OPEN

European Journal of Clinical Nutrition (2017) **71**, 245–251

www.nature.com/ejcn

ORIGINAL ARTICLE

Fatty acid intake and its dietary sources in relation with markers of type 2 diabetes risk: The NEO study

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OBJECTIVE: The aim of this study was to examine the relations between intakes of total, saturated, mono-unsaturated, poly-unsaturated and trans fatty acids (SFA, MUFA, PUFA and TFA), and their dietary sources (dairy, meat and plant) with markers of type 2 diabetes risk.
SUBJECTS/METHODS: This was a cross-sectional analysis of baseline data of 5675 non-diabetic, middle-aged participants of the Netherlands Epidemiology of Obesity (NEO) study. Associations between habitual dietary intake and fasting and postprandial blood glucose and insulin, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), HOMA of β-cell function (HOMA-B) and Disposition Index were assessed through multivariable linear regression models with adjustments for demographic, lifestyle and dietary factors.
RESULTS: Mean (s.d.) intakes in percent of energy (En%) were 34.4 (5.8) for total fatty acids, 12.4 (2.9) for SFA, 12.2 (2.4) for MUFA, 6.9 (1.9) for PUFA and 0.6 (0.2) for TFA. As compared with carbohydrates, only SFA was weakly inversely associated with fasting insulin, HOMA-IR and HOMA-B. When stratified by dietary source, all fatty acids from meat were positively associated with fasting insulin — total fatty acids$_{meat}$ (per 5 En%: 10.0%; 95% confidence interval: 4.0, 16.3), SFA$_{meat}$ (per 1 En%: 3.7%; 0.4, 7.2), MUFA$_{meat}$ (per 1 En%: 5.0%; 2.0, 8.1), PUFA$_{meat}$ (per 1 En%: 17.3%; 6.0, 29.7) and TFA$_{meat}$ (per 0.1 En%: 10.5%; 3.2, 18.3). Similarly, all fatty acids from meat were positively associated with HOMA-IR and HOMA-B and inversely with Disposition Index.
CONCLUSIONS: Our study suggests that the relations between fatty acid intakes and markers of type 2 diabetes risk may depend on the dietary sources of the fatty acids. More epidemiological studies on diet and cardiometabolic disease are needed, addressing possible interactions between nutrients and their dietary sources.

European Journal of Clinical Nutrition (2017) **71,** 245–251; doi:10.1038/ejcn.2016.204; published online 9 November 2016

INTRODUCTION

The worldwide prevalence of type 2 diabetes mellitus (diabetes) continues to grow.[1] Risk factors for developing diabetes include overweight, physical inactivity and an unhealthy diet. Evidence-based lifestyle modification programmes involve diet; yet limited evidence is available on the role of individual dietary factors in the development of diabetes.[2] With respect to dietary fat, an expert consultation of the Food and Agriculture Organization of the United Nations concluded in 2010, that the strength of the evidence for high intake of saturated fatty acids (SFAs) as a risk factor for diabetes is 'possible', and for trans fatty acid (TFA) intake is 'probable'. Furthermore, it was concluded that the strength of the evidence for the intake of poly-unsaturated fatty acids (PUFA) as a beneficial factor is 'probable', and insufficient evidence was available for mono-unsaturated fatty acids (MUFA).[3] Suggested underlying mechanisms for the relation between increased PUFA and diabetes include increased insulin sensitivity by altering phospholipid composition of cell membranes, which in turn may alter the function of insulin receptors,[4] and increased insulin sensitivity through short-term alterations of gene expression and enzyme activities.[5]

In intervention studies replacing dietary SFA with MUFA, one reported an improved insulin sensitivity,[6] whereas others reported no effect on fasting insulin or insulin sensitivity.[7–9] In intervention studies that replaced dietary SFA with omega-6 PUFA, liver fat accumulation was reduced[10,11] and insulin sensitivity improved.[10,12] In prospective cohort studies, dietary intakes of total fat, SFA and MUFA were not associated with the incidence of diabetes.[13–19] Higher intakes of PUFA were associated with a lower[15,16] or with no[14,17–19] risk of diabetes, but were associated with a lower risk among the studies that investigated the replacement of SFA by PUFA.[14–16] In totality, the scientific evidence on the relations between dietary fatty acids and risk of diabetes and its underlying features is limited and inconclusive. In addition, some recent studies indicate that the food from which fatty acids originate, such as dairy and meat, may explain the inconsistency in reported relations between dietary fat and risk of coronary heart disease[20] and diabetes.[21]

The primary objective of the present analysis was to examine the relations between the dietary intakes of total fatty acids, SFA, MUFA, PUFA and TFA with blood markers of diabetes risk. The secondary objective was to examine the possible influence of the major dietary sources of fatty acids, that is, dairy, meat and plant sources on these associations.

SUBJECTS AND METHODS

Design and study population

The Netherlands Epidemiology of Obesity (NEO) study is a population-based prospective cohort study in 6673 individuals, included between

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Received 17 May 2016; revised 27 July 2016; accepted 18 September 2016; published online 9 November 2016

2008 and 2012. Details of the study design and population are described elsewhere.[22] Men and women aged between 45 and 65 years with a self-reported body mass index (BMI) of 27 kg/m^2 or higher living in the greater area of Leiden were eligible to participate in the NEO study. In addition, all inhabitants aged between 45 and 65 years from one municipality (Leiderdorp) were invited irrespective of their BMI, allowing for a reference distribution of BMI in the general population. The study was approved by the medical ethics committee of the Leiden University Medical Center (LUMC), and all participants gave written informed consent.

The present study is a cross-sectional analysis of baseline data. We excluded participants who reported to have diabetes, used diabetes medication, had undiagnosed diabetes (fasting glucose of 7.0 mmol/l or higher) ($n = 749$), as well as participants with missing data for glucose or insulin ($n = 225$), or not fasting at baseline ($n = 20$). Furthermore, we excluded participants with missing dietary intake data ($n = 4$).

Dietary assessment

Habitual diet was assessed using a validated semiquantitative self-administered 125-item food frequency questionnaire.[23,24] Dietary intakes were calculated from the 2011 version of the Dutch food composition table.[25] Intakes of fatty acids from major dietary sources were categorized into dairy, meat, plant and other food groups (see Supplementary Table S1 for a detailed list of dietary sources and Supplementary Information S1 for details on dietary assessment). In a random subsample of 110 men and 119 women, the relative validity of the FFQ against two 24-h dietary recalls was assessed. Correlation coefficients corrected for within-person variation for total fatty acids, SFA, MUFA and PUFA were around 0.5 and increased to 0.6 after energy-adjustment, except for the correlation coefficient for PUFA which remained 0.5.

