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Abstract

Background: Substitution of carbohydrates with fat in a diet for type 2 diabetes patients is still debated.

Objective: This study aimed to investigate the association between dietary carbohydrate intake and isocaloric substitution with (i) total fat, (ii) saturated fatty acids (SFA), (iii) mono-unsaturated fatty acids (MUFA) and (iv) poly-unsaturated fatty acids (PUFA) with all-cause and cardiovascular (CVD) mortality risk and 5-year weight change in patients with type 2 diabetes.

Methods: The study included 6,192 patients with type 2 diabetes from 15 cohorts of the European Prospective Investigation into Cancer and Nutrition (EPIC). Dietary intake was assessed at recruitment with country-specific food-frequency questionnaires. Cox and linear regression were used to estimate the associations with (CVD) mortality and weight change, adjusting for confounders and using different methods to adjust for energy intake.

Results: After a mean follow-up of 9.2 y ±SD 2.3 y, 791 (13%) participants had died, of which 268 (4%) due to CVD. Substituting 10 gram or 5 energy % of carbohydrates by total fat was associated with a higher all-cause mortality risk (HR 1.07 [1.02-1.13]), or SFAs (HR 1.25 [1.11-1.40]) and a lower risk when replaced by MUFAs (HR 0.89 [0.77-1.02]). When carbohydrates were substituted with SFAs (HR 1.22 [1.00-1.49]) or PUFAs (HR 1.29 [1.02-1.63]) CVD mortality risk increased. The 5-year weight was lower when carbohydrates were substituted with total fat or MUFAs. These results were consistent over different energy adjustment methods.

Conclusions: In diabetes patients, substitution of carbohydrates with SFAs was associated with a higher (CVD) mortality risk and substitution by total fat was associated with a higher all-cause mortality risk. Substitution of carbohydrates with MUFAs may be associated with lower mortality risk and weight reduction. Instead of promoting replacement of carbohydrates

97 by total fat, dietary guideline should continue focusing on replacement by fat-subtypes;
98 especially SFAs by MUFAs.
99
100 Keywords: carbohydrates, fatty acids, mortality risk, body weight, type 2 diabetes,
101 substitution

INTRODUCTION

Type 2 diabetes patients have a 2 to 3 fold higher risk of cardiovascular disease (CVD)(1). However, the percentage of carbohydrates in the diet is still debated(2). Initially, carbohydrates were avoided due to their postprandial glucose and insulin raising effects. However, carbohydrates are likely replaced by fat, which may result in unfavorable changes in blood lipid levels, and perhaps an increased mortality risk. Recently, dietary diabetes guidelines have abandoned their recommendations on lowering fat intake(4). because low-carbohydrate compared to low-fat diets improved glycated hemoglobin, fasting glucose, and triglycerides(2, 3) without harmful effects in the short term(5).

In recent literature, the associations of SFAs with CVD risk are debated. For the general population, two meta-analyses of prospective studies could not detect a significant positive association between SFA intake and CVD risk(6),(7). This lack of association may be because SFAs were replaced with carbohydrates, which was not addressed by these meta-analyses. Among diabetes patients, prospective studies investigating the substitution of carbohydrates with fats in the diet are scarce. We are aware of only one study among women with type 2 diabetes that showed a 22% CVD risk reduction with isocaloric substitution of 5 energy% SFA with carbohydrates(8). It is still largely unknown whether substitution of carbohydrates by other types of fat affects (CVD) mortality.

The substitution of carbohydrates with different types of fat may also affect body weight. Overweight is very common among type 2 diabetes patients and weight loss could reduce mortality by 25% in these patients(9). In short term studies, both iso-caloric low-carbohydrate and low-fat calorie-restricted diets were effective on weight loss in patients with type 2 diabetes, without harmful effects from low-carbohydrate diets on blood lipids(5). Long term effects have only been studied in the general population. Two observational studies in the EPIC cohort showed that a higher proportion of fat and fat subtypes(10) at the expense of

energy from carbohydrates was not associated with weight change(11). We are not aware of any such long-term studies among diabetes patients.

