolic syndrome components, % (n)				
Abdominal obesity	47.0 (289)	38.1 (237)	46.6 (288)	<0.01
Hypertriglyceridemia	5.6 (35)	5.1 (32)	4.8 (30)	0.81
Low HDL-cholesterol	2.6 (16)	4.2 (26)	2.2 (14)	0.10

High blood pressure	87.8 (545)	86.8 (541)	86.7 (539)	0.82
High fasting plasma glucose	28.6 (177)	31.7 (196)	34.8 (216)	0.07
Intervention group, % (n)				
MedDiet+EVOO	37.1 (231)	34.3 (214)	32.3 (201)	0.32
MedDiet+nuts	33.8 (210)	35.3 (220)	34.0 (212)	
Low-fat control diet	29.1 (181)	30.3 (189)	33.7 (210)	
Energy intake, kcal/day	2358 ± 534	2279 ± 521	2332 ± 538	0.03
Food consumption, g/day[c]				
Vegetables	335 ± 145	330 ± 133	348 ± 151	0.09
Fruits	392 ± 211	388 ± 202	366 ±194	0.05
Eggs	19 ± 11	20 ± 10	21 ± 12	<0.01
Legumes	23 ± 17	21 ± 11	20 ± 10	<0.01
Dairy	421 ± 241	384 ± 216	360 ± 212	<0.01
Fish	100 ± 47	102 ± 43	105 ± 45	0.10
Cereals	232 ± 92	234 ± 82	225 ± 79	0.11

Biscuits	25 ± 30	24 ± 29	21 ± 24	0.07
Nuts	13 ± 16	12 ± 13	11 ± 14	0.01
Olive oil	43 ± 18	42 ± 16	40 ± 16	0.03
Alcohol	10 ± 16	10 ± 13	10 ± 14	0.61

Data are expressed as means (standard deviation) or medians [IRQ, interquartile range] for continuous variables and percentages and numbers (n) for categorical variables.

Abbreviations: T, Tertile; BMI, Body mass index; MedDiet, Mediterranean diet, EVOO, extra-virgin olive oil.

[a]Tertile cut-offs are based on energy-adjusted daily average of total meat intake.

[b]P values for differences between tertiles were calculated by chi-square or ANOVA tests for categorical and continuous variables, respectively.

[c]All dietary variables were adjusted for total energy intake.

Table 2. Hazard ratios (95% confidence intervals) of metabolic syndrome incidence across average energy-adjusted tertiles of total meat, red meat and processed red meat, red meat, processed red meat and poultry and rabbit consumption during the follow-up [a]

	Meat consumption (g/day)			
	T1[a]	**T2**	**T3**	**P-trend**
Total meat, median g/day [b]	87.0	120.6	158.9	
Metabolic syndrome incidence, % (n)	49.2 (306)	42.1 (262)	58.1 (362)	<0.01
Crude model	1.00 ref.	0.82 (0.69-0.97)	1.31 (1.12-1.54)	<0.01
Multivariable model 1	1.00 ref.	0.83 (0.70-0.98)	1.32 (1.12-1.55)	<0.01
Multivariable model 2	1.00 ref.	0.95 (0.80-1.13)	1.29 (1.09-1.53)	0.01
Multivariable model 3	1.00 ref.	0.93 (0.78-1.11)	1.23 (1.03-1.45)	0.02
Red meat and processed red meat, median g/day [c]	38.4	62.9	96.4	
Metabolic syndrome incidence, % (n)	45.5 (283)	44.3 (276)	59.6 (371)	
Crude model	1.00 ref.	0.96 (0.81-1.14)	1.61 (1.37-1.89)	<0.01
Multivariable model 1	1.00 ref.	0.97 (0.82-1.15)	1.67 (1.41-1.97)	<0.01
Multivariable model 2	1.00 ref.	1.03 (0.87-1.23)	1.57 (1.32-1.86)	<0.01
Multivariable model 3	1.00 ref.	0.98 (0.82-1.17)	1.46 (1.22-1.74)	<0.01
Red meat, median g/day [d]	19.5	39.3	67.5	
Metabolic syndrome incidence, % (n)	47.9 (298)	44.1 (275)	57.3 (357)	<0.01
Crude model	1.00 ref.	0.89 (0.75-1.05)	1.38 (1.17-1.63)	<0.01
Multivariable model 1	1.00 ref.	0.89 (0.75-1.05)	1.43 (1.21-1.68)	<0.01
Multivariable model 2	1.00 ref.	0.91 (0.77-1.09)	1.32 (1.10-1.57)	<0.01

Multivariable model 3	1.00 ref.	0.86 (0.72-1.02)	1.27 (1.06-1.52)	<0.01
Processed red meat, median g/day [e]	12.3	22.4	35.3	
Metabolic syndrome incidence, % (n)	46.0 (286)	45.1 (281)	58.3 (363)	<0.01
Crude model	1.00 ref.	0.96 (0.81-1.14)	1.44 (1.22-1.69)	<0.01
Multivariable model 1	1.00 ref.	0.97 (0.82-1.14)	1.46 (1.24-1.72)	<0.01
Multivariable model 2	1.00 ref.	1.06 (0.89-1.26)	1.42 (1.20-1.68)	<0.01
Multivariable model 3	1.00 ref.	1.06 (0.89-1.26)	1.37 (1.15-1.62)	<0.01
Poultry and rabbit, median g/day [f]	28.9	58.6	79.4	
Metabolic syndrome incidence, % (n)	56.4 (351)	43.2 (269)	49.8 (310)	<0.01
Crude model	1.00 ref.	0.67 (0.57-0.79)	0.79 (0.67-0.93)	<0.01
Multivariable model 1	1.00 ref.	0.67 (0.57-0.78)	0.78 (0.66-0.92)	<0.01
Multivariable model 2	1.00 ref.	0.76 (0.64-0.90)	0.85 (0.72-1.01)	0.03
Multivariable model 3	1.00 ref.	0.74 (0.63-0.88)	0.83 (0.70-0.99)	0.02

Abbreviations: T, Tertile.

Multivariable model 1 adjusted for intervention group, sex, age (years), leisure time physical activity (METs-min/day), BMI (kg/m^2), current smoker (yes/no), former smoker (yes/no). Multivariable model 2 additionally adjusted for average consumption quintiles of vegetables (g/d), fruit (g/d), legumes (g/d), cereals (g/d), fish (g/d), dairy products (g/d), alcohol (g/d and quadratic term), biscuits (g/d), olive oil (g/d) and nuts (g/d). Multivariable model 3 additionally adjusted for the prevalence of metabolic syndrome components at baseline: abdominal obesity (yes/no), hypertriglyceridemia (yes/no), low HDL-cholesterol (yes/no), hypertension (yes/no) and high fasting plasma glucose (yes/no). All models were stratified by recruitment centre.

[a]Tertile cut-offs are based on energy-adjusted daily average of total meat, red meat and processed red meat, red meat, processed red meat and poultry and rabbit .[b]Includes all meat products: chicken, turkey, rabbit, pork, beef, veal, lamb, several types of sausages and processed red meat. [c]Includes pork, veal, lamb, several types of sausages and processed red meat. [d]Includes pork, beef, veal and lamb. [e]Includes several types of sausages and processed red meat. [f]Includes chicken, turkey and rabbit.

Table 3. Hazard ratios (95% CI) of metabolic syndrome components (abdominal obesity, hypertriglyceridemia, low HDL-cholesterol, high blood pressure and high fasting plasma glucose) across energy-adjusted tertiles of specific meat consumption[a]