Markers of diabetes risk

Participants were invited for a baseline visit after an overnight fast of at least 10 h. Within 5 min after drawing a fasting blood sample, all participants consumed a liquid mixed meal (400 ml) that contained 2.5MJ, of which 16 percent of energy (En%) was derived from protein, 50 En% from carbohydrates and 34 En% from fat. Subsequently, blood samples were drawn after 30 and 150 min.[22] From these, fasting and postprandial blood glucose and insulin, HbA1c, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), HOMA of β-cell function (HOMA-B) and Disposition index were determined. In Supplementary Information S1, a detailed overview of laboratory methods and calculations is given.

Non-dietary factors

Participants completed questionnaires on demography, lifestyle and medical history, and underwent an extensive physical examination by trained research nurses.[22] Use of diabetes medication or statins was categorized into yes or no (reference), and family history of diabetes was reported as having any parent or sibling with diabetes or no (reference). Ethnicity was grouped into white (reference) or other. Tobacco smoking was reported as never smoker (reference), former smoker or current smoker. The level of education was grouped into low (reference), middle and high education, and usual physical activity was expressed in metabolic equivalents of task (MET)—hours per week.[26] Height (m) and waist circumference (cm) were measured by tape measures. Body weight (kg) and body fat (%) were measured by a bioelectrical impedance balance (TBF-310; Tanita Europe BV, Amsterdam, The Netherlands). BMI was calculated as weight (kg)/height (m)^2.

Statistical analysis

Details of statistical procedures are given in Supplementary Information S1. All results are based on weighted analyses to represent a population-based study with a normal BMI distribution, that is, adjusted for the oversampling of persons with a BMI $\geqslant$ 27 kg/m^2.

Potential energy under-reporters[27,28] were identified on the basis of disparities between reported energy intakes and predicted energy requirements, using the ratio of Goldberg's cutoff point for energy intake (EI) to basal metabolic rate (BMR).[29,30] Participants with EI:BMR under the cutoff point (< 1.09) were considered energy under-reporters (Supplementary Information S1).

Participants' characteristics were presented by quartiles of SFA intake (%En). We performed linear regression to examine the associations between dietary intake of total fatty acids, SFA, MUFA, PUFA and TFA and the markers of type 2 diabetes risk. In model 1 we adjusted for age and sex. In model 2, we additionally adjusted for total energy intake, alcohol consumption and dietary fiber intake. In model 3 we included additional adjustments for total body fat, tobacco smoking, educational level, physical activity, family history of diabetes, use of statins, energy underreporting,[31] intake of coffee, sugar-sweetened beverages and dietary cholesterol. Finally, in model 4 we adjusted for protein intake, and in the models for SFA, MUFA, PUFA and TFA we also adjusted each for intakes of the other fatty acids. The coefficients in model 1 can be interpreted as the difference in markers of diabetes risk when a specific fatty acid is consumed on top of the diet. The coefficients in models 2 and 3 can be interpreted as the difference in outcome when isocalorically substituting an unspecified part of the diet (average rest of the diet) by a specific fatty acid (energy substitution), and the coefficients from model 4 can be interpreted as the difference in outcome when isocalorically substituting carbohydrates by a specific fatty acid (carbohydrate substitution).[32] In secondary analyses, dietary fatty acid intake was stratified according to dairy, meat and plant sources. The regression models were additionally adjusted for the intake of fatty acids from the other dietary sources.

Because data on markers of diabetes risk were not normally distributed, we transformed these to the natural logarithm (ln). For interpretation of the results, regression coefficients were back transformed and expressed as the percentage change with 95% confidence intervals (CI) (Supplementary Information S1).

In sensitivity analyses, we repeated all analyses with fatty acid intakes in equally sized quartiles, after exclusion of 403 participants with extreme energy intakes (< 500 or > 3500 kCal for women and < 800 or > 4000 kCal for men), with protein as substitution nutrient, and without weighting toward the normal BMI distribution. Lastly, we repeated all analyses with further specification of fatty acids from dairy (cheese, milk and milk products, butter), plant-based foods (fruit/vegetables/cereal, nuts, oils/fats/condiments) and other dietary sources (candy bars, fish). All analyses were performed with the STATA Statistical Software (Statacorp, College Station, TX, USA), version 12.1.

RESULTS

The present analyses included 5675 participants, with a mean (s.d.) age of 56 years[6] and a mean BMI of 26.1 (4.2) kg/m^2, and 44% were men (Table 1). Median (25th, 75th percentile) baseline concentrations of fasting glucose, fasting insulin and HbA1c of all participants were 5.3 (5.0, 5.6) mmol/l, 7.5 (5.2, 11.3) mU/l and 5.3 (5.1, 5.4)%, respectively (Supplementary Table S2 presents baseline levels of all markers of diabetes risk). Mean habitual intake of total fatty acids was 34.4 (5.8) En%, of which 39% was SFA, 38% was MUFA, 21% was PUFA and 2% was TFA (Table 2).

After adjusting for age and sex (model 1), the intakes of total fatty acids, MUFA, PUFA and TFA, but not SFA, were weakly positively associated with HbA1c, fasting insulin, insulin$_{AUC}$, HOMA-IR and HOMA-B (Table 3). The energy substitution model adjusted for demographic and lifestyle factors (model 3) attenuated the observed estimates for total fatty acids, MUFA and TFA. As compared with the average rest of the diet, PUFA intake was weakly positively associated with glucose$_{AUC}$, fasting insulin, insulin$_{AUC}$, HOMA-IR, HOMA-B and weakly inversely associated with the Disposition Index. The intake of TFA was weakly inversely associated with glucose$_{AUC}$. In the carbohydrate substitution model (model 4), the observed estimates for PUFA and TFA were attenuated. Also, SFA intake was, as compared with carbohydrates, weakly inversely associated with fasting insulin, HOMA-IR and HOMA-B. Per 1 En% SFA intake, fasting insulin was 1.4% lower (95% CI: -2.7, -0.1), HOMA-IR was 1.4% lower (-2.8, 0.0) and HOMA-B was 1.5% lower (-2.9, -0.2).