This study aimed to investigate the association between dietary carbohydrate intake and substitution with different types of fat with all-cause and CVD mortality risk in type 2 diabetes patients. We used different methods of energy adjustment (the residual, nutrient density, and energy-partition methods) because each method has a different interpretation(12). As secondary endpoints, we investigated the associations with subsequent weight (and waist circumference) change.

METHODS

Study population

Within the European Prospective Investigation into Cancer and Nutrition (EPIC)(13), a subcohort was defined of participants with a confirmed diagnosis of diabetes mellitus at recruitment as has been described earlier(14). The following EPIC-centers have contributed to this project: Florence, Varese, Ragusa, Turin, and Naples (Italy), Navarra and San Sebastian (Spain), Bilthoven and Utrecht (the Netherlands), Heidelberg and Potsdam (Germany), Malmö and Umeå (Sweden), and Aarhus and Copenhagen (Denmark). Self-reports of diabetes at recruitment were confirmed by a second source of information, i.e. contact to a medical specialist or practitioner, self-reported use of medication for diabetes treatment, repeated self-report of diagnosis during follow-up or record linkage to a diabetes registry or a glycated hemoglobin (HbA1c) level above 42 mmol/mol (6%). The study was conducted according to the guideline laid down in the Declaration of Helsinki and was approved by a local ethical review committee of each centre and of the International Agency for Research on Cancer in Lyon, France. All subjects provided written informed consent.

Of 7,048 initial self-reports, 5,542 diabetes diagnoses were confirmed. A further 870 prevalent diabetes cases without self-reported diabetes at recruitment were identified as a result of verification efforts in other projects within EPIC. This led to a subcohort comprising 6,412 individuals with confirmed diabetes at recruitment(14). After exclusion of participants with missing dietary information (N=42), participants in the highest or lowest 1% of the ratio of total energy intake/estimated energy requirement (N=177), and one deceased participant with missing date of death, the analytical example for cardiovascular and all-cause mortality analysis included 6,192 participants (3,355 men and 2,837 women).

For the analysis on weight change, participants with missing baseline weight or extreme anthropometry at recruitment or follow-up [height <130 cm, BMI <16 kg/m^2, waist circumference <40 or >160 cm and waist circumference <60 cm with BMI >25 kg/m^2] were excluded on top of the above mentioned exclusions. Furthermore, participants with extreme weight change >5 kg/year (N=2) or those without follow-up data on weight or BMI (N=2,067 this included the cohorts of Turin and Ragusa and parts of the cohort in Naples (all in Italy)) were excluded. This analytical sample included 4,123 participants (2,267 men and 1,856 women). For 1,898 participants waist change could be analyzed.

Dietary assessment

In EPIC, usual dietary intake during the previous year was assessed at recruitment by means of self-administered country-specific validated dietary questionnaires(13), either quantitative dietary questionnaires with individual portion sizes (in France, Spain, the Netherlands, Germany and Italy, except Naples) or semi-quantitative food frequency questionnaires (in Denmark, Naples (Italy), Sweden, and the UK), that were developed and validated locally(15). Correlation coefficients for the relative validity for carbohydrate measured with food frequency questionnaires varied from 0.46 to 0.76 in women and from 0.40 to 0.84 in

men; for fat correlations varied from 0.41 to 0.63 in women and 0.31 to 0.67 in men(15). Dietary fat intake data consisted of fat intake [g/d] for total fat and types of fat, including SFAs, MUFAs, and PUFAs. Further, we used carbohydrate, protein and alcohol intake [g/d] and total energy intake [kcal/d] for the analysis.

Basal metabolic rate was estimated using the Schofield equations. Participants with a ratio of energy intake to basal metabolic rate of <1.14 were defined as energy under-reporters, whereas those with a ratio >2.40 were classified as energy over-reporters according to the Goldberg cut-offs.

