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Effect of *trans*-fatty acid intake on insulin sensitivity and intramuscular lipids—a randomized trial in overweight postmenopausal women

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ABSTRACT

Intake of industrially produced *trans*-fatty acids (TFA) has been linked to increased risk of type 2 diabetes mellitus in observational studies. We investigated the causality of this association by examining if a high intake of TFA impairs measures of glucose homeostasis and induces intramuscular lipid deposition in abdominally obese women. In a double-blind, parallel dietary intervention study, 52 healthy but overweight postmenopausal women were randomized to receive either partially hydrogenated soybean oil (15 g/d TFA) or a control oil (mainly oleic and palmitic acid) for 16 weeks. Three markers of glucose homeostasis and 4 markers of lipolysis were derived from glucose, insulin, C-peptide, nonesterified fatty acid, and glycerol concentrations during a 3-hour frequent sampling oral glucose tolerance test. Intramuscular lipids were assessed by magnetic resonance spectroscopy. Forty-nine women completed the study. Insulin sensitivity (assessed by $ISI_{composite}$), β-cell function (the disposition index), and the metabolic clearance rate of insulin were not significantly affected by the dietary intervention. Neither was the ability of insulin to suppress plasma nonesterified fatty acid and glycerol during oral glucose ingestion nor the intramuscular lipid deposition. In conclusion, high TFA intake did not affect glucose metabolism over 16 weeks in postmenopausal overweight women. A study population with a stronger predisposition to insulin resistance and/or a longer duration of exposure may be required for insulin sensitivity to be affected by intake of industrial TFA.

1. Introduction

Intake of *trans*-fatty acids (TFA) is associated to an increased risk of type 2 diabetes mellitus according to data from the prospective Nurses' Health Study. Replacement of 2 percentage of energy (E%) from TFA by polyunsaturated fatty acids was estimated to decrease the risk of type 2 diabetes mellitus by 40% [1]. Whereas others have failed to find such an association in

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Author contributions: NTB, SBH, TML, SS, and AA designed the study; NTB and EC conducted the data collection; and NTB performed the data analyses and wrote the manuscript. All authors were involved in interpretation of the data and review of the manuscript.

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large prospective cohort studies with slightly lower median TFA intakes (~1.3 E% vs 2 E% in the Nurses' Health Study) [2,3], an Iranian cross-sectional study points in the same direction [4].

A few controlled intervention studies have investigated the effect of TFA intake on measures of glucose homeostasis, but the results are inconclusive. In lean and healthy young subjects, a high intake of either ruminant or industrial TFA does not seem to impair insulin and glucose metabolism in short-term studies of 4 to 5 weeks' duration [5-7]. Recently, it was shown that approximately 5 g/d of TFA (ruminant as well as industrial) did not affect peripheral insulin sensitivity in moderately overweight women either [8]. In contrast, meals with high TFA contents have been shown to produce higher postprandial insulin and C-peptide concentrations than similar meals with *cis*-fatty acids in overweight subjects and type 2 diabetes mellitus patients [9,10].

We hypothesized that industrial TFA might affect substrate metabolism in several ways that would increase the risk of developing type 2 diabetes mellitus: A high TFA intake might (1) induce peripheral insulin resistance, possibly through increased deposition of intramuscular lipids [11,12]; (2) impair β-cell function; and (3) increase lipolysis by decreasing the inhibitory action of insulin.

To explore these hypotheses, we conducted a dietary intervention study examining the effect of long-term exposure to high daily amounts of industrial TFA on glucose homeostasis and markers of lipolysis in healthy but abdominally obese postmenopausal women. We anticipated that a 16-week intervention period would be sufficiently long to allow for incorporation of TFA into muscle structural lipids with possible influence on development of insulin resistance [13] and for induction of intramuscular lipid accumulation [11].

2. Methods

This work is based on a dietary intervention study examining the effect of a high intake of TFA on multiple risk markers for cardiovascular disease and type 2 diabetes mellitus [14]. In this article, we report the results for insulin sensitivity, β-cell function, markers of lipolysis, and intramuscular lipids. The measurements reported here were collected at baseline (week 0) and at the end of the dietary intervention (week 16).

2.1. Study design

The dietary intervention study had a randomized, double-blind, parallel design. Fifty-two healthy, moderately overweight postmenopausal women were allocated to 26 g/d of partially hydrogenated soybean oil with approximately 60% TFA (n = 25) or control oil (CTR; n = 27) for 16 weeks. The participants were randomized to the 2 diets stratified by waist circumference. The computer-generated randomization sequences with random permuted blocks of 4 were kept undisclosed to the investigators until after study completion. All study personnel and participants were blinded to the treatment assignment for the duration of the study.