In the full models stratified by dietary source, as compared with carbohydrates, all fatty acids from meat were positively associated with fasting insulin; per 5 En% of total fatty acids$_{meat}$ 10.0% higher (95% CI: 4.0, 16.3), per 1 En% SFA$_{meat}$ 3.7% higher (0.4, 7.2), per 1 En% MUFA$_{meat}$ 5.0% higher (2.0, 8.1), per 1 En% PUFA$_{meat}$ 17.3% higher (6.0, 29.7) and per 0.1 En% TFA$_{meat}$ 10.5% higher (3.2, 18.3). Similarly, as compared with carbohydrates, all fatty acids from meat were positively associated with HOMA-IR and HOMA-B and inversely associated with the Disposition Index (Figure 1). For SFA from dairy and plant sources, weak associations were observed.

Table 1. Baseline characteristics of 5675 participants in the NEO study, 45–65 years without type 2 diabetes, by quartiles of SFA intake

	Quartiles of SFA intake, %En (range)			
	Q1 (2.4–10.4)	*Q2 (10.4–12.0)*	*Q3 (12.0–14.0)*	*Q4 (14.0–27.5)*
Sex (% men)	44[a]	46	44	40
Age (years)	56.0 ± 5.9	55.6 ± 6.0	55.3 ± 6.0	55.7 ± 6.1
Ethnicity (% white)	92	96	98	96
Education level (% low)	33	32	35	37
Body mass index (kg/m^2)	26.1 ± 4.1	26.0 ± 3.8	26.2 ± 4.4	26.2 ± 4.6
Total body fat (%)	31.3 ± 8.5	31.1 ± 8.1	31.4 ± 8.9	32.0 ± 8.7
Waist circumference (cm)	91.2 ± 12.8	91.3 ± 12.1	91.7 ± 13.3	91.8 ± 13.6
Physical activity (MET hours/week)	125 ± 59	118 ± 56	121 ± 59	121 ± 62
Smoking (% never)	34	41	41	39
Family history of diabetes (%)	27	27	29	31
Statin use (%)	11	8	7	6
Mean daily dietary intakes				
Total energy (MJ/day)	8.7 ± 2.6	9.2 ± 2.6	9.7 ± 3.0	10.5 ± 3.6
Total energy/BMR	1.4 ± 0.4	1.5 ± 0.4	1.5 ± 0.4	1.7 ± 0.6
Energy under-reporter (%)	21	15	12	10
Total fatty acids (%En)	28.5 ± 4.3	33.2 ± 3.5	35.7 ± 3.8	39.3 ± 4.2
SFA (%En)	9.1 ± 1.1	11.2 ± 0.4	12.9 ± 0.6	16.1 ± 2.1
MUFA (%En)	10.5 ± 2.1	12.0 ± 2.0	12.6 ± 2.0	13.3 ± 2.0
PUFA (%En)	6.5 ± 1.7	7.1 ± 1.7	7.2 ± 2.0	6.6 ± 1.9
TFA (%En)	0.4 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.7 ± 0.2
Protein (%En)	14.6 ± 2.3	14.5 ± 2.0	14.3 ± 2.2	14.2 ± 2.5
Carbohydrate (%En)	47.9 ± 6.1	45.4 ± 5.1	43.8 ± 5.0	41.1 ± 5.5
Alcohol (%En)	4.7 (1.4–9.2)	3.4 (1.3–6.9)	3.0 (1.0–5.6)	2.1 (0.6–5.1)
Dietary fiber (%En)	2.2 ± 0.4	2.0 ± 0.3	1.8 ± 0.3	1.7 ± 0.3
Dietary cholesterol (mg/day)[b]	182 ± 58	194 ± 50	209 ± 58	237 ± 76
Coffee (g/day)[b]	406 (174–638)	406 (406–638)	406 (406–638)	406 (200–638)
Sugar-sweetened beverages (g/day)[b]	7 (0–53)	13 (0–53)	18 (0–95)	13 (0–53)

Abbreviations: BMR, basal metabolic rate; %En, percentage of energy; MET, metabolic equivalents of task; MUFA, mono-unsaturated fatty acids; NEO, Netherlands Epidemiology of Obesity study; PUFA, poly-unsaturated fatty acids; SFA, saturated fatty acids; TFA, trans fatty acids. [a]Individual data were weighted to the BMI distribution of the general Dutch population. Mean ± s.d.; %; Median (25th-75th percentile). [b]Values were adjusted for total energy intake.

Table 2. Habitual intake of types of dietary fatty acids from total diet and stratified by dairy, meat and plant sources in 5675 participants in the NEO study, 45–65 years without type 2 diabetes

	Total diet	*Dairy*	*Meat*	*Plant*
Total fatty acids (%En)	34.2 ± 5.6[a]	6.4 ± 3.9	4.0 ± 2.3	14.5 ± 5.2
SFA (%En)	12.3 ± 2.9	4.2 ± 2.6	1.4 ± 0.8	3.0 ± 1.3
MUFA (%En)	12.1 ± 2.3	1.4 ± 0.9	1.6 ± 0.9	5.6 ± 2.2
PUFA (%En)	6.8 ± 1.8	0.2 ± 0.1	0.5 ± 0.3	4.8 ± 1.9
TFA (%En)	0.54 ± 0.16	0.17 ± 0.13	0.06 ± 0.04	0.09 ± 0.06

Abbreviations: %En, percentage of energy; SFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids; NEO, Netherlands Epidemiology of Obesity Study; PUFA, poly-unsaturated fatty acids; TFA, trans fatty acids. [a]Individual data were weighted to the BMI distribution of the general Dutch population. Mean ± s.d.

SFA_{dairy} was inversely associated with fasting insulin (per 1 En%: −1.5%; −3.0, −0.1) and HOMA-B (per 1 En%: −1.6%; −3.1, −0.1); and SFA_{plant} was inversely associated with HOMA-IR (per 1 En%: −2.5%; −5.0, 0.0). Results were essentially the same when fatty acids were compared with the average rest of the diet in the energy substitution model (Supplementary Figure S1). Further specification of fatty acids from different dietary sources only showed inverse associations between all fatty acids derived from fruit/vegetables/cereal products and $insulin_{AUC}$ (Supplementary Table S3).

Results were essentially the same after repeating the analyses with fatty acid intakes in quartiles, after exclusion of participants with extreme energy intakes, and with protein instead of carbohydrates as the substitution nutrient. In sensitivity analyses without weighting the analysis toward the normal BMI distribution, associations were similar, but with smaller confidence intervals (Supplementary Figure S2).