Assessment of anthropometric measures and weight change

At recruitment, body weight [kg] and height [cm] were measured without shoes according to standardized procedures(16). Waist circumference [cm] was measured either at the narrowest torso circumference or at the midpoint between the lower ribs and iliac crest. Weight and waist measurements were corrected to account for protocol differences between centers as previously described(16). For normally dressed participants without shoes 1.5 kg for weight and 2.0 cm for waist circumference were subtracted from the original measurement; for participants in light clothing without shoes 1 kg was subtracted from the weight. BMI was calculated as body weight [kg] divided by height squared [m^2].

At follow-up, weight and waist circumferences [cm] were self-reported in all centers. Weight change [g/y] was calculated by subtracting the recruitment weight from the follow-up weight, subsequently dividing this by the years of follow-up. This result was multiplied by 5 for the 5-year weight change [g/5y]. The same applies for the calculation of 5-year waist change.

Measurements of non-dietary factors

Lifestyle- and health related variables were collected at recruitment using a general questionnaire. Physical activity was indexed into four categories (inactive, moderately inactive, moderately active and active) based on the validated Cambridge Physical Activity Index(17) and information on smoking status was coded into three categories (never, former, current). Smoking intensity was assessed in eight categories (never; former smokers divided in three categories: quit less or equal to 10 years, quit 11-20 years, and quit over 20 years; current smokers, also divided three categories: smoking 1-15, 16-25, and over 25 cigarettes a day, and one category with current pipe, cigar or occasional smokers). Education was assessed in four categories: primary education, lower vocational education, advances elementary education and intermediate vocational education. Diabetes duration was calculated from the date of the confirmed diagnosis as mentioned above or by self-reported age at diagnosis. Insulin use was defined by self-reported diabetes related medication at recruitment.

Cardiovascular mortality

Information on vital status, cause and date of death, were obtained by using follow-up mailings and subsequent inquiries to municipal registries, regional health departments, physicians, or by record linkages with local, regional, or central cancer registries, boards of health, or hospitals (Germany), or death indexes (other countries). Mortality data were coded according to the International Classification of Diseases (ICD-10). For CVD mortality (ICD-10 [I00-I99]), primary and secondary cause of death were combined.

Statistical analysis

Baseline characteristics are presented by tertiles of carbohydrate intake using mean and standard deviation for continuous variables and percentages for categorical variables.

The associations of dietary carbohydrate intake substituted by (i) total fat, (ii) SFAs, (iii) MUFAs and (iv) PUFAs, and all-cause and CVD mortality were explored in separate models using Cox regression. Linear regression was used to explore the association between dietary carbohydrate intake and 5-year weight (and waist circumference) change. For all regression analyses sex, age, energy-intake, baseline BMI [kg/m^2], duration of diabetes [y], insulin use [no/yes], education (four categories), physical activity index (four categories), smoking status at recruitment (three categories), and country were considered as confounding factors. In the linear regression for weight and waist circumference change analyses, length of follow-up was also included. In fat subtype analyses, mutual adjustments were made for all fat subtypes. For subjects with missing values on education (n=273), smoking status (n=21), smoking intensity (n=106), physical activity index (n=342), or duration of diabetes [y](n=410) values were imputed with multiple imputation in which 5 duplicate datasets were sampled, with the missing values replaced by imputed values. The results of these imputations were pooled with Rubin's rules.

As total energy intake is known to be correlated with cardiovascular disease, energy adjustment is needed. To model this 'isocaloric substitution' three different energy-adjustment approaches were used: nutrient residual (energy-adjusted), energy partition and multivariate nutrient density methods (12).

- In the nutrient residual method, the residuals from the regression of absolute intake of total fat, protein and alcohol intake on total energy intake were obtained and then rescaled by adding the mean population energy intake in the regression equations. As total energy intake is an important predictor of the disease, total energy intake was also included in the model. The rescaled residuals were divided by 10 to generate intakes per 10g/d. These nutrient

residuals by definition provide a measure of nutrient intake uncorrelated with total energy intake.

- For the energy partition method, we included the absolute fat intake and other macronutrients (absolute carbohydrate, protein and alcohol intakes per 10 g/d). Since all other macronutrients were included as covariate in the model, total energy should be omitted in this model. In this model the coefficient for the fat intake represents the full effect of the nutrient unconfounded by other sources of energy.
- In the nutrient density method, the nutrient densities from total fat, protein, alcohol and total energy (per 5 energy%/d) were included as covariates. Next, for all three energy-adjustment methods total fat was additionally divided into SFAs, MUFAs and PUFAs. The coefficient for the nutrient density term represents the relation of the nutrient composition of the diet with disease, holding total energy intake constant. Thus this method is an 'isocaloric' analysis which controls for confounding by energy-intake.

Interactions between dietary carbohydrate intake and 1) sex, 2) age, 3) BMI and 4) smoking status were tested in the mortality sample using the nutrient residual method, and adjusted for all former mentioned possible confounders. Furthermore, interaction with glycemic index was tested in all samples. Interactions were tested by adding an interaction term to all different models. This interaction term consisted of the term of interest (f.e. age or glycemic index based on glucose) multiplied by total fat intake per 10 gram and all fat types respectively.

Sensitivity analyses were performed by excluding type 1 diabetes patients, excluding those with prevalent chronic diseases (cancer, cardiovascular disease, stroke and cancer) at recruitment, excluding over- and under-reporters of energy intake, adjusting for smoking

intensity with eight categories and for healthy dietary habits by adjusting for dietary vitamin C and fiber intakes. Finally, we used a meta-analytic approach to investigate heterogeneity across countries (STATA 11 metan procedure) by pooling the multivariate-adjusted HRs per country using the DerSimonian and Laird random effects model and testing for heterogeneity using a chi-square test. Analyses were performed using the SPSS 20.0 statistic software package and P-values <0.05 were considered significant.

RESULTS

Baseline characteristics

Baseline characteristics of the study population are shown in **Table 1**. Compared to subjects in the highest tertile of carbohydrate intake, the subjects in the lowest tertile consumed more dietary fat and alcohol; they were more likely male and current smokers. Age, BMI, duration of diabetes, insulin use and education was not different over the tertiles (Table 1). After a mean follow-up period of 9.2 y ± SD 2.3y, 791 (13%) participants had died, of which 268 (4%) due to CVD. In the analytical sample of weight change the mean weight was 75.7 kg (SD 14.7) for females and 85.6 kg (SD 13.5) for males with an average weight change of -0.53 kg/5y (SD 6.1) for females and -1.1 kg/y (SD 6.3) for males. In the waist circumference change sample the average waist circumference was 90.8 (SD 13.3) cm for females and 99.7 (SD 11.3) cm for males with an average change of 4.5 (SD 6.4) cm/5y for females and 2.2 (SD 5.8) cm/5y for males (**Supplemental table 1**).

Table 1. Characteristics of the population according to percentage of energy from carbohydrate (n=6192) for (cardiovascular and all-cause) mortality analysis.

	Total	Tertiles of percentage of energy from carbohydrates		
		1	2	3

N	6192	2064	2064	2064
Sex: male %(n)	54.2% (3355)	68.1% (1405)	51.9% (1072)	42.5% (878)
Age [y][1]	57.4 (6.7)	56.9 (6.4)	57.7 (6.7)	57.7 (6.8)
BMI [kg/m^2]	28.8 (4.9)	28.8 (4.7)	29.0 (5.1)	28.7 (4.9)
Waist circumference [cm]	96.8 (13.0)	97.9 (12.8)	96.3 (13.0)	94.7 (12.3)
Duration of diabetes [y][2]	4.7 (1.9-10.1)	4.6 (2.2-10.1)	4.8 (2.0-10.1)	4.5 (1.7-10.0)
Insulin use %(n)	22.3% (1383)	22.2% (459	22.6% (466)	22.2% (458)
Education %(n)				
Lower education	71.2% (4411)	67.6% (1395)	73.3% (1513)	72.8% (1503)
Physical activity index %(n)				
Active	37.2% (2301)	39.2% (809)	36.1% (745)	36.1% (746)
Tobacco status %(n)				
Current smoker	25.1% (1553)	32.3% (666)	24.3% (502)	18.7% (385)
Carbohydrates [energy%]	42.7 (7.3)	34.9 (3.9)	42.7 (1.7)	50.6 (4.0)
Protein [energy%]	18.0 (3.3)	18.8 (3.6)	18.3 (3.1)	17.0 (2.9)
Fat [energy%]	34.7 (6.2)	38.3 (6.6)	35.3 (4.6)	30.4 (4.2)
Saturated fat [energy%]	13.1 (3.3)	14.2 (3.8)	13.5(3.0)	11.7 (2.6)
Monounsaturated fat [energy%]	12.7 (3.4)	14.6 (3.7)	12.8 (2.7)	10.8 (2.4)
Polyunsaturated fat [energy%]	6.0 (2.1)	6.5 (2.5)	6.1 (1.9)	5.4 (1.6)
Alcohol [energy%][2]	2.2 (0.2-6.7)	6.0 (1.3-13.1)	2.0(0.3-5.8)	0.7(0.1-2.6)
Glycemic Index	55.1 (3.0)	54.8 (4.1)	55.3(3.7)	55.4(3.9)
Total energy [kcal]	2074 (639)	2215 (665)	2067 (616)	1940 (606)

[1]Mean ± SD (all such values); [2]median (interquartiles), all such values. Lower education is primary education or lower vocational education; Active is moderately active or active; 410 duration of diabetes; 273 education, 21 smoking state 342 physical activity index were imputed.

All-cause and CVD mortality

Substitution of 10 g/d or 5 energy%/d of carbohydrates by total fat was associated with higher all-cause mortality risk (HR nutrient residual method 1.07 [1.02-1.13]), and even stronger when carbohydrates were substituted by SFAs (HR nutrient residual method 1.25 [1.11-1.40]). These findings were consistent with all three energy adjustment methods. On the other hand, substitution of carbohydrates by MUFAs, was associated with lower all-cause mortality risk, but this was only statistically significant with the energy partition method (HR 0.98 [0.97-1.00]). Substitution by PUFAs tended to be associated with higher all-cause mortality risk, but this did not reach significance with all three energy-adjustment methods (**Table 2**).

312 **Table 2**. Hazard ratio [95% CI] of all-cause and cardiovascular mortality for replacing
313 carbohydrate intake with major types of fat estimated from various models for energy
314 adjustment

Energy adjustment models	HR[1]	
	All-cause mortality	CVD mortality
Nutrient residual model (with energy in the model)		
Total fat (10g)	1.07[2] [1.02-1.13]	1.06 [0.96-1.16]
Saturated fat (10 g)	1.25[2] [1.11-1.40]	1.22[2][1.00-1.49]
Monounsaturated fat (10 g)	0.89 [0.77-1.02]	0.85 [0.67-1.08]
Polyunsaturated fat (10 g)	1.13 [0.97-1.32]	1.29[2] [1.02-1.63]
Energy-partition model		
Total fat (10 g)	1.05[2][1.02-1.09]	1.04 [0.97-1.10]
Saturated fat (10 g)	1.21[2] [1.10-1.34]	1.18 [0.99-1.41]
Monounsaturated fat (10 g)	0.87[2][0.76-1.00]	0.82 [0.65-1.04]
Polyunsaturated fat (10 g)	1.01 [0.95;1.28]	1.25 [0.99-1.56]
Multivariate nutrient density model (with energy in the model)		
Total fat (5 en%)	1.08[2] [1.02-1.16]	1.03 [0.93-1.15]
Saturated fat (5 en%)	1.29[2] [1.13-1.48]	1.24 [0.98-1.57]
Monounsaturated fat (5 en%)	0.86 [0.73-1.02]	0.77 [0.58-1.02]
Polyunsaturated fat (5 en%)	1.20 [1.00-1.45]	1.37[2] [1.03-1.81]

315 N=6,192 with 791 cases all-cause mortality and 268 cases in cardiovascular mortality.
316 [1]Hazard ratio (HR) respectively Beta, adjusted for energy intake, protein intake (per 10g / 5
317 energy%), alcohol intake (per 10 gram / 5 energy%), age at recruitment, body mass index,
318 duration of diabetes, insulin use (no/yes), education level (four categories), physical activity
319 index (four categories), tobacco status (three categories), sex, and country. In the analysis of
320 fat subtypes, mutual adjustments were made for all fat subtypes
321 [2]$P<0.05$
322
323 For CVD mortality we found similar results. Substitution of carbohydrates by total fat
324 tended to be associated with higher CVD mortality risk and substitution by MUFAs tended to
325 be associated with lower CVD mortality risk, although both associations were not statistically
326 significant. Substitution by SFAs was associated with higher CVD mortality risk, but this was

only significant with the nutrient residual method (HR 1.22 [1.00-1.49]). Substitution by PUFAs was significantly associated with higher risk of CVD mortality in the nutrient residual method (HR 1.29 [1.02-1.63]) and the nutrient density method (HR 1.37 [1.03-1.81]). This association was borderline significant with the energy partition method.

Weight and waist change

All three energy-adjustment methods consistently showed borderline significantly lower 5-year weight when carbohydrates were substituted by total fat, except with the residual model, which was non-significant (β -98 [-234; 37] g), and significantly lower 5-year weight when carbohydrates were replaced by MUFAs (β -537 [-834; -241] g). Substituting carbohydrates by SFAs and PUFAs were not associated with differences in weight change (**Table 3**).

Substitutions of fat or different fatty acids were not associated with differences in 5-year waist circumference change (Table 3).

340 **Table 3.** Beta [95% CI] of 5-year weight and waist change for replacing carbohydrate intake
341 with major types of fat estimated from various models for energy adjustment

Energy adjustment models	Beta[1]	
Nutrient residual model (with energy in the model)	5-year weight change [g/5y]	5-year waist change [cm/5y]
Total fat (10 g)	-98 [-234; 37]	-0.20 [-0.41; 0.01]
Saturated fat (10 g)	132 [-160; 423]	-0.40 [-0.92; 0.11]
Monounsaturated fat (10 g)	-537[2] [-834; -241]	-0.10 [-0.71; 0.51]
Polyunsaturated fat (10 g)	231 [-125; 588]	0.04 [-0.61; 0.70]
Energy-partition model		
Total fat (10 g)	-16[2] [-27; -6]	-0.01[-0.03; 0.01]
Saturated fat (10 g)	9 [-20; 38]	-0.03 [-0.09; 0.02]
Monounsaturated fat (10 g)	-66[2] [-98; -33]	-0.00 [-0.07; 0.07]
Polyunsaturated fat (10 g)	20 [-18; 57]	0.02 [-0.05; 0.09]
Multivariate nutrient density model (with energy in the model)		
Total fat (5 en%)	-163[2] [-326; -1]	-0.23 [-0.49; 0.03]
Saturated fat (5 en%)	178 [-174; 529]	-0.53 [-1.16; 0.10]
Monounsaturated fat (5 en%)	-819[2] [-1175; -462]	-0.11 [-0.83; 0.62]
Polyunsaturated fat (5 en%)	355 [-82; 793]	0.15 [-0.67; 0.97]

342 N=4,123 for 5-year weight change; N=1,898 for 5-year waist change.

343 [1]Beta, adjusted for energy intake, protein intake (per 10g / 5 energy%), alcohol intake (per 10
344 gram / 5 energy%), age at recruitment, body mass index, duration of diabetes, insulin use
345 (no/yes), education level (four categories), physical activity index (four categories), tobacco
346 status (eight categories), sex, country and follow-up time. In the analysis of fat subtypes,
347 mutual adjustments were made for all fat subtypes

348 [2]$P<0.05$

349

350 **Interactions and sensitivity analyses**

351 No interactions were present with age (p=0.44), smoking status (p=0.97), BMI at recruitment
352 (p=0.60) or GI of the diet (p>0.19). The interaction with sex (p=0.05) was borderline
353 significant. However, stratified analyses for sex showed no substantially different results.

354 Sensitivity analyses (HR [95%CI] for substitution of carbohydrates by total fat on all-
355 cause mortality in the nutrient residual method) excluding patients with type 1 diabetes

(N=288 excluded; HR 1.08 [1.02;1.14]); or energy over- and under-reporters (n=1454 excluded; HR 1.07 [1.05;1.10]); or people with chronic illness at recruitment (N=419 excluded; HR 1.07 [1.02;1.13]) showed similar results. Sensitivity analyses with smoke intensity instead of smoking status strengthened the association between substitution of carbohydrates with total fat and all-cause mortality (HR 1.09 [1.06-1.12]). Sensitivity analysis adjusting for vitamin C and fiber slightly attenuated these results (HR 1.04 [0.97;1.11] for total fat ; HR 1.25 [1.02;1.37] for SFA; HR 0.83 [0.70;0.99] for MUFA) (**Supplemental table 2**). When pooling HRs for different countries, significant heterogeneity was present for estimates of total fat (residual model: χ^2 =13.1; p=0.02) and SFAs (nutrient density: χ^2 =14.8; p=0.01 and residual model: χ^2 =14.5; p=0.01), but not the other fat types (p>0.09). A correlation matrix between energy, carbohydrates, protein, total fat and fat subtypes showed the lowest correlations for PUFA (**Supplemental table 3**).

DISCUSSION

In this European prospective study among individuals with diabetes, isocaloric substitution of 10 gram of carbohydrates with SFA was associated with a 25% higher risk of all-cause and a 22% higher risk of CVD mortality. Furthermore, substitution with total fat was associated with a higher all-cause mortality risk, whereas substitution with MUFAs decreased this risk. Unexpectedly, substitution with PUFAs was associated with a higher CVD mortality risk. Substitutions with total fat and MUFAs were associated with lower weight.

The findings on substitution with SFA are in line with a prospective study among women with type 2 diabetes, which found a 22% CVD risk reduction with isocaloric substitution of 5 energy% SFA by carbohydrates(8). In the general population, meta-analyses of prospective cohort studies showed that substituting carbohydrates with SFA were not associated with CVD risk (RR 1.07[0.96;1.19])(6) and substitution of SFA with carbohydrates was not associated with CHD mortality (HR 0.96[0.82;1.13])(18). These associations all pointed in the same direction, but were stronger among diabetes patients for CVD risk (RR 1.22[1.02;1.39])(8) and for (CVD) mortality in our study. Therefore, substitution of SFAs with carbohydrates might be relevant for diabetes patients.

Substitution with total fat showed a marginally elevated risk for all-cause mortality. For CVD mortality we observed a similar association, but not statistically significant; probably due to the low number of CVD deaths. Substitution with total fat was not associated with a higher risk for CVD mortality in women with diabetes as well(8). As total fat is the sum of the different fat subtypes; associated with higher or lower CVD mortality risk, this likely explains the more modest association of substituting carbohydrates with total fat with (CVD) mortality. Therefore, it may be more important to focus on fat subtypes.

Indeed, our study showed that substitution of carbohydrates by MUFAs was associated with a lower (CVD) mortality risk. Tanasescu found that substitution of SFA with MUFA was

associated with a lower CVD risk than substitution with carbohydrates. This suggested that substitution of carbohydrates by MUFA might be associated with a lower CVD risk(8). Our study confirms this finding.

Unexpectedly, substitution by PUFAs was associated with a higher CVD mortality risk. Sensitivity analyses leaving out energy over- and under-reporters strengthened our findings in terms of narrower CIs (Supplemental table 2). This result could be due to the underlying types of PUFAs. N-6 PUFAs may promote inflammation and may thus augment many diseases, particularly if consumed in amounts unbalanced to n-3 PUFAs, whereas n-3 PUFAs seem to counter these adverse effects. A meta-analysis found that PUFA intake was associated with a higher CHD mortality risk, but not with CHD events. Here, the authors stressed the unreliability of the dietary fat sources of the observational data(19). The significant association was mainly caused by a Finnish study which showed a significantly higher risk of CHD mortality with higher intake of n-3 PUFAs from fish(20). In our study, stratified analyses by different regions also showed a significantly higher risk for CVD mortality for substitution of 10 gram carbohydrates by PUFA in the northern region (Sweden and Denmark) HR 1.17[1.01;1.35]; whereas the results in the southern region (Italy and Spain) HR 1.09[0.90;1.32], and the middle region (the Netherlands and Germany) HR 0.94[0.81;1.09] were not significant. However, other meta-analyses showed that substitution of SFAs with PUFAs was associated with a lower CHD mortality risk(18, 21). Therefore, we do not expect that the underlying types of PUFAs can completely explain our findings.

Since weight loss could reduce mortality in diabetes patients by 25%(9), the associations between substitution of carbohydrates with fat(types) and weight change could perhaps explain the results found for mortality. Especially for MUFAs these results are in line with each other. We found that substitution by total fat and especially MUFAs was associated with a lower 5-year weight. Other studies did not find associations between different subtypes

418 of fat and weight change in the general population(10, 11) nor in diabetes patients(22). The
419 significant associations of substitution of carbohydrates with fat subtypes we observed might
420 be due to stronger associations in diabetes patients. The fact that participants with type 2
421 diabetes from our study lost weight overall, whereas weight gain was shown for the total
422 EPIC cohort(11) could also play a role. This could be caused by under-reporting of self-
423 reported follow-up weight or intentional weight loss(23). Furthermore, it is well established
424 that diabetes patients tend to be more katabolic with loss of lean tissue, which might explain
425 the loss of body weight, but increase of waist circumference(24). Healthier dietary habits
426 associated with certain fat subtypes could also play a role. However, adjustment for fiber and
427 vitamin C as indicators of a healthy diet did not completely explain these associations.
428 The quality of the carbohydrates that are replaced might also play a role, since GI of
429 the diet has been associated with CVD risk(25). We therefore investigated the interaction of
430 GI of the diet with fat substitution, but we could not detect a significant interaction. This
431 indicated that quality of the carbohydrates did not influence our results to a large extent. A
432 previous study in this population also showed no association of GI with (CVD) mortality(26).
433 Energy adjustment is needed if total energy intake is related to disease risk(12). In
434 general, we did not observe different associations depending on the energy adjustment
435 method, in line with Hu et al(27). Both the residual and nutrient density methods have an
436 isocaloric substitution interpretation. As the nutrient residual method attempts to overcome
437 the potential underreporting of dietary fat intake, this may be an advantage of this method.
438 The nutrient density model, expressed as energy percent, is useful because public health
439 recommendations are generally expressed in terms of energy percentages. In the partition
440 model, total energy is partitioned into all nutrients that bear energy and it represents the effect
441 of “adding saturated fat”, which includes both its energy and non-energy effects(27). This
442 method thus addresses a different research question.

Strengths of the study are its prospective design with long follow-up, the large sample size, inclusion of both men and women, and inclusion of 15 cohorts across European countries with widely varying dietary fat intakes.

There are certain limitations. The use of self-reported dietary questionnaires potentially resulted in the underreporting of fat intake(28). However, the use of energy-adjusted fat intake minimizes such potential misclassification(12) and a sensitivity analysis excluding potential under-reporters showed similar results(29). We assessed dietary intake only at recruitment and did not examine any changes in intake during follow-up, which might vary over time. However, excluding participants most likely to have changed their diets (those with chronic disease at recruitment and cases occurring in the first two years) did not alter our findings. Furthermore, assessment of the long-term reproducibility of the FFQ showed fairly high correlation between measurements at recruitment and at follow-up (correlation coefficients: 0.41–0.77)(30). As mentioned above, we had no data on type of PUFA. At follow-up, weight and waist circumferences were self-reported, which may lead to potential underestimation from self-report. However, results in the two centers (which are not part of this study) with measured weight and waist circumferences were in agreement with the rest of the cohort(11). Finally, heterogeneity between countries was present for some estimates, but we had insufficient power for certain countries for stratified analyses.

To conclude, isocaloric substitution of carbohydrates with saturated fat was associated with higher all-cause and CVD mortality risk. Substitution by MUFAs may be associated with lower all-cause mortality risk and substitution by PUFAs showed a higher risk of CVD mortality. Instead of promoting substitution of carbohydrates by total fat, dietary guidelines should continue focusing on substitution by fat-subtypes; especially SFAs by MUFAs.

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