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Total and subtypes of dietary fat intake and risk of type 2 diabetes mellitus in the PREDIMED Study

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ABSTRACT

Background: The associations between dietary fat intake and cardiovascular disease have been evaluated in several studies, but less is known about its influence on the risk of diabetes.

Objective: To examine the associations between total dietary fat and specific types of fat and the incidence of type 2 diabetes mellitus. We also examined the associations between food sources rich in saturated fatty acids and diabetes risk.

Methods: A prospective cohort analysis of 3,349 individuals free of diabetes at baseline but who were at high cardiovascular risk from the PREvención con DIeta MEDiterránea (PREDIMED) study was conducted. Detailed dietary information was assessed at baseline and yearly during the follow-up using a food frequency questionnaire. Hazard ratios (HRs) and 95% confidence intervals(CIs) for type 2 diabetes according to yearly updated fat intake were estimated with the use of multivariable Cox proportional hazards models.

Results: We documented 266 incident cases during 4.3 years of follow-up. Polyunsaturated and monounsaturated fat intake were not significantly associated with the risk of type 2 diabetes. Total fat intake was not associated with higher risk of type 2 diabetes in multivariable model 1, but when the model was additionally adjusted for baseline glucose a significant association was observed. After multivariable adjustment, participants in the highest quartile of saturated fat and animal fat intake had higher risk of diabetes compared to the lowest quartile (HR: 2.19; 95%CI, 1.28, 3.73; P for trend=0.01; 2.00; 95%CI: 1.29, 3.09; *P* trend <0.01, respectively). The intake of 1 serving of butter and cheese was associated with higher diabetes risk while whole-yogurt intake was associated with lower risk.

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Conclusions: Saturated and animal fats were significantly associated with higher risk of type 2 diabetes whereas no significant associations were found for monounsaturated and polyunsaturated fat in a Mediterranean population at high cardiovascular risk.

Keywords: dietary fat, fat subtypes, saturated fat, monounsaturated fat, ω-3 fatty acids, type 2 diabetes, PREDIMED Study.

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BACKGROUND

The global epidemic of type 2 diabetes (T2D) has become a public health challenge in the past few decades. In 2015, 415 (8.8%) million adults worldwide suffered from T2D, and it is estimated that these rates will increase to 642 million (10.4%) in 2040 (1). Importantly, T2D accounted for 14.5% of deaths in 2015 and it has become a serious burden for health systems of many countries (1). Accruing evidence has demonstrated that the combination of several unhealthy lifestyle factors, including a Western-style diet, reduced physical activity, smoking, overweight, and obesity explained nearly 90% of T2D cases (2). Of note, dietary fats, and especially the type of fat consumed, have been in the spotlight of research because of their effects on health. Although the 2015 Dietary Guidelines for Americans encouraged the consumption of vegetable fats and oils and discouraged the consumption of animal fats (3), past research has mainly focused on evaluating the associations between the quality of fats and the risk of cardiovascular disease (4), and less is known about its influence on the risk of T2D.

The existing findings on the associations between T2D and types of fat intake remain inconsistent. Previous observational studies have indicated that total fat intake was not associated with higher incidence of T2D (5–8) but results for specific types of fat have been controversial. For example, the consumption of polyunsaturated fatty acids (PUFA) was associated with lower risk of T2D in the Nurses' Health Study (NHS) (6) but no association was found in the Iowa Women's Health Study (7) despite both studies included middle-aged women and the diet was evaluated using food frequency questionnaires. On the other hand, although saturated fatty acids (SFA) have been related to insulin resistance (9), no significant associations were found between SFA intake and the incidence of T2D in several epidemiologic studies (10). Dietary SFA

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represents a heterogeneous category of fatty acids that can be obtained from different food sources, including dairy products, meats, processed meats, and eggs, among others. Because of the complexity of this fatty acids and the food matrix in which they are present, SFA can have different biological effects on human health (11). Recently, a meta-analysis of randomized controlled trials has shown that consuming more unsaturated fats (MUFA and PUFA) in place of either carbohydrates or SFA may improve glycated hemoglobin A1C (HbA1C) and homeostasis model assessment for insulin resistance (HOMA-IR). PUFA consumption, in particular, showed additional benefits on insulin secretion capacity (12).

Previous data from the PREDIMED study have demonstrated that a dietary pattern high in vegetable fat, mainly nuts and olive oil, decreased the risk of T2D and its complications (13,14), but the associations between dietary fat and subtypes of fat intake on the incidence of T2D have not been evaluated before in Mediterranean individuals at high cardiovascular risk. Therefore, we aimed to investigate the associations between total dietary fat and specific types of dietary fat in relation to the risk of T2D in nondiabetic participants from the PREDIMED study. We also examined the associations between animal food sources rich in SFA and T2D risk.

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METHODS

Study population

The present study is a prospective cohort analysis of individuals free of T2D at baseline in the framework of the PREDIMED Study. The PREDIMED study (registered at http://www.controlled-trials.com as ISRCTN35739639) was a multicenter, parallel-group, randomized clinical trial aimed at evaluating the effects of the Mediterranean Diet (MedDiet) on the primary prevention of cardiovascular disease in individuals at high cardiovascular risk (PREDIMED website: http://www.predimed.es) (15,16). From October 2003 until June 2009, 7,447 participants were recruited. Participants in the PREDIMED Study were men (aged 55–80 years) and women (aged 60–80 years) free of cardiovascular disease at baseline but who were at high risk because they had either T2D or at least three of the following cardiovascular risk factors: current smoking, hypertension, hypercholesterolemia, low high-density lipoprotein cholesterol, overweight/obesity, or family history of premature coronary heart disease. Exclusion criteria were the presence of any severe chronic illness, alcohol or drug abuse, body mass index (BMI) $\geq$40 kg/m^2, and allergy or intolerance to olive oil or nuts (16). For this analysis, we further excluded those participants who had T2D at baseline (n=3,614), individuals who lacked measures of blood glucose control (n=292), without follow-up (n=94), who had implausible daily energy intake (<500 or >3500kcal/d for women and <800 or > 4000kcal/d for men) or who had not completed the baseline Food Frequency Questionnaire (FFQ) (n=98). The final analyses included 3,349 individuals free of T2D at baseline. The institutional review boards of all the recruiting centers approved all procedures. Written informed consent was obtained from all study participants.

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Ascertainment of type 2 diabetes mellitus

The primary endpoint for the present analysis was T2D incidence, diagnosed according to American Diabetes Association criteria (17), namely fasting plasma glucose levels of ≥7.0 mmol/L (≥126.1 mg/dL) or 2-h plasma glucose levels of ≥11.1 mmol/L (≥200.0 mg/dL) after an oral dose of 75 g of glucose or new use of oral/insulin medication. A review of all medical records of participants was completed yearly in each center by physician-investigators who were blinded to the intervention. When new-onset T2D cases were identified on the basis of a medical diagnosis reported in the medical charts or on a glucose test during routine biochemical analyses (done at least once per year), these reports were sent to the PREDIMED Clinical Events Committee, whose members were also blinded to treatment allocation. Only when a second test using the same criteria and repeated within the next 3 months was available, the new T2D case was definitively confirmed by the adjudication committee (13).

Dietary assessment

Dietary intake was measured using a validated semi-quantitative FFQ that trained dietitians completed in a face-to-face interview with the participant at baseline and yearly during the follow-up (18). This questionnaire, which has been validated in a population at high cardiovascular risk from Spain (18), included 137 food items and a 9 level scale incremental frequencies of consumption for each food items (never or almost never; 1–3 times/month; 1, 2–4, and 5–6 times/week; and 1, 2–3, 4–6, and >6 times/day). We used Spanish food composition tables to estimate energy and nutrient intake (19).

Other covariates assessment

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At baseline and yearly during the follow-up, a questionnaire about lifestyle, educational achievement, medical history, and medication use was administered. Physical activity was assessed using the validated Spanish version of the Minnesota Leisure-Time Physical Activity questionnaire (20). Trained personnel took anthropometric and blood pressure measurements. We used calibrated scales and a wall-mounted stadiometer to measure weight and height, respectively, with participants in light clothing and no shoes; we used a validated oscillometer [Omron HEM705CP, Hoofddorp, Netherlands] to measure blood pressure, in triplicate with a 5-minute interval between each measurement and we recorded the mean of these three values. Participants were considered to be hypercholesterolemic or hypertensive if they had previously been diagnosed as such, and/or they were being treated with cholesterol-lowering, or antihypertensive agents, respectively.

Statistical analysis

For each participant, we calculated the follow-up time as the interval between the date of randomization and the date of T2D diagnosis, death from any cause, or the date of the last contact visit, whichever came first. The percentages of energy intake from total fat and specific dietary fats were calculated using yearly updated measurements to better represent the long-term diet. We used data from baseline to the last FFQ before the onset of T2D to categorize participants into quartiles of dietary fat (MUFA, PUFA, SFA, *trans* fat, animal fat, vegetal fat, marine ω-3 fatty acids, non-marine ω-3 fatty acid and ω-6 linoleic acid). Baseline characteristics were presented for the total non-diabetic population of the PREDIMED study and according to extreme quartiles of total dietary fat and subtypes of fat intake as the mean (SD) for quantitative traits and *n*(%) for categorical variables. We have calculated the correlations between MUFA

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and SFA with different food groups as well as fat type-adjusted residuals of SFA and fat type-adjusted residuals of MUFA.

We used multivariable time-dependent Cox proportional hazards models to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) of T2D comparing participants in each quartile with those in the lowest quartile. To assess a linear trend, we assigned the median intake within each quartile and modeled the variable as continuous. In addition to modeling percentage of energy from total and specific fat as quartiles, we also evaluated them as continuous. Multivariable model 1 was adjusted for age, sex, intervention group, BMI (kg/m^2), smoking status (never, former, or current smoker), educational level (primary education, secondary education, or academic/graduate), leisure-time physical activity (metabolic equivalent task minutes/d), baseline hypertension or use of antihypertensive medication (yes/no), yearly updated total energy intake (kcal/d), alcohol intake (g/d), updated quartiles of fiber, protein intake, and dietary cholesterol. Model 2 for specific subtypes of fat also included as covariates updated quartiles of the other subtypes of fat. Model 3 was further adjusted for potential mediators of the associations including hypercholesterolemia or use of lipid-lowering drugs (yes/no) and fasting plasma glucose (mg/dL) at baseline, respectively. All models were stratified by recruitment center. We have also presented the main results for MedDiet group and control group separately. We have evaluated the associations between baseline dietary fat intake and the risk of incident type 2 diabetes as a secondary analysis. To test the robustness of our findings, we conducted sensitivity analysis excluding those participants who developed T2D during the first year of follow-up (n=39).

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We evaluated the effects of specific types of fats by expressing them as a percentage of total energy. When all types of fats, protein, alcohol and total energy, as well as the other covariates, were included simultaneously in the models (models 2 and 3), the coefficient from these models can be interpreted as the estimated differences in risk of substituting a certain percentage of energy from total fat or specific types of fat for carbohydrates.

Finally, we have also investigated the association between the intake of one serving of animal food rich in SFA (processed red meat, red meat, eggs, whole-fat milk, butter, whole-fat yogurt and cheese) and the risk of T2D. The models were adjusted for the non-dietary covariates listed above and intakes of total energy, alcohol, vegetables, fruits, legumes, cereals, fish, meat, dairy, olive oil, nuts and biscuits (g/d) (except if the exposure was included in these food groups).

Data were analyzed using a commercially available software program Stata 12.1 (StataCorp) and statistical significance was set at a 2-tailed P value <0.05.

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Results

During a median follow-up of 4.3 years, we documented 266 incident cases of T2D. At baseline, participants with higher total fat intake had lower blood glucose levels, lower intake of total energy and higher intake of all subtypes of fat. Participants with higher SFA and *trans* fat intake were more likely to smoke, to be less physically active, and consumed less dietary fiber (**Table 1**). Baseline characteristics of the study population according to quartiles of animal and vegetable fat intake, and specific subtypes of PUFA intake are described in **Supplemental table 1**. At baseline, the mean intake of total fat in percentage of energy in the MedDiet groups was 38.33±6.30, and in the control group 37.95±6.56. At year 3, total fat intake in the MedDiet group increased to 40.71±5.49, and in control group decreased to 37.40±6.44. The means and SDs of total fat and subtypes of fat intake at baseline and during the follow-up by intervention group are presented in **Supplemental table 2**. Spearman correlations between MUFA and SFA, as well as type-adjusted residuals for these fats, and food groups are presented in **Supplemental table 3**. The correlation coefficient between MUFA and SFA was 0.40. The respective coefficients for type-adjusted residuals of SFA and cheese, red meat and processed meat were 0.43, 0.36, 0.30, respectively.

No significant associations were found for total fat intake and type 2 diabetes in multivariable models adjusted for cardiovascular risk factors and dietary factors; but when the model was further adjusted for baseline glucose, higher total fat intake was weakly associated with the risk of T2D, although the P for trend was non-significant (P trend = 0.06) (**Table 2**). Higher intake of SFA was associated with higher risk of T2D in all the multivariable models. After adjusting for plasma glucose at baseline, the HR of developing T2D for higher intake of SFA, as compared to

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the lowest quartile, was 2.19 (95% CI: 1.28, 3.73; *P* trend = 0.01). No significant associations were observed for MUFAs, PUFAs or *trans* fat and the risk of T2D. These findings were consistent with the analysis of fat intake as a continuous variable per each 5% increase in energy increase. A 5% energy increment from SFAs intake was associated with 2-fold higher risk of T2D (HR: 2.14; 95% CI: 1.30, 3.52; *P* trend < 0.01) **(Supplemental Table 4)**.

Animal fat intake was strongly associated with a higher risk of T2D (HR: 2.00; 95% CI: 1.29, 3.09; *P* trend < 0.01) after adjusting for baseline fasting plasma glucose (**Table 3**). Although vegetable fat showed a trend towards a higher risk of T2D in model 3 adjusted for baseline plasma glucose (HR: 1.50; 95% CI: 0.99, 2.25; *P* trend = 0.09), no significant associations were found in the models not adjusted for plasma glucose, using a continuous variable and in sensitivity analysis. Per each 5% increase in energy intake from animal fat the risk of T2D increased by 26% (HR: 1.26; 95% CI: 1.04, 1.53; *P* trend = 0.02) (Supplemental Table 4). No significant associations were found between quartiles of marine ω-3 fatty acid, non-marine ω-3 fatty acid, linoleic acid intake and T2D. When the intake of marine ω-3 fatty acid was modeled as a continuous variable, we found an inverse association with T2D incidence (Supplemental Table 4). When separating the analysis for intervention group (**Supplemental Table 5**), no significant associations were found for total fat, MUFA, PUFA, trans fatty acid and n-3, n-6 fatty acids and T2D. Participants in the higher quartile of animal fat intake had higher risk of T2D than its counterparts in the lower quartile in the two MedDiet and control groups.

Figure 1 shows the risk of T2D by the intake of one serving of food animal sources rich in SFA. Increasing the intake of 12g of butter and 30g of cheese intake was associated with higher risk of T2D [HR (95%CI): 2.42 (1.42, 4.13); P <0.01; and 1.32(1.15, 1.52), P <0.01, respectively)

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whereas the intake of whole-fat yogurt was associated with a lower risk (HR: 0.65; 95% CI, 0.45, 0.94; P=0.02). No significant associations between, read meat, processed meat, eggs or whole-fat milk and diabetes were observed.

The associations between baseline SFA and baseline animal fat with T2D risk were not significant [Multivariable model 3 for 4^{th} Q vs. 1^{st} Q of SFA, HR (95% CI): 1.16 (0.67, 1.99); and respectively for animal fat: 1.24 (0.78, 1.98)].

When we conducted sensitivity analysis by excluding those participants who developed T2D during the first year of follow-up (n=39) the results were consistent with those of the primary analysis. SFA and animal fat were consistently associated with higher risk of T2D [Multivariable model 3 for 4^{th} Q vs. 1^{st} Q of SFA, HR (95% CI): 2.46 (1.38, 4.38); *P* trend =0.01; and respectively for animal fat: 1.87 (1.18, 2.97); *P* trend = 0.01)] whereas 5% increase in energy from marine ω-3 fatty acids was associated with lower risk (HR: 0.32; 95% CI: 0.13, 0.77; P value < 0.01).

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DISCUSSION

In this prospective study of participants at high cardiovascular risk, we found that SFA and animal fat intake, but not the intake of others subtypes of fat, were strongly associated with the risk of T2D after controlling for recognized classical potential confounders and for plasma glucose levels at baseline. Butter and cheese intake, food sources rich in SFA, were associated with higher incidence of T2D whereas whole-fat yogurt intake was associated with lower risk. These findings suggest a different role of SFA on the risk of T2D depending on the food matrix in which they are consumed.

Despite the fact that previous studies have been inconsistent in terms of the association between SFA and T2D, we found a strong positive association between SFA and T2D. Participants who had higher SFA consumption, had about 2-fold higher risk of T2D compared to their counterparts with lower intakes of SFA, and per each 5% increase in energy intake from SFA intake the risk of T2D increased substantially. These findings are in agreement with the Food and Agriculture Organization (FAO) of the United Nations Report, which concluded that SFA might be associated with insulin resistance and T2D (21). Findings from the NHS also indicated that SFA intake was associated with 34% higher risk of diabetes in multivariable models adjusted for diet, but the association was weakened after adjustment for BMI (22). In two other prospective studies, incident T2D and conversion to T2D were positively associated with SFA consumption (23,24). On the other hand, null associations between SFA intake and type 2 diabetes have been shown in long-term cohorts and in a recent meta-analysis of observational studies (10). However, some of the studies included in the meta-analysis were small or did not include mutual adjustment for other types of fatty acids. In the Women's Health Initiative, reducing SFA, when

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replaced with carbohydrates, did not reduce the risk of type 2 diabetes after 8.1 years of follow-up (25). A number of reasons may account for this findings including that, compared to other trials, participants were not at higher risk of diabetes at baseline, and that other trials have included physical activity and weight loss as part of the intervention (25). More recently, a meta-analysis of randomized controlled trials has demonstrated that replacing 5% of energy from carbohydrates with SFA had no significant effect on fasting glucose but lowered fasting insulin. Replacing SFA with PUFA significantly lowered glucose, HbA1c, and HOMA (12). Together, it is important to consider the replacement nutrient when assessing the associations between dietary fat intake and chronic diseases. Of note, in our population of elderly Mediterranean individuals at high cardiovascular risk, the intake of refined carbohydrates and added sugars is considerably low compared to other populations, therefore, higher intake of SFA at expenses of lowering the intake of carbohydrates, may explain the observed harmful effects of SFA on type 2 diabetes.

The main contributors of the animal sources of SFA intake in our population were cheese (22.9%), red meat and processed meat (17.6% and 8.0%), followed by eggs and other dairy products (ranging from 1% to 5% of the animal SFA intake). Moderate correlations were observed for type-adjusted residuals of SFA and red meat, processed meat and total cheese; but not for adjusted residuals of MUFA and these food groups. Results from the present study and previous findings in the PREDIMED Study (26) suggest that dairy products, food sources of SFA, are inversely associated with T2D. Nevertheless, the effect differs depending on the dairy product consumed and one of the reasons may be the different type of SFA that these products contain. Individual studies and meta-analysis have shown that higher dairy fat biomarkers were inversely association with the risk of type 2 diabetes (27) but red meats and processed meats were associated with and increased risk (28). We found that butter and cheese consumption was

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associated with higher risk of T2D whereas whole-yogurt intake was associated with a lower risk. Although cheese consumption was inversely associated with the risk of diabetes in some studies (29,30) not all studies agreed (31). Indeed, there is evidence suggesting that in men, cheese intake was associated with a 5% higher T2D risk in a meta-analysis of two prospective studies (31). Because we did not differentiate between the type of cheese consumed and the intake of cheese is often combined with refined carbohydrates this may explain the increased risk of T2D observed in our study, however, clinical trials are needed to confirm these associations. An inverse association between butter and T2D (RR = 0.96, 95% CI = 0.93, 0.99; P = 0.021) has been recently reported in a meta-analysis (32). Butter is a source of animal fat and *trans* fatty acids, and it has been previously observed that substituting butter for olive oil is beneficial for T2D prevention (33). In our population, the intake of olive oil is much higher than butter intake which may have led to the observed results. Although higher risk of T2D with the consumption of red meat and processed meat has been demonstrated in previous studies (28,34), contrary to our hypothesis, we did not find significant associations between processed meat, red meat and T2D in the present analysis, possibly residual confounding may have blunted the potential associations. However, total meat intake and processed meat intake was associated with higher risk of metabolic syndrome and its components (including high fasting glucose) in our previous analysis (35).

We observed a lack of association between total fat intake and the risk of T2D after adjusting for cardiovascular risk factors and dietary factors; but a trend to an increased risk was observed when plasma glucose was included in the model, which may be a potential mediator of the associations. However, non-significant associations were found when separating the analysis by intervention group. Although conflicting results have been found for total fat intake and T2D

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(36), in three previous prospective studies with a follow-up ranging from 6 to 14y, including the NHS (6); the Iowa Womens' Health Study (7); and the Australian Longitudinal Study on Women's Health (8), total dietary fat intake was not significantly associated with the risk of diabetes. In line with our results, in two of these previous studies, MUFA intake was not significantly associated with the risk of T2D incidence (6,7). Total PUFA intake was not associated with incident T2D in our population, but possibly the mutual adjustment of PUFA for other types of fat may have diluted the potential associations. Despite other previous studies found similar findings (8), since we now know that the quality of fat is more important than the quantity of fat consumed, high intake of PUFA and MUFA in place of SFA and *trans* fat should be recommended for chronic disease prevention and may also be beneficial for the risk of T2D (37). Notably, we also found that total animal fat intake was associated with higher risk of T2D. In this sense, our results support the current dietary recommendations that favour plant-based fat diets over animal fats (37), encouraging the intake of healthy vegetable fat, such as olive oil or nuts. We found a non-significant suggestive trend of an increased risk of T2D by higher intake of vegetable fat, this may be explained because besides fruits, vegetables, and nuts, this food group also included other vegetable oils (like coconut and palm oil), margarine and processed pastry which may have driven the positive trend on an increased T2D risk in our population.