DISCUSSION

In this cross-sectional analysis in a non-diabetic middle-aged Dutch population, we observed that dietary SFA intake, when isocalorically compared with carbohydrate intake, was weakly inversely associated with fasting insulin, HOMA-IR and HOMA-B. Total fatty acids, PUFA, MUFA and TFA intake were not associated with any marker of type 2 diabetes risk. In contrast, stratification by the major dietary sources of fatty acids revealed that all fatty acids from meat were adversely associated with markers of insulin resistance and secretion, whereas fatty acids from dairy and plant sources were not.

To our knowledge, this is the first observational study that systematically studied both the intake of all fatty acids and their dietary sources in relation to blood markers of diabetes risk. Insulin resistance and insulin secretion are key underlying features of abnormal glucose metabolism and seem to occur early in the pathogenesis of diabetes.[33,34] Our primary findings are not in line with conclusions from an FAO/WHO expert panel that total SFA intake is a 'possible' risk factor for diabetes, and that the strength of the evidence for high total PUFA intake as a beneficial factor is 'probable'.[3] Our stratified findings, however, are supported by recent research suggesting that the type of food that delivers dietary fat may be relevant for the relationship with

Table 3. Associations of the intake of types of dietary fatty acids with markers of type 2 diabetes risk in 5675 participants in the NEO study, 45–65 years without type 2 diabetes

	Expressed per	*Percentage (95% CI)*[a]			
		Model 1[b]	*Model 2*	*Model 3*	*Model 4*
Fasting glucose					
Total FA	5 En%	0.0 (−0.4, 0.4)	0.0 (−0.4, 0.5)	0.1 (−0.4, 0.5)	0.1 (−0.4, 0.5)
SFA	1 En%	0.0 (−0.2, 0.1)	−0.1 (−0.3, 0.1)	0.0 (−0.2, 0.1)	0.0 (−0.2, 0.2)
MUFA	1 En%	0.1 (−0.1, 0.3)	0.0 (−0.2, 0.2)	0.0 (−0.2, 0.2)	0.1 (−0.1, 0.4)
PUFA	1 En%	0.0 (−0.2, 0.2)	0.1 (−0.1, 0.3)	0.2 (0.0, 0.4)	−0.1 (−0.3, 0.2)
TFA	0.1 En%	0.0 (−0.2, 0.3)	0.0 (−0.3, 0.3)	−0.2 (−0.5, 0.1)	−0.2 (−0.6, 0.1)
$Glucose_{AUC}$					
Total FA	5 En%	0.4 (−0.3, 1.0)	0.2 (−0.6, 1.0)	0.4 (−0.5, 1.3)	0.5 (−0.4, 1.4)
SFA	1 En%	0.0 (−0.3, 0.2)	−0.3 (−0.6, 0.0)	−0.1 (−0.5, 0.2)	0.1 (−0.3, 0.6)
MUFA	1 En%	0.3 (0.0, 0.6)	0.1 (−0.3, 0.4)	0.1 (−0.2, 0.5)	0.1 (−0.4, 0.5)
PUFA	1 En%	0.3 (−0.1, 0.7)	0.6 (0.2, 1.0)	0.7 (0.2, 1.1)	0.3 (−0.2, 0.8)
TFA	0.1 En%	−0.1 (−0.5, 0.4)	−0.5 (−1.0, 0.0)	−0.6 (−1.2, −0.1)	−0.6 (−1.3, 0.1)
HbA1c					
Total FA	5 En%	0.23 (0.07, 0.40)	−0.13 (−0.32, 0.05)	−0.08 (−0.27, 0.11)	−0.11 (−0.30, 0.09)
SFA	1 En%	0.08 (0.02, 0.14)	−0.07 (−0.14, 0.00)	−0.04 (−0.12, 0.03)	0.00 (−0.10, 0.09)
MUFA	1 En%	0.07 (−0.01, 0.15)	−0.06 (−0.15, 0.03)	−0.04 (−0.13, 0.05)	−0.05 (−0.17, 0.07)
PUFA	1 En%	0.10 (0.00, 0.19)	0.04 (−0.05, 0.13)	0.06 (−0.04, 0.16)	0.03 (−0.10, 0.16)
TFA	0.1 En%	0.14 (0.03, 0.25)	−0.05 (−0.17, 0.07)	−0.09 (−0.22, 0.04)	−0.12 (−0.27, 0.03)
Fasting insulin					
Total FA	5 En%	2.8 (0.0, 5.6)	−0.9 (−3.9, 2.2)	0.4 (−1.9, 2.6)	0.3 (−2.0, 2.5)
SFA	1 En%	0.1 (−1.1, 1.3)	−2.1 (−3.5, −0.8)	−0.9 (−2.1, 0.2)	−1.4 (−2.7, −0.1)
MUFA	1 En%	1.4 (0.0, 2.8)	0.0 (−1.5, 1.4)	0.4 (−0.7, 1.5)	0.8 (−0.6, 2.2)
PUFA	1 En%	1.9 (0.7, 3.2)	1.9 (0.6, 3.3)	2.3 (0.9, 3.6)	0.2 (−1.4, 1.8)
TFA	0.1 En%	2.9 (1.2, 4.6)	0.9 (−1.4, 3.1)	−0.1 (−2.0, 1.7)	0.9 (−1.2, 2.9)
$Insulin_{AUC}$					
Total FA	5 En%	1.8 (0.2, 3.5)	−0.5 (−2.4, 1.5)	0.0 (−2.1, 2.0)	0.1 (−2.0, 2.1)
SFA	1 En%	0.0 (−0.7, 0.7)	−1.4 (−2.3, −0.6)	−0.6 (−1.5, 0.3)	−0.4 (−1.6, 0.8)
MUFA	1 En%	0.8 (0.0, 1.6)	0.1 (−0.8, 0.9)	−0.1 (−1.0, 0.9)	−0.3 (−1.4, 0.9)
PUFA	1 En%	1.5 (0.5, 2.5)	1.5 (0.4, 2.6)	1.5 (0.4, 2.7)	0.9 (−0.4, 2.2)
TFA	0.1 En%	1.2 (0.0, 2.4)	−0.3 (−1.7, 1.0)	−0.6 (−1.9, 0.6)	−0.1 (−1.8, 1.5)
HOMA-IR					
Total FA	5 En%	2.8 (−0.2, 5.9)	−0.8 (−4.1, 2.4)	0.4 (−2.0, 2.9)	0.3 (−2.1, 2.8)
SFA	1 En%	0.0 (−1.3, 1.3)	−2.2 (−3.7, −0.8)	−0.9 (−2.1, 0.3)	−1.4 (−2.8, 0.0)
MUFA	1 En%	1.5 (0.0, 3.0)	0.0 (−1.6, 1.5)	0.4 (−0.7, 1.6)	0.9 (−0.6, 2.4)
PUFA	1 En%	1.9 (0.6, 3.2)	2.0 (0.6, 3.5)	2.4 (1.0, 3.9)	0.2 (−1.5, 1.8)
TFA	0.1 En%	2.9 (1.1, 4.7)	0.8 (−1.6, 3.3)	−0.4 (−2.3, 1.6)	0.6 (−1.5, 2.8)
HOMA-B					
Total FA	5 En%	3.4 (1.5, 5.3)	−0.4 (−2.7, 1.9)	0.0 (−2.4, 2.4)	−0.1 (−2.5, 2.3)
SFA	1 En%	0.5 (−0.4, 1.4)	−1.5 (−2.7, −0.4)	−0.8 (−2.0, 0.4)	−1.5 (−2.9, −0.2)
MUFA	1 En%	1.5 (0.6, 2.5)	0.3 (−0.7, 1.3)	0.2 (−0.8, 1.3)	0.5 (−0.9, 2.0)
PUFA	1 En%	1.8 (0.6, 3.1)	1.3 (0.1, 2.6)	1.6 (0.3, 2.8)	0.3 (−1.3, 1.9)
TFA	0.1 En%	2.7 (1.1, 4.3)	0.6 (−1.3, 2.5)	0.4 (−1.5, 2.4)	1.5 (−0.6, 3.7)
Disposition Index					
Total FA	5 En%	−1.1 (−2.8, 0.6)	−0.1 (−2.1, 1.8)	−0.8 (−2.5, 0.9)	−0.9 (−2.6, 0.9)
SFA	1 En%	0.1 (−0.6, 0.7)	0.9 (0.1, 1.7)	0.3 (−0.4, 1.1)	0.3 (−0.6, 1.2)
MUFA	1 En%	−0.8 (−1.6, 0.1)	−0.1 (−1.0, 0.8)	−0.5 (−1.3, 0.3)	−0.7 (−1.7, 0.2)
PUFA	1 En%	−0.6 (−1.4, 0.3)	−1.1 (−2.0, −0.2)	−1.5 (−2.4, −0.5)	−0.1 (−1.2, 1.0)
TFA	0.1 En%	−0.7 (−1.7, 0.3)	0.2 (−1.1, 1.5)	0.8 (−0.4, 2.0)	0.5 (−0.9, 1.9)

Abbreviations: AUC, area under the curve; B, β-cell function; CI, confidence interval; %En, percentage of energy; FA, fatty acids; HbA1c, glycated hemoglobin; HOMA, Homeostatic Model Assessment; IR, insulin resistance; MUFA, mono-unsaturated fatty acids; NEO, Netherlands Epidemiology of Obesity study; PUFA, poly-unsaturated fatty acids; SFA, saturated fatty acids; TFA, trans fatty acids. [a]Linear regression coefficients of the ln-transformed markers of type 2 diabetes risk were back transformed and expressed as percentage with 95% CI. Individual data were weighted to the BMI distribution of the general Dutch population. [b]Model 1 adjusted for: age (years), sex (M/F); model 2 (energy substitution): additionally adjusted for total energy intake (kJ/day), alcohol (En%), dietary fiber (En%); model 3: additionally adjusted for total body fat (%), smoking (never/former/current smoker), education level (low/middle/high), physical activity (MET hours/week), family history of diabetes (no/yes), statin use (no/yes), energy under-reporter (no/yes), coffee (g/day), sugar-sweetened beverages (g/day), dietary cholesterol (mg/day); model 4 (carbohydrate substitution): additionally adjusted for protein (En%), and all other fatty acids (En%).

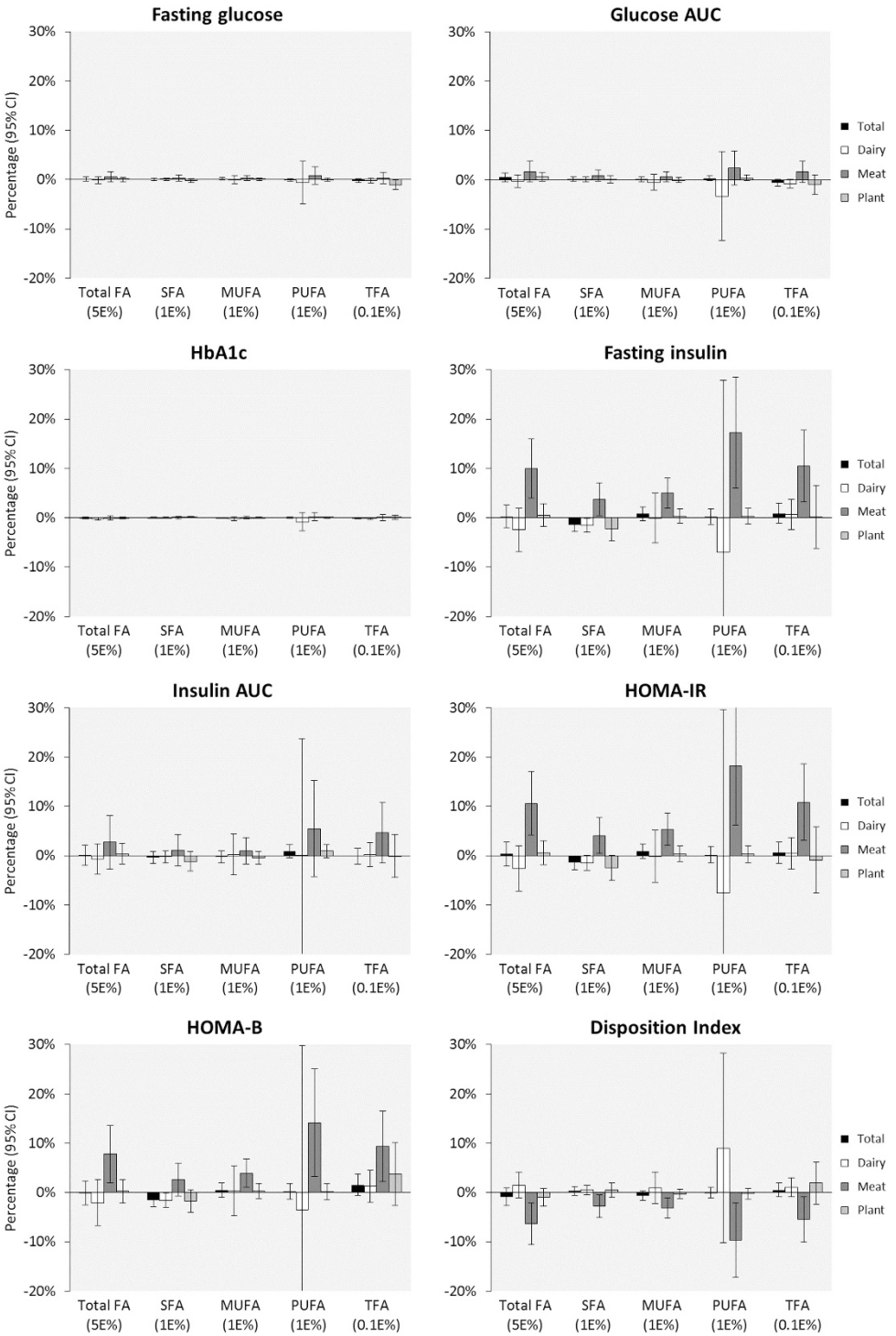

Figure 1. Associations (percentage (95% CI)) of the intake of types of dietary fatty acids and their dietary sources (dairy, meat and plant) with markers of type 2 diabetes risk in 5675 participants in the NEO study, 45–65 years without type 2 diabetes. AUC, area under the curve; B, β-cell function; CI, confidence interval; %En, percentage of energy; FA, fatty acids; HbA1c, glycated hemoglobin; HOMA, Homeostatic Model Assessment; IR, insulin resistance; MUFA, mono-unsaturated fatty acids; NEO, Netherlands Epidemiology of Obesity Study; PUFA, poly-unsaturated fatty acids; SFA, saturated fatty acids; TFA, trans fatty acids. Regression coefficients of the ln-transformed markers of type 2 diabetes risk were back transformed and expressed as percentage with 95% CI. Individual data were weighted to the BMI distribution of the general Dutch population. Linear regression models were adjusted for age (years), sex (M/F), total energy intake (kJ/day), alcohol (En%), dietary fiber (En%), total body fat (%), smoking (never/former/current smoker), education level (low/middle/high), physical activity (MET hours/week), family history of diabetes (no/yes), statin use (no/yes), energy under-reporter (no/yes), coffee (g/day), sugar-sweetened beverages (g/day), dietary cholesterol (mg/day), protein (En%) and all other fatty acids (En%).

diabetes.[21,35,36] Stratifying our analyses revealed that higher intakes of all fatty acids from meat were adversely associated with markers of insulin resistance and secretion, whereas the fatty acids from dairy and plant sources were not. Sensitivity analyses showed that these associations were robust. Our data suggest that the dietary source of fat is a more important determinant than the type of fatty acid in the relation between dietary fat intake and markers of diabetes risk.

There are several possible explanations for the associations between intakes of fatty acids from meat and markers of insulin resistance and secretion. First, it is conceivable that intake of fatty acids from meat reflects the intake of other active compounds in meat. High correlations between nutrients from the same dietary source preclude attributing observed associations to the specific nutrient under study, or to other compounds from the same dietary source. For example, associations between meat intake and risk of diabetes have been explained by components such as dietary cholesterol, protein, heme-iron, advanced glycation end products and preservatives such as sodium and nitrites/nitrates.[17,36,37] In our analysis, it was not possible to further explore this, because the food frequency questionnaire did not specify for types of meat consumed (red/white or processed/unprocessed).[38] Another explanation is that intake of fat from meat reflects other dietary or lifestyle habits related to diabetes risk, which may confound the observed associations. A higher consumption of meat is generally associated with higher BMI and waist circumference, lower physical activity and a lower education level.[39] Despite our extensive adjustment for a range of diet and lifestyle factors, we cannot fully exclude the presence of residual confounding by unmeasured or incompletely measured factors.

Our study adds evidence to the possible interactions between specific fatty acids and their dietary sources in their association with cardiometabolic disease. Earlier research showed that associations between SFA and incident cardiovascular disease depended on the dietary source.[20] It was observed that the consumption of SFA from dairy was inversely associated with cardiovascular disease risk, whereas the consumption of SFA from meat was associated with increased cardiovascular disease risk.[20] This study did, however, not address other types of dietary fat. Another recent prospective analysis observed that high-fat but not low-fat dairy products were associated with lower diabetes risk, and that both high- and low-fat meat products increased diabetes risk.[21] The authors suggested that fat in dairy could partly contribute to previously observed associations between dairy intake and reduced diabetes risk, and that meat intake was associated with increased risk, independently of the fat content.[21] Moreover, a meta-analysis indicated that sources of MUFA should be taken into account when evaluating cardiovascular risk.[40] When stratifying for dietary source, MUFA from olive oil resulted in significant inverse associations with all-cause mortality and cardiovascular disease events, whereas MUFA from mixed animal and vegetable sources did not.[40] Studies across populations are needed to better assess the interaction between fatty acids and their dietary sources.

In the present study, we used energy substitution models in which specific fatty acids were isocalorically compared with carbohydrates and with each other. An advantage of substitution models is that they mimic effects of exchanging nutrients against each other, as is the case in dietary intervention studies.[32,41] In addition, substitution models make the results less dependent on the composition of the average rest of the diet;[32] therefore, results can be compared with other observational studies that used substitution models. However, a limitation is that results from our substitution models can be difficult to translate into practice. For example, in real life it is impossible to replace SFA from meat with total carbohydrate, while keeping other fatty acids from meat constant. Therefore, we repeated all analyses with protein as comparison nutrient and we repeated all analyses without specifying the carbohydrate substitution model. The results showed little differences and confirmed the robustness of the observed associations between fatty acids from meat and risk markers of diabetes.

Strengths of our study are the large population size and the detailed information on diet, markers of diabetes risk and potential confounding factors. Studying blood markers of type 2 diabetes risk (including glucose and HbA1c status) rather than studying clinically diagnosed type 2 diabetes offers the benefits of continuous over discrete data and avoids diagnostic misclassification. A limitation is that we could not measure insulin sensitivity by the hyperinsulinemic–euglycemic clamp technique, which is considered the gold-standard. Instead, we measured fasting insulin, HOMA-IR, HOMA-B and postprandial Disposition Index to assess hepatic insulin sensitivity, insulin secretion and β-cell function accounting for variations in whole-body insulin sensitivity. These markers, in particular measured after a mixed meal challenge, have been confirmed as an adequate substitute for the clamp technique in large population studies.[42–44] Another limitation is the cross-sectional and observational nature of our study, leaving room for reverse causation and residual confounding.

In conclusion, in this non-diabetic population, when isocalorically compared with carbohydrates, total dietary SFA intake was weakly inversely associated with markers of insulin resistance and secretion, whereas other fatty acids were not. Our results suggest that the relations between the intake of fatty acids and markers of insulin resistance and secretion may depend on the dietary source of fatty acids. More epidemiological studies on diet and cardiometabolic disease are needed addressing possible interactions between nutrients and their dietary sources.

CONFLICT OF INTEREST

AJW, MA and PLZ are employed by Unilever; Unilever markets food products made of vegetable oils, including margarines and dressings. The remaining authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We express our gratitude to all individuals who participate in the Netherlands Epidemiology in Obesity study. We are grateful to all participating general practitioners for inviting eligible participants. We furthermore thank all research nurses for collecting the data and I de Jonge, MSc for all data management of the NEO study. We thank Nutricia Research, Utrecht, the Netherlands, for providing the mixed meal and Unilever Research & Development, Vlaardingen, the Netherlands for supporting the dietary intake validation study. The NEO study is led by the NEO study group (Supplementary Information S2). The NEO study is supported by the participating departments, the Division and the Board of Directors of the Leiden University Medical Center, and by the Leiden University, Research Profile Area 'Vascular and Regenerative Medicine'.

REFERENCES

1 International Diabetes Federation. *IDF Diabetes Atlas* 7th edition. Brussels, Belgium: International Diabetes Federation, 2015. http://www.diabetesatlas.org.
2 Schellenberg ES, Dryden DM, Vandermeer B, Ha C, Korownyk C. Lifestyle interventions for patients with and at risk for type 2 diabetes: a systematic review and meta-analysis. *Ann Intern Med* 2013; **159**: 543–551.
3 Foods and Agricultural Organization, World Health Organization. Fats and fatty acids in human nutrition. Report of an expert consultation. FAO Food Nutr Pap 2010 Report No. 91, pp 1–166.
4 Storlien LH, Pan DA, Kriketos AD, O'Connor J, Caterson ID, Cooney GJ *et al.* Skeletal muscle membrane lipids and insulin resistance. *Lipids* 1996; **31**(Suppl): S261–S265.
5 Riserus U, Willett WC, Hu FB. Dietary fats and prevention of type 2 diabetes. *Progr Lipid Res* 2009; **48**: 44–51.
6 Vessby B, Uusitupa M, Hermansen K, Riccardi G, Rivellese AA, Tapsell LC *et al.* Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: the KANWU study. *Diabetologia* 2001; **44**: 312–319.

7 Bos MB, de Vries JHM, EJM Feskens, van Dijk SJ, DWM Hoelen, Siebelink E *et al.* Effect of a high monounsaturated fatty acids diet and a Mediterranean diet on serum lipids and insulin sensitivity in adults with mild abdominal obesity. *Nutr Metab Cardiovasc Dis* 2010; **20**: 591–598.
8 Jebb SA, Lovegrove JA, Griffin BA, Frost GS, Moore CS, Chatfield MD *et al.* Effect of changing the amount and type of fat and carbohydrate on insulin sensitivity and cardiovascular risk: The RISCK (Reading, Imperial, Surrey, Cambridge, and Kings) trial. *Am J Clin Nutr* 2010; **92**: 748–758.
9 Tierney AC, McMonagle J, Shaw DI, Gulseth HL, Helal O, Saris WHM *et al.* Effects of dietary fat modification on insulin sensitivity and on other risk factors of the metabolic syndrome-LIPGENE: a European randomized dietary intervention study. *Int J Obes* 2011; **35**: 800–809.
10 Bjermo H, Iggman D, Kullberg J, Dahlman I, Johansson L, Persson L *et al.* Effects of n-6 PUFAs compared with SFAs on liver fat, lipoproteins, and inflammation in abdominal obesity: a randomized controlled trial. *Am J Clin Nutr* 2012; **95**: 1003–1012.
11 Rosqvist F, Iggman D, Kullberg J, Cedernaes J, Johansson HE, Larsson A *et al.* Overfeeding polyunsaturated and saturated fat causes distinct effects on liver and visceral fat accumulation in humans. *Diabetes* 2014; **63**: 2356–2368.
12 Summers LKM, Fielding BA, Bradshaw HA, Ilic V, Beysen C, Clark ML *et al.* Substituting dietary saturated fat with polyunsaturated fat changes abdominal fat distribution and improves insulin sensitivity. *Diabetologia* 2002; **45**: 369–377.
13 Micha R, Mozaffarian D. Saturated fat and cardiometabolic risk factors, coronary heart disease, stroke, and diabetes: a fresh look at the evidence. *Lipids* 2010; **45**: 893–905.
14 Harding AH, Day NE, Khaw KT, Bingham S, Luben R, Welsh A *et al.* Dietary fat and the risk of clinical type 2 diabetes: The European Prospective Investigation of Cancer-Norfolk Study. *Am J Epidemiol* 2004; **159**: 73–82.
15 Meyer KA, Kushi LH, Jacobs J, Folsom AR. Dietary fat and incidence of type 2 diabetes in older Iowa women. *Diabetes Care* 2001; **24**: 1528–1535.
16 Salmeron J, Hu FB, Manson JE, Stampfer MJ, Colditz GA, Rimm EB *et al.* Dietary fat intake and risk of type 2 diabetes in women. *Am J Clin Nutr* 2001; **73**: 1019–1026.
17 Van Dam RM, Willett WC, Rimm EB, Stampfer MJ, Hu FB. Dietary fat and meat intake in relation to risk of type 2 diabetes in men. *Diabetes Care* 2002; **25**: 417–424.
18 Hodge AM, English DR, O'Dea K, Sinclair AJ, Makrides M, Gibson RA *et al.* Plasma phospholipid and dietary fatty acids as predictors of type 2 diabetes: interpreting the role of linoleic acid. *Am J Clin Nutr* 2007; **86**: 189–197.
19 Kroger J, Zietemann V, Enzenbach C, Weikert C, Jansen EH, Doring F *et al.* Erythrocyte membrane phospholipid fatty acids, desaturase activity, and dietary fatty acids in relation to risk of type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. *Am J Clin Nutr* 2011; **93**: 127–142.
20 De Oliveira Otto MC, Mozaffarian D, Kromhout D, Bertoni AG, Sibley CT, Jacobs Jr DR *et al.* Dietary intake of saturated fat by food source and incident cardiovascular disease: the Multi-Ethnic Study of Atherosclerosis. *Am J Clin Nutr* 2012; **96**: 397–404.
21 Ericson U, Hellstrand S, Brunkwall L, Schulz CA, Sonestedt E, Wallstrom P *et al.* Food sources of fat may clarify the inconsistent role of dietary fat intake for incidence of type 2 diabetes. *Am J Clin Nutr* 2015; **101**: 1065–1080.
22 de Mutsert R, den Heijer M, Rabelink TJ, Smit JW, Romijn JA, Jukema JW *et al.* The Netherlands Epidemiology of Obesity (NEO) study: study design and data collection. *Eur J Epidemiol* 2013; **28**: 513–523.
23 Siebelink E, Geelen A, de Vries JH. Self-reported energy intake by FFQ compared with actual energy intake to maintain body weight in 516 adults. *Br J Nutr* 2011; **106**: 274–281.
24 Verkleij-Hagoort AC, de Vries JHM, Stegers MPG, Lindemans J, NTC Ursem, RPM Steegers-Theunissen. Validation of the assessment of folate and vitamin B12 intake in women of reproductive age: the method of triads. *Eur J Clin Nutr* 2007; **61**: 610–615.
25 National Institute for Public Health and the Environment (RIVM). NEVO: Dutch Food Composition Database, 2011.
26 Wendel-Vos GCW, Schuit AJ, Saris WHM, Kromhout D. Reproducibility and relative validity of the short questionnaire to assess health-enhancing physical activity. *J Clin Epidemiol* 2003; **56**: 1163–1169.
27 Heitmann BL, Lissner L, Osler M. Do we eat less fat, or just report so? *Int J Obes* 2000; **24**: 435–442.
28 Paalanen L, Mannisto S, Virtanen MJ, Knekt P, Rasanen L, Montonen J *et al.* Validity of a food frequency questionnaire varied by age and body mass index. *J Clin Epidemiol* 2006; **59**: 994–1001.
29 Black AE. Critical evaluation of energy intake using the Goldberg cut-off for energy intake: basal metabolic rate. A practical guide to its calculation, use and limitations. *Int J Obes* 2000; **24**: 1119–1130.
30 Goldberg GR, Black AE, Jebb SA, Cole TJ, Murgatroyd PR, Coward WA *et al.* Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording. *Eur J Clin Nutr* 1991; **45**: 569–581.
31 Mendez MA, Popkin BM, Buckland G, Schroder H, Amiano P, Barricarte A *et al.* Alternative methods of accounting for underreporting and overreporting when measuring dietary intake-obesity relations. *Am J Epidemiol* 2011; **173**: 448–458.
32 Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr* 1997; **65**(Suppl): 122S–1228S.
33 Tabak AG, Jokela M, Akbaraly TN, Brunner EJ, Kivimaki M, Witte DR. Trajectories of glycaemia, insulin sensitivity, and insulin secretion before diagnosis of type 2 diabetes: an analysis from the Whitehall II study. *Lancet* 2009; **373**: 2215–2221.
34 Weyer C, Bogardus C, Mott DM, Pratley RE. The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. *J Clin Invest* 1999; **104**: 787–794.
35 Gijsbers L, Ding EL, Malik VS, GJ de, Geleijnse JM, Soedamah-Muthu SS. Consumption of dairy foods and diabetes incidence: a dose-response meta-analysis of observational studies. *Am J Clin Nutr* 2016; **103**: 1111–1124.
36 Pan A, Sun Q, Bernstein AM, Manson JE, Willett WC, Hu FB. Changes in red meat consumption and subsequent risk of type 2 diabetes mellitus: three cohorts of US men and women. *JAMA Intern Med* 2013; **173**: 1328–1335.
37 Feskens EJ, Sluik D, van Woudenbergh GJ. Meat consumption, diabetes, and its complications. *Curr Diab Rep* 2013; **13**: 298–306.
38 Feunekes GIJ, Van Staveren WA, de Vries JHM, Burema J, Hautvast JGAJ. Relative and biomarker-based validity of a food-frequency questionnaire estimating intake of fats and cholesterol. *Am J Clin Nutr* 1993; **58**: 489–496.
39 Pan A, Sun Q, Bernstein AM, Schulze MB, Manson JE, Willett WC *et al.* Red meat consumption and risk of type 2 diabetes: 3 cohorts of US adults and an updated meta-analysis. *Am J Clin Nutr* 2011; **94**: 1088–1096.
40 Schwingshackl L, Hoffmann G. Monounsaturated fatty acids, olive oil and health status: a systematic review and meta-analysis of cohort studies. *Lipids Health Dis* 2014; **13**: 154.
41 Jakobsen MU, O'Reilly EJ, Heitmann BL, Pereira MA, Bälter K, Fraser GE *et al.* Major types of dietary fat and risk of coronary heart disease: a pooled analysis of 11 cohort studies. *Am J Clin Nutr* 2009; **89**: 1425–1432.
42 Maki KC, Kelley KM, Lawless AL, Hubacher RL, Schild AL, Dicklin MR *et al.* Validation of insulin sensitivity and secretion indices derived from the liquid meal tolerance test. *Diabetes Technol Ther* 2011; **13**: 661–666.
43 Rijkelijkhuizen JM, Girman CJ, Mari A, Alssema M, Rhodes T, Nijpels G *et al.* Classical and model-based estimates of beta-cell function during a mixed meal vs. an OGTT in a population-based cohort. *Diabetes Res Clin Pract* 2009; **83**: 280–288.
44 Otten J, Ahren B, Olsson T. Surrogate measures of insulin sensitivity vs the hyperinsulinaemic-euglycaemic clamp: a meta-analysis. *Diabetologia* 2014; **57**: 1781–1788.

Supplementary Information accompanies this paper on European Journal of Clinical Nutrition website (http://www.nature.com/ejcn)