	T1	T2	T3	P- trend
Total Meat[b]				
Abdominal obesity	1.00 ref.	0.87 (0.69-1.09)	1.34 (1.07-1.68)	0.01
Hypertriglyceridemia	1.00 ref.	0.94 (0.80-1.09)	1.21 (1.03-1.41)	0.01
Low HDL-cholesterol	1.00 ref.	0.90 (0.77-1.06)	1.29 (1.10-1.50)	<0.01
High blood pressure	1.00 ref.	0.76 (0.52-1.12)	0.88 (0.59-1.31)	0.64
High fasting plasma glucose	1.00 ref.	0.87 (0.72-1.05)	1.21 (1.00-1.46)	0.04
Red and processed red meat[c]				
Abdominal obesity	1.00 ref.	1.19 (0.96-1.49)	1.73 (1.36-2.18)	<0.01
Hypertriglyceridemia	1.00 ref.	1.02 (0.87-1.19)	1.47 (1.26-1.72)	<0.01
Low HDL-cholesterol	1.00 ref.	1.08 (0.92-1.26)	1.45 (1.24-1.70)	<0.01
High blood pressure	1.00 ref.	0.95 (0.66-1.37)	1.25 (0.84-1.88)	0.28
High fasting plasma glucose	1.00 ref.	0.99 (0.82-1.19)	1.28 (1.05-1.56)	0.01
Red meat[d]				
Abdominal obesity	1.00 ref.	1.07 (0.86-1.33)	1.40 (1.19-1.88)	<0.01
Hypertriglyceridemia	1.00 ref.	0.88 (0.76-1.03)	1.25 (1.08-1.46)	<0.01
Low HDL-cholesterol	1.00 ref.	0.99 (0.86-1.16)	1.36 (1.17-1.59)	<0.01
High blood pressure	1.00 ref.	0.78 (0.55-1.12)	1.05 (0.71-1.54)	0.69
High fasting plasma glucose	1.00 ref.	1.07 (0.89-1.29)	1.18 (0.97-1.43)	0.09
Processed red meat[e]				
Abdominal obesity	1.00 ref.	0.83 (0.66-1.03)	1.50 (1.21-1.86)	<0.01
Hypertriglyceridemia	1.00 ref.	0.89 (0.77-1.04)	1.26 (1.09-1.46)	<0.01
Low HDL-cholesterol	1.00 ref.	0.90 (0.77-1.04)	1.25 (1.08-1.45)	<0.01

High blood pressure	1.00 ref.	0.94 (0.66-1.34)	0.97 (0.66-1.41)	0.88
High fasting plasma glucose	1.00 ref.	0.96 (0.80-1.15)	1.23 (1.02-1.48)	0.02
Poultry and rabbit[f]				
Abdominal obesity	1.00 ref.	0.72 (0.59-0.89)	0.81 (0.65-1.01)	0.03
Hypertriglyceridemia	1.00 ref.	0.69 (0.59-0.80)	0.78 (0.67-0.91)	<0.01
Low HDL-cholesterol	1.00 ref.	0.70 (0.61-0.82)	0.83 (0.71-0.96)	<0.01
High blood pressure	1.00 ref.	0.69(0.48-0.99)	0.68 (0.47-0.97)	0.02
High fasting plasma glucose	1.00 ref.	0.74 (0.62-0.88)	0.83 (0.69-0.99)	0.01

Abbreviations: CI, confidence interval, T, tertile.

[a]Tertile cut-offs are based on energy-adjusted daily average meat intake.

The metabolic syndrome components were defined according to updated harmonizing criteria.

Cox regression models adjusted for intervention group, sex, age (year), leisure time physical activity (METs-min/day), BMI (kg/m^2), current smoker (yes/no), former smoker (yes/no), quintiles of average consumption of vegetables (g/d), fruit (g/d), legumes (g/d), cereals (g/d), fish (g/d) dairy (g/d), biscuits (g/d), olive oil (g/d) and nuts (g/d), and alcohol (g/d) (continuous and quadratic term). All models were stratified by recruitment center.

[b]Includes all meat products: chicken, turkey, rabbit, pork, beef, veal, lamb, several types of sausages and processed red meat.

[c]Includes pork, beef, veal, lamb, several types of sausages and processed red meat.

[d]Includes pork, beef, veal and lamb.

[e]Includes several types of sausages and processed red meat.

[f]Includes chicken, turkey and rabbit.

FIGURE LEGEND

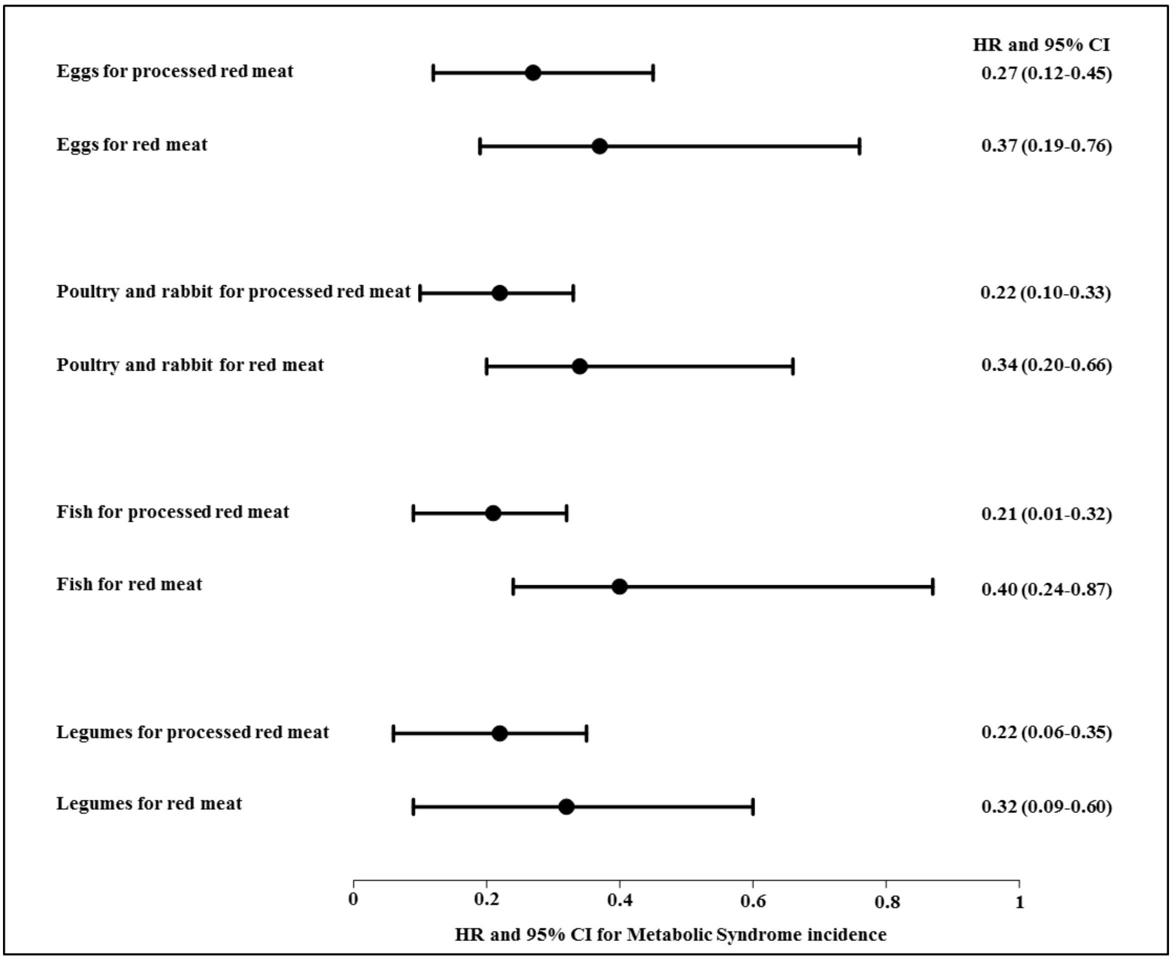

Figure 1. HR and 95% CI of metabolic syndrome for replacing red meat and processed red meat with poultry and rabbit, fish, legumes and eggs. Cox regression model adjusted for age (years), sex, leisure time physical activity (METs min/day), BMI (kg/m^2), current smoker (yes/no), former smoker (yes/no) at baseline, daily average consumption quintiles of vegetables (g/d), fruit (g/d), legumes (g/d) (except when substitution with legumes was analyzed), cereals (g/d), fish (g/d) (except when substitution with fish was analyzed), dairy products (g/d), biscuits (g/d), olive oil (g/d) and nuts (g/d) and alcohol (as continuous variable in g/d and adding the quadratic term), and for the prevalence of metabolic syndrome components at baseline: abdominal obesity (yes/no),

hypertriglyceridemia (yes/no), low HDL-cholesterol (yes/no), hypertension (yes/no) and high fasting plasma glucose (yes/no).