Our data suggests that 1% increase in energy intake from marine ω-3 fatty acids was associated with about 50% lower risk of T2D but no significant associations were found when analyzed as quartiles of intake or for other subtypes of PUFAs. Previous data regarding the associations with marine ω-3 fatty acids were inconsistent, and a meta-analysis including 16 prospective cohort studies and more than 25,670 cases of diabetes concluded that consumption of seafood ω-3 fatty acids was not significantly associated with T2D risk (per 250 mg/d, RR=1.04; 95%CI, 0.97,

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1.10) (38). Contrary to our findings, consumption of ω-3 plant sources of fatty acids has been associated with 11% lower risk of T2D per each 0.5 g/d (38). In addition, the results for linoleic acid in the present study are consistent with a meta-analysis of five prospective cohort studies showing no significant associations between the intake of ω-6 fatty acids and diabetes (39).

Finally, no association between *trans* fat and T2D was observed in our population, perhaps because the intake of this type of fat is very low in Spain and especially in elderly Mediterranean population who consumed few amounts of processed food. In agreement with these results, a meta-analysis including six prospective cohort studies found no association between *trans* fat intake and T2D (HR: 1.10; 95%CI: 0.95, 1.27), although the authors reported that the interpretation of these findings is complicated because of the heterogeneity between the included studies (10).

Dietary fats could affect insulin resistance and consequently the risk of diabetes through several mechanisms that are yet not well understood. Dietary fatty acids may play a differential role on diabetes onset through the mediation of cell-membrane fatty acid composition and functions, including membrane fluidity, ion permeability, insulin receptor binding and affinity (40). For instance, a greater saturated fatty acid content of membrane phospholipids increases insulin resistance (40). Moreover, increased serum SFA has recently been shown to be associated with insulin resistance, elevated serum glucose concentration, and tissue inflammation (41). Palmitic acid might activate inflammatory cytokines and pose specific lipotoxicity to pancreatic β cells (42). Similar effects may be true for animal fat but further research is needed to help in the understanding of these mechanisms. On the other hand, MUFAs and PUFAs have been shown to

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have beneficial effects on serum lipids, inflammation, blood pressure, insulin resistance, endothelial function and glycemic control (43–46).

Findings from the present study cannot prove causality and it is difficult to rule out residual confounding. We adjusted for several known risk factors for T2D, including several dietary factors, but measurement errors are inevitable in estimates of food and nutrients. Finally, results from a Mediterranean population at high cardiovascular risk may not be generalizable to more diverse populations. Because most developed countries have had dietary guidelines recommending the reduction of SFA intake for several decades, we acknowledge that it is difficult to disentangle between the health consciousness of the population for reducing SFA intake versus a true effect of SFA. The strengths of our study include the prospective design, the use of repeated measures of diet and lifestyle, and the accurate and blind assessment of incident case of T2D.

CONCLUSIONS

In summary, the present data suggests that SFAs and animal fat intake were strongly associated with higher risk of T2D incidence in a Mediterranean population at high cardiovascular risk whereas no significant associations were observed for monounsaturated and polyunsaturated fat. Some animal food sources rich in SFA, such as cheese and butter were associated with higher risk of T2D while others like whole-yogurt were associated with a lower risk. These findings may contribute to give a deeper insight to the recommendations for dietary guidelines in order to advice on the type of dietary fat to be consumed at a population level.

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Table 1. Baseline Characteristics According to Total Dietary Fat and Specific Types of fat Intake

		Total Fat		MUFAs		PUFAs		SFAs		*trans* fat	
	Total population	Q1	Q4	Q1	Q4	Q1	Q4	Q1	Q4	Q1	Q4
Participants, n	3349	838	837	838	837	838	837	838	837		
Age, y	67(6)	67(6)	66(6)	67(6)	67(6)	67(6)	67(6)	67(6)	66(6)	67(6)	66(6)*
Women, n (%)	2082(62.2)	481 (57.4)	583 (69.7)*	508 (60.6)	571 (68.2)*	523 (62.4)	516 (61.7)	497 (59.3)	574 (68.6)*	543 (64.8)	542 (64.8)*
BMI, kg/m^2	30.0(3.6)	29.8(3.3)	30.3(3.9)*	29.8(3.4)	30.3(3.8)*	30.2(3.5)	29.8(3.7)	29.6(3.4)	30.5(3.8)*	29.6(3.4)	30.2(3.7)*
Smoking status, n (%)											
Never	2092(62.5)	513 (61.2)	557 (66.6)	509 (60.7)	556 (66.4)	523 (62.4)	523 (62.5)	528 (63.0)	537 (64.2)*	557 (66.5)	525 (62.7)*
Former	732 (21.9)	183 (21.8)	159 (19.0)	193 (23.0)	159 (19.0)	177 (21.1)	193 (23.1)	180 (21.5)	159 (19.0)*	164 (19.6)	171 (21.9)*
Current	525 (15.7)	142 (17.0)	121 (14.5)	136 (16.2)	122 (14.6)	138 (16.5)	121 (14.5)	130 (15.5)	141 (16.9)*	117 (14.0)	141 (15.7)*
Intervention group, n (%)											
MedDiet + EVOO	1114(33.3)	283 (33.8)	278 (33.2)	268 (32.0)	269 (32.1)	294 (35.1)	255 (30.5)*	286 (34.1)	276 (33.0)	270 (32.2)	267 (31.9)
MedDiet + nuts	1165(34.8)	263 (34.4)	304 (36.3)	269 (32.1)	298 (35.6)	240 (28.6)	327 (39.1)*	281 (33.5)	280 (33.5)	279 (33.3)	297 (35.5)
Control group	1070(32.0)	292 (34.8)	255 (30.5)	301 (35.9)	270 (32.3)	304 (36.3)	255 (30.5)*	271 (32.3)	281 (33.6)	289 (34.5)	273 (32.6)
Education, n (%)											
Primary	2540(75.8)	656 (78.3)	624 (74.6)	650 (77.6)	609 (72.8)	643 (76.7)	636 (76.0)	649 (77.5)	617 (73.7)	667 (79.6)	616 (73.6)
Secondary	541 (16.2)	108 (12.9)	148 (17.7)	120 (14.3)	165 (19.7)	128 (15.3)	140 (16.7)	115 (13.7)	153 (18.3)	109 (13.0)	148 (17.7)
University/graduate	268 (8.0)	74 (8.8)	65 (7.8)	68 (8.1)	63 (7.5)	67 (8.0)	61 (7.3)	74 (8.8)	67 (8.0)	62 (7.4)	73 (8.7)
Fasting blood glucose (mg/dL)	98.2(14.9)	99.0(16.5)	97.1(13.7)*	99.2(16.8)	97.6(14.3)	98.8±(14.3)	98.9(15.2)	99.0(16.3)	98.5(16.5)	98.4(15.0)	98.9(17.0)
Physical activity, MET-min/d	232(222)	246(250)	223(201)	242(250)	225(200)	222(228)	256(240)*	253(239)	207(207)*	254(234)	207(205)*
Hypertension, n (%)	3092(92.3)	774 (92.4)	776 (92.7)	780 (93.1)	764 (91.3)	776 (92.6)	786 (93.9)	768 (91.7)	781 (93.3)	762 (90.9)	773 (92.4)
Hypercholesterolemia, n (%)	2857(85.3)	732 (87.4)	705 (84.2)	727 (86.8)	709 (84.7)	693 (82.7)	718 (85.8)	750 (89.5)	680 (81.2)*	732 (87.4)	691 (82.6)*
Energy and nutrient intake											
Total energy intake, kcal/d	2261(523)	2292(565)	2175(446)*	2307(548)	2141(421)*	2219(532)	2331(516)*	2266(548)	2282(504)	2176(519)	2289(532)*
Carbohydrates, % of energy	42.9(6.9)	50.2(5.5)	35.8(4.4)*	49.6(5.8)	36.6(4.8)*	47.4(6.4)	40.0(6.5)*	48.5(6.3)	38.2(5.6)*	45.3(7.1)	40.6(6.4)*
Protein, % of energy	16.3(2.7)	16.6(2.8)	16.01(2.4)*	16.7(2.7)	15.8(2.3)*	16.7(3.0)	15.8(2.4)*	16.0(2.7)	16.7(2.7)*	16.2(2.8)	16.4(2.6)
Total fat, % of energy	38.2(6.4)	30.0(3.1)	46.3(3.0)*	30.9(4.2)	45.5(3.7)*	33.0(5.2)	41.7(5.7)*	32.3(5.2)	43.2(5.0)*	35.9(6.7)	40.8(5.8)*
MUFAs, % of energy	19.0(4.2)	14.3(2.3)	23.8(2.8)*	13.7(1.8)	24.5(2.0)*	16.6(3.3)	19.6(4.4)*	16.0(3.6)	21.3(3.9)*	18.4(4.5)	19.9(4.1)*
PUFAs, % of energy	6.1(2.0)	4.8(1.5)	7.2(2.0)*	5.6(2.2)	6.6(1.6)*	4.1(0.5)	8.8(1.6)*	5.7(2.0)	6.3(1.8)*	5.9(1.9)	6.2(2.0)*
SFAs, % of energy	9.7(2.2)	7.8(1.6)	11.5(2.0)*	8.3(2.0)	11.0(2.0)*	9.1(2.3)	9.8(2.1)*	7.1(0.9)	12.6(1.4)*	8.0(1.7)	11.6(2.1)*
trans Fat, % of energy	0.22(0.13)	0.17(0.11)	0.27(0.15)*	0.19(0.12)	0.24(0.15)*	0.20(0.13)	0.23(0.14)*	0.13(0.08)	0.34(0.15)*	0.08(0.02)	0.41(0.11)*
Animal fat	13.9(4.3)	12.0(3.5)	15.7(4.3)*	13.0(4.0)	14.7(4.1)*	13.9(4.3)	13.3(3.8)*	10.2(2.6)	18.0(3.8)*	11.1(3.3)	16.6(4.3)*
Vegetal fat	24.2(6.2)	17.9(3.9)	30.5(4.8)*	17.9(4.2)	30.8(4.3)*	19.1(4.8)	28.4(5.5)*	22.1(5.7)	25.2(6.3)*	24.9(6.6)	24.1(6.2)

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Marine ω-3 fatty acids, % of energy	0.32(0.19)	0.30(0.18)	0.34(0.19)*	0.30(0.19)	0.33(0.19)*	0.27(0.17)	0.34(0.20)*	0.32(0.18)	0.31(0.19)	0.35(0.21)	0.29(0.18)*
Non-Marine ω-3 fatty, % of energy	0.55(0.23)	0.44(0.17)	0.66(0.25)*	0.50(0.23)	0.60(0.21)*	0.40(0.09)	0.78(0.29)*	0.50(0.24)	0.60(0.20)*	0.53(0.25)	0.59(0.22)*
ω-6, Linoleic acid	5.0(1.8)	3.9(1.4)	6.1(1.9)*	4.6(2.1)	5.5(1.4)*	3.2(0.5)	7.5(1.6)*	4.7(1.8)	5.2(1.7)*	4.8(1.7)	5.3(1.9)*
Dietary fiber, g/d	25.3(8.6)	28.85(10.1)	21.6(6.2)*	29.1(10.1)	21.7(6.1)*	25.6(9.0)	26.3(9.5)*	29.4(10.3)	21.9(6.7)*	27.2(9.7)*	23.4(7.6)*
Alcohol, g/d	9.11(14.9)	11.5(18.7)	6.2(10.0)*	10.2(17.4)	6.7(10.5)*	10.2(17.7)	8.8(13.5)	11.3(18.8)	6.4(10.0)*	8.8(14.9)	7.5(11.4)*

Abbreviations: MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; SFAs, saturated fatty acids; EVOO, extra-virgin olive oil; MedDiet, Mediterranean Diet; MET-min, metabolic equivalent task minutes; Q, quartile. Mean ± SD (all such values). All quartiles were included in the analyses. *P value <0.05 for comparisons between quartiles of dietary fat subtypes. Pearson's chi-square test for categorical variables or 1-factor ANOVA for continuous variables. Baseline hypertension or use of antihypertensive medication (yes/no), hypercholesterolemia or use of lipid-lowering drugs (yes/no).

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Table 2. Risk of Type 2 Diabetes According to Updated Quartiles of Total Dietary Fat and Specific Types of Fat

	Quartiles				
	1 (lowest)	2	3	4 (highest)	P-trend
Total fat					
Cases/person-years	55/3395.5	71/3459.9	66/3470.1	74/3471.8	
Median, % of energy	32.0	37.5	41.5	46.4	
Multivariable model 1	1 (ref.)	1.30 (0.89, 1.88)	1.27 (0.87, 1.85)	1.38 (0.93, 2.06)	0.12
Multivariable model 2	-	-	-	-	-
Multivariable model 3	1 (ref.)	1.54 (1.03, 2.30)	1.30 (0.87, 1.96)	1.58 (1.03, 2.42)	0.06
Monounsaturated fat					
Cases/person-years	65/3390.1	74/3458.7	59/3466.5	68/3481.9	
Median, % of energy	15.2	18.8	21.6	24.9	
Multivariable model 1	1 (ref.)	1.14 (0.80, 1.61)	0.92 (0.63, 1.35)	1.02 (0.69, 1.49)	0.85
Multivariable model 2	1 (ref.)	1.03 (0.72, 1.47)	0.78 (0.52, 1.18)	0.77 (0.50, 1.19)	0.15
Multivariable model 3	1 (ref.)	1.00 (0.69, 1.46)	0.79 (0.51, 1.22)	0.80 (0.50, 1.26)	0.24
Polyunsaturated fat					
Cases/person-years	68/3399.5	59/3457.5	66/3478.9	73/3461.3	
Median, % of energy	4.4	5.5	6.7	8.6	
Multivariable model 1	1 (ref.)	0.91 (0.63, 1.31)	1.05 (0.73, 1.51)	1.17 (0.81, 1.70)	0.36
Multivariable model 2	1 (ref.)	0.92 (0.63, 1.35)	1.08 (0.74, 1.58)	1.19 (0.81, 1.76)	0.31
Multivariable model 3	1 (ref.)	1.00 (0.67, 1.48)	1.15 (0.78, 1.70)	1.24 (0.82, 1.85)	0.31
Saturated fat					
Cases/person-years	45/3421.1	65/3474.4	65/3438.7	91/3463.0	
Median, % of energy	7.0	8.6	9.8	11.7	
Multivariable model 1	1 (ref.)	1.52 (1.01, 2.28)	1.53 (0.99, 2.35)	2.00 (1.31, 3.04)	<0.01
Multivariable model 2	1 (ref.)	1.52 (0.98, 2.37)	1.58 (0.98, 2.55)	2.21 (1.31, 3.72)	<0.01
Multivariable model 3	1 (ref.)	1.63 (1.03, 2.58)	1.61 (0.97, 2.66)	2.19 (1.28, 3.73)	0.01
***trans* Fat**					
Cases/person-years	45/3486.1	73/3434.8	71/3440.7	77/3435.7	
Median, % of energy	0.06	0.12	0.19	0.32	
Multivariable model 1	1 (ref.)	1.57 (1.05, 2.33)	1.45 (0.96, 2.18)	1.55 (1.02, 2.37)	0.16
Multivariable model 2	1 (ref.)	1.39 (0.92, 2.10)	1.16 (0.75, 1.79)	1.10 (0.68, 1.79)	0.72
Multivariable model 3	1 (ref.)	1.49 (0.97, 2.28)	1.22 (0.77, 1.93)	1.21 (0.73, 2.01)	0.94

Time-dependent Cox regression models were used to assess the risk of type 2 diabetes incidence by quartiles of updated measurements of total dietary fat and specific types of fat intake. Multivariable model 1 was adjusted for age (y), sex, intervention group, yearly updated total energy intake (kcal/d), alcohol intake (continuous, adding a quadratic term), yearly updated quartiles of fiber, protein intake, and dietary cholesterol, BMI (kg/m2), smoking status (never, former, or current smoker), educational level (primary education, secondary education, or academic/graduate), leisure-time physical activity (metabolic equivalent task minutes/d) and baseline hypertension or use of antihypertensive medication (yes/no). Model 2 for specific subtypes of fat also included as covariates the other subtypes of fat in quartiles. Model 3 was further adjusted for hypercholesterolemia or use of lipid-lowering drugs

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(yes/no) and fasting plasma glucose at baseline (mg/dL). All models were stratified by recruitment center. Extremes of total energy intake (>4000 or < 800 kcal/d in men and >3500 or <500 kcal/d in women) were excluded.

Table 3. Risk of Type 2 Diabetes According to Updated Quartiles of Animal and Vegetable Fat Intake and Specific subtypes of Polyunsaturated Fat Intake

	Quartiles				
	1 (lowest)	2	3	4 (highest)	P-trend
Animal fat					
Cases/person-years	49/3450.0	65/3453.5	54/3449.8	98/3445.0	
Median, % of energy	8.4	11.3	13.8	17.3	
Multivariable model 1	1 (ref.)	1.34 (0.89, 2.00)	1.15 (0.75, 1.77)	2.00 (1.32, 3.04)	<0.01
Multivariable model 2	1 (ref.)	1.37 (0.91, 2.05)	1.20 (0.78, 1.85)	2.17 (1.42, 3.30)	<0.01
Multivariable model 3	1 (ref.)	1.45 (0.94, 2.23)	1.27 (0.81, 2.00)	2.00 (1.29, 3.09)	<0.01
Vegetable fat					
Cases/person-years	68/3387.7	67/3475.0	60/3464.3	71/3470.2	
Median, % of energy	18.9	24.8	28.8	33.9	
Multivariable model 1	1 (ref.)	1.03 (0.72, 1.47)	0.92 (0.63, 1.35)	1.21 (0.81, 1.79)	0.47
Multivariable model 2	1 (ref.)	1.09 (0.76, 1.56)	1.05 (0.71, 1.55)	1.44 (0.97, 2.14)	0.11
Multivariable model 3	1 (ref.)	1.13 (0.78, 1.63)	1.02 (0.68, 1.53)	1.50 (0.99, 2.25)	0.09
Marine ω-3 fatty acids					
Cases/person-years	81/3456.2	73/3449.5	53/3438.7	59/3452.9	
Median, % of energy	0.15	0.25	0.35	0.59	
Multivariable model 1	1 (ref.)	0.99 (0.71, 1.39)	0.68 (0.47, 0.99)	0.86 (0.59, 1.25)	0.32
Multivariable model 2	1 (ref.)	1.04 (0.74, 1.47)	0.74 (0.51, 1.07)	0.91 (0.62, 1.34)	0.48
Multivariable model 3	1 (ref.)	1.08 (0.75, 1.54)	0.76 (0.51, 1.14)	0.92 (0.61, 1.39)	0.53
Non-Marine ω-3 fatty acids					
Cases/person-years	69/3388.9	67/3455.2	60/3487.0	70/3466.1	
Median, % of energy	0.36	0.47	0.63	0.88	
Multivariable model 1	1 (ref.)	0.92 (0.65, 1.31)	0.87 (0.59, 1.27)	1.14 (0.79, 1.65)	0.53
Multivariable model 2	1 (ref.)	0.82 (0.56, 1.19)	0.69 (0.44, 1.07)	0.86 (0.53, 1.38)	0.50
Multivariable model 3	1 (ref.)	1.01 (0.68, 1.51)	0.99 (0.61, 1.61)	1.18 (0.70, 2.00)	0.70
ω-6, Linoleic acid					
Cases/person-years	68/3396.1	53/3464.1	75/3474.8	70/3462.3	
Median, % of energy	3.5	4.6	5.6	7.3	
Multivariable model 1	1 (ref.)	0.81 (0.55, 1.19)	1.19 (0.83, 1.72)	1.10 (0.75, 1.60)	0.38
Multivariable model 2	1 (ref.)	0.92 (0.61, 1.39)	1.45 (0.95, 2.24)	1.23 (0.76, 1.98)	0.34
Multivariable model 3	1 (ref.)	0.89 (0.58, 1.38)	1.25 (0.78, 1.98)	1.01 (0.60, 1.69)	0.97

Time-dependent Cox regression models were used to assess the risk of type 2 diabetes incidence by quartile of updated measurements of total animal and vegetable fat, marine and non-marine ω-3 fatty acids and ω-6 linoleic acid intake. Multivariable model 1 was adjusted for age (y), sex, intervention group, yearly updated total energy intake (kcal/d), alcohol intake (continuous, adding a quadratic term), yearly updated quartiles of fiber, protein intake, and dietary cholesterol, BMI (kg/m2), smoking status (never, former, or current smoker),

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educational level (primary education, secondary education, or academic/graduate), leisure-time physical activity (metabolic equivalent task minutes/d) and baseline hypertension or use of antihypertensive medication (yes/no). Model 2 for animal and vegetable fat was further adjusted for each other, and for subtypes of polyunsaturated fatty acids was further adjusted for each other. Model 3 was further adjusted for hypercholesterolemia or use of lipid-lowering drugs (yes/no) and for fasting plasma glucose at baseline (mg/dL). All models were stratified by recruitment center. Extremes of total energy intake (>4000 or < 800 kcal/d in men and >3500 or <500 kcal/d in women) were excluded.

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FIGURE LEGENDS

Figure 1. Multivariate adjusted HRs (95% CI) of incident type 2 diabetes by increasing the consumption of 1 serving of food sources rich in saturated fat

HR of type 2 diabetes according to increasing one serving consumption of food sources rich in saturated fat: processed red meat, red meat, eggs, whole-fat milk, butter, whole-fat yogurt and cheese. Multivariable model was adjusted for age (y), sex, intervention group, BMI (kg/m^2), smoking status (never, former, or current smoker), educational level (primary education, secondary education, or academic/graduate), leisure time physical activity (metabolic equivalent task minutes/d), baseline hypertension or use of antihypertensive medication (yes/no), hypercholesterolemia or use of lipid-lowering drugs (yes/no), fasting plasma glucose (mg/dL), yearly updated total energy intake (kcal/d), alcohol intake (continuous, adding a quadratic term) and vegetables, fruits, legumes, cereals, fish, meat, dairy, olive oil, nuts and biscuits (all in g/d) (except if the exposure was included in these food groups). The analyses were stratified by recruitment center.

[1] includes offal, ham, sausages, pâté, hamburgers and bacon.

[2] includes pork, veal, beef and lamb.

[3] includes petit Suisse, ricotta, cottage, spreadable, and semi-cured/cured cheeses.