The subjects were recruited from Copenhagen, Denmark, and the surrounding areas by advertising in the local media. Subject inclusion criteria were as follows: healthy; body mass index between 25 and 32 kg/m^2; waist circumference greater than 80 cm; postmenopausal (self-reported) for the last 12 months; and aged 45 to 70 years. The exclusion criteria were as follows: known diabetes or other chronic diseases; history of cardiovascular disease; smoking; hypertension (>160/100 mm Hg); fasting plasma triglycerides greater than 3 mmol/L; fasting plasma low-density lipoprotein (LDL) cholesterol greater than 6 mmol/L; fasting plasma glucose greater than 7 mmol/L; use of hormones or antihypertensive, antilipidemic, or anticholesterolemic drugs; weight changes greater than 3 kg for 2 months before screening; participation in strenuous physical activity for more than 10 h/wk; and abnormalities in routine biochemical and hematological tests. The subjects were not allowed to donate blood during the study and 3 months before screening or to use fish oil as dietary supplements during the study and 1 month before commencement.

All participants gave their informed consent to the study, which was carried out at the University of Copenhagen, Denmark, was approved by the Municipal Ethical Committee of The Capital Region of Denmark (H-B_2007-089), and was registered at clinicaltrials.gov as NCT00655902.

2.2. Intervention and compliance

We compared the effect of consuming 15.7 g/d of TFA from partially hydrogenated soybean oil with that of a control oil consisting of a 50/50% mix of palm oil and high oleic sunflower oil. The test fats (kindly supplied by AarhusKarlshamn, Aarhus, Denmark) mainly differed in the content of TFA, palmitic (C16:0), oleic (*cis*-C18:1n-9), and linoleic acid (*cis*-C18:2n-6). The TFA fat consisted of 60.4% TFA (of which ~98% was *trans*-C18:1 and 2% *trans*-C18:2 isomers), 19.6% *cis*-C18:1, 12.7% C16:0, and 6.2% C18:0; and the control fat consisted of 61.4% *cis*-C18:1, 26.7% C16:0, 6.6% C18:2, and 3.8% C18:0. Both fats contained minor amounts of C14:0, C20:0, and C22:0.

The fats were incorporated into bread rolls providing a total of 600 kcal/d (41 E% from fat), equivalent to 28% of the subjects' energy requirements on average. A clinical dietician instructed the participants how to let the bread rolls isocalorically substitute food items from their habitual diets.

Dietary intake was measured using 3-day weighed food records at baseline and in the last week of the intervention. The only significant dietary differences between diet groups during the intervention were the contributions of energy from monounsaturated fatty acids (MUFA) and TFA, indicating that the diets were overall comparable apart from the fatty acid composition. The intake of TFA was higher (7.0 ± 0.2 E% [mean ± SEM] vs 0.3 ± 0.0 E%) and the intake of MUFA was lower (10.3 ± 0.4 E% vs 13.4 ± 0.8 E%) in the TFA group compared with the CTR group.

Self-reported compliance, as assessed by means of study diaries, showed that 98% of all test bread rolls were consumed, with no difference between diet groups. Incorporation of fatty acids into phospholipids of red blood cells was used as an objective compliance measure. Red blood cells from all TFA-fed subjects were enriched in *trans*-C18:1 residues after the dietary intervention, whereas those of control subjects were not (data not shown).

2.3. Measurements

At each visit, the subjects were told to be 10-hour fasted (except for 0.5 L water). They were instructed to avoid vigorous exercise and alcohol consumption on the day before and to consume similar carbohydrate-rich evening meals on the evening before each visit. Body weight and height were measured by standard procedures.

We assessed insulin sensitivity by use of frequent sampling 3-hour oral glucose tolerance tests (OGTTs) where subjects ingested a solution of 75 g glucose dissolved in 300 mL water. Venous blood samples were collected before and during the OGTT at the following time points: –10, 0, 10, 20, 30, 45, 60, 75, 90, 105, 120, 150, and 180 minutes for measurement of plasma glucose and serum insulin and C-peptide. Samples for measurement of plasma glycerol and nonesterified fatty acids (NEFA) were collected at –10, 0, 30, 60, 90, and 120 minutes.

Intra- and extramyocellular lipids (in psoas major) were measured by H^1 magnetic resonance (MR) spectroscopy [15] using Achieva 3.0-T MRI system (Philips Medical Systems) and a sense cardiac coil. A single-voxel PRESS sequence with the spectroscopy volumes of 11 mm × 11 mm × 11 mm was used for acquisition. The MR scanner's software was used to fit the acquired spectrum to obtain spectroscopic areas of water and intra- and extramyocellular lipids. Consequently, content of the lipids in percentage relative to water was calculated.

2.4. Laboratory analyses

Fasting and OGTT plasma glucose concentrations were analyzed by a colorimetric assay (Ortho-Clinical Diagnostics; Johnson & Johnson, Birkerød, Denmark) for the Vitros 5.1 FS analyzer (intraassay coefficient of variation: 5.1%). Fasting and OGTT serum insulin and C-peptide concentrations were analyzed by a solid-phase, enzyme-labeled chemiluminescent immunometric assay and a solid-phase, 2-site chemiluminescent immunometric assay, respectively (Siemens Medical Solutions Diagnostics, Ballerup, Denmark), for the IMMULITE 2500 analyzer (intraassay coefficients of variation: 4.2% and 2.6%, respectively).

Fasting and OGTT plasma glycerol concentrations were analyzed by a direct colorimetric assay (Randox, Crumlin, United Kingdom) and plasma NEFA concentrations were measured by an enzymatic colorimetric assay (Trichem, Frederikssund, Denmark), both for the Pentra 400 Analyzer (Horiba ABX Diagnostics, Montpellier, France).

Red blood cell phospholipid fatty acid composition was assessed as an objective compliance measure. The fatty acid composition of these phospholipids as well as of dietary fats was analyzed by gas chromatography (on an Agilent 6890 GC, Agilent Technologies, Little Falls, DE, USA) of fatty acid methyl esters on high-polarity cyano-silica columns (Varian, Walnut Creek, CA) and a flame ionization detector.

2.5. Calculations

Areas under the curves (AUCs) were calculated by means of the trapezoidal rule, and incremental AUCs (iAUCs) represent AUCs greater than the fasting value.

Three central parameters to describe insulin sensitivity, clearance of insulin, and β-cell function, respectively, during oral glucose ingestion were chosen:

1. Insulin sensitivity was estimated by use of the insulin sensitivity index $ISI_{composite}$ as proposed by Matsuda and DeFronzo, which has been shown to correlate closely with the *M* value of the hyperinsulinemic euglycemic clamp in individuals displaying a broad range of insulin sensitivity [16]. $ISI_{composite}$ was calculated as = 10 000/(fasting glucose · fasting insulin · mean $glucose_{t0\text{-}30\text{-}60\text{-}90\text{-}120}$ · mean $insulin_{t0\text{-}30\text{-}60\text{-}90\text{-}120}$)^{½}, where glucose is measured in milligrams per deciliter and insulin in microunits per milliliter.
2. The insulin metabolic clearance rate (MCRi) was estimated as the changes in prehepatic insulin secretion relative to plasma insulin concentration during the OGTT. The calculation of MCRi has previously been described in detail [17] and is based on the following principle: As C-peptide is co-secreted equimolarly with insulin from the pancreatic β-cells, but is not absorbed by the liver before entering the blood stream, the C-peptide concentrations during the OGTT allow for the estimation of the prehepatic insulin secretion rate (ISR). The estimation was performed by deconvolution of the C-peptide concentration using the computer program ISEC [18], which has been validated to calculate prehepatic ISR during intravenous glucose tolerance tests [19], meal tests [20], and OGTTs [21]. The ISR-AUC is an expression of the total amount of prehepatic insulin secreted during the OGTT. Because the insulin-AUC represents both insulin secretion and clearance, the MCRi can be calculated as the ratio of ISR-AUC to insulin-AUC.
3. β-Cell function was expressed by the disposition index that estimates the β-cell responsiveness taking into account ambient insulin sensitivity. A detailed description of these end points has been given by Haugaard et al [17]. Briefly, the index for β-cell responsiveness (or insulin secretory capacity), B_{total}, was calculated as the change in ISR per unit change in glucose concentration by performing cross-correlation analysis between plasma glucose concentrations and ISR during the OGTT. The disposition index was calculated as the product of B_{total} and $ISI_{composite}$.

Four integrated parameters to measure and describe lipolysis during oral glucose ingestion were chosen. First, the plasma concentrations of NEFA and glycerol at their nadir period during the OGTT (90-120 min); $NEFA_{mean,\ 90\text{-}120\ min}$; and $glycerol_{mean,\ 90\text{-}120\ min}$ were taken as independent markers of remnant lipolysis. Second, the ability of insulin to suppress both NEFA and glycerol during oral glucose ingestion was estimated as the change in NEFA and glycerol, respectively, relative to the integer of changes in insulin concentration during the OGTT ($\Delta NEFA/insulin_{iauc}$ and $\Delta glycerol/insulin_{iauc}$). The change in NEFA and glycerol during the OGTT (ΔNEFA and Δglycerol) was calculated as the change from *t* = 0 minute to the mean of values at *t* = 90 and 120 minutes as a percentage of the value at *t* = 0 minute.

2.6. Statistical analyses

The study size was estimated based on the effect of TFA on the LDL cholesterol to high-density lipoprotein (HDL) cholesterol ratio, which was one of the 3 primary end points of this study (the others being hepatic fat and insulin sensitivity). The results for blood lipids and hepatic fat have been reported previously [14]. It was estimated that a minimum of 48 subjects was required to detect an absolute difference of 0.5 in the LDL/HDL cholesterol ratio between diet groups with a statistical power of 80% and a 2-sided significance level of 5%. Fifty-two participants were recruited to allow for an estimated dropout rate of 10%.

Data were analyzed using SAS version 9.1 (SAS Institute, Cary, NC). The primary analysis included participants who completed the intervention (n = 49). The statistical significance level was defined as $P < .05$.

At baseline, characteristics of participants in the 2 diet groups were compared with the use of 2-tailed unpaired t tests or Kruskal-Wallis χ^2 tests for skewed data. Analysis of covariance (ANCOVA) was used to assess the baseline-adjusted difference between diet groups for variables measured at weeks 0 and 16. For variables with repeated measures over time (OGTT data), a mixed model of repeated measures examining the effect of diet and time (with time denoting the measurement time points during the OGTT) and their interactions was applied, again with the baseline value as a covariate. A Gaussian covariance structure was used for all variables. Variance homogeneity and normality were investigated by residual plots, histograms, and Shapiro-Wilk test; and data were log transformed when needed.

3. Results

Baseline characteristics for the 49 completers of the study (24 of 25 randomized to the TFA group and 25 of 27 randomized to the CTR group) are presented in Table 1 [22]. Body weight increased by 1.1 ± 0.2 kg in both diet groups during the dietary intervention, with no significant difference between diets ($P = .8$).

There was no effect of diet on fasting concentrations of glucose, insulin, C-peptide, glycerol, and NEFA (Table 2). Responses for glucose, insulin, C-peptide, and ISR during the 3-hour OGTT before and after the intervention are depicted in Fig. 1. At week 16, there were neither significant diet by time (in min) interactions nor significant effects of diet in repeated-measures analysis for any of these variables when adjusting for the response at baseline. The AUC values were not significantly different either (Fig. 1).

As expected, the baseline relationship between $ISI_{composite}$ and B_{total} fitted a hyperbola (n = 30, $r = 0.52$, $P = .004$) for subjects with *normal glucose tolerance* (NGT; defined as 2-hour glucose <7.8 mmol/L), whereas a shift to the left ($P = .06$) was seen for subjects with *impaired glucose tolerance* (IGT; 2-hour glucose ≥7.8 mmol/L).

None of the OGTT-derived parameters describing glucose homeostasis or lipolysis changed significantly in either diet group during the 16 weeks of intervention. Thereby, after the intervention, there was no difference between diet groups in $ISI_{composite}$, disposition index, MCRi, nadir concentrations of NEFA/glycerol, or the ability of insulin to suppress NEFA/glycerol when adjusting for baseline values (Table 2).

Intra- and extramyocellular lipids did not change during the dietary intervention in either study group (Table 2); neither did the ratio between the 2 (data not shown).

Table 1 – Subject characteristics at baseline for completers in the TFA and CTR groups

	TFA	CTR
n	24	25
Age (y)	58.5 ± 4.6	58.8 ± 5.5
Height (cm)	165.3 ± 5.5	166.9 ± 5.2
Weight (kg)	78.7 ± 7.1	78.4 ± 8.6
Body mass index (kg/m^2)	28.8 ± 1.7	28.1 ± 2.2
Waist circumference (cm)	97.1 ± 7.3	95.5 ± 6.8
Systolic blood pressure (mm Hg)	118 ± 12	118 ± 11
Diastolic blood pressure (mm Hg)	81 ± 8	82 ± 6
No. of NFG/IFG/type 2 diabetes mellitus (fasting) [a]	18/6/0	20/5/0
No. of NGT/IGT/type 2 diabetes mellitus (OGTT) [b,c]	11/11/2	19/6/0
Fasting blood parameters		
p-Glucose (mmol/L)	5.2 ± 0.6	5.3 ± 0.4
s-Insulin (pmol/L)	24 (16-37)	37 (25-54)
s-C-peptide (pmol/L)	563 (471-674)	598 (505-708)
p-Glycerol (pmol/L)	96 ± 38	84 ± 42
p-NEFA (μmol/L)	732 ± 162	693 ± 195
p-Triglycerides (mmol/L)	1.13 ± 0.50	1.19 ± 0.50
p-Cholesterol (mmol/L)	5.4 ± 0.9	5.5 ± 1.0
OGTT-derived parameters		
$ISI_{composite}$ [c]	7.3 (5.0-10.7)	5.3 (3.8-7.4)
B_{total} [c]	2.3 ± 0.2	3.0 ± 0.3 *
Disposition index [c]	14.9 (9.6-23.2)	14.6 (11.1-19.1)
MCRi (L/min) [c]	3.4 ± 0.3	2.7 ± 0.2 *
$NEFA_{mean,\ 90\text{-}120\ min}$ (μmol/L)	37 (28-50)	42 (35-53)
$\Delta NEFA/insulin_{iAUC}$ (%·L/[min pmol])	4.2 (3.2-5.5)	2.9 (2.1-4.0) *
$Glycerol_{mean,\ 90\text{-}120\ min}$ (pmol/L)	21 ± 3	28 ± 4
$\Delta Glycerol/insulin_{iAUC}$ (%·L/[min pmol]) [d]	3.7 ± 0.5	2.4 ± 0.3 *
Intramuscular lipids		
Intramyocellular lipids (%) [e]	1.6 (1.1-2.3)	1.5 (1.1-1.9)
Extramyocellular lipids (%) [e]	5.2 (4.0-6.7)	4.3 (3.5-5.4)
Total myocellular lipids (%) [e]	7.3 (6.0-9.0)	6.0 (5.0-7.2)

Values are means ± SD or geometric means (95% confidence intervals) for skewed data; completers only. NFG indicates normal fasting glucose; p, plasma; s, serum.

[a] Normal fasting glucose, fasting plasma glucose <5.6 mmol/L; IFG, fasting plasma glucose ≥5.6 to 6.9 mmol/L; type 2 diabetes mellitus, fasting plasma glucose ≥7.0 mmol/L [22].

[b] Normal glucose tolerance, 2-hour glucose <7.8 mmol/L during an OGTT; IGT, 2-hour glucose ≥7.8 and <11.1 mmol/L; type 2 diabetes mellitus, 2-hour glucose ≥11.1 mmol/L [22].

[c] n = 23 in the TFA group.

[d] n = 22 in the TFA group.

[e] Assessed by magnetic resonance spectroscopy of psoas major; n = 23 in the TFA group and n = 22 in the control group due to a few erroneous scans.

* $P < .05$ vs TFA group by 2-tailed unpaired t tests or Kruskal-Wallis χ^2 tests for skewed data.

Table 2 – Glucose, insulin, and lipid metabolic data and intramuscular lipids in the TFA and CTR groups after the 16-week dietary intervention

	TFA (n = 24)		CTR (n = 25)		TFA – CTR	
	Week 16 [a]	Change from baseline [b]	Week 16 [a]	Change from baseline [b]	Mean difference at week 16 [c]	P [d]
Fasting blood parameters						
p-Glucose (mmol/L)	5.3 (5.2 to 5.4)	0.02 ± 0.05	5.2 (5.1 to 5.3)	−0.10 ± 0.07	0.13 (−0.04 to 0.30)	.13
s-Insulin (pmol/L) [e]	32 (25 to 39)	0.0 (−5.3 to 9.9)	33 (27 to 41)	1.1 (−3.6 to 8.8)	−4 (−30 to 31)%	.77
s-C-peptide (pmol/L) [e]	605 (550 to 665)	22 (−54 to 127)	584 (532 to 641)	22 (−63 to 108)	4 (−9 to 18)%	.60
p-Glycerol (pmol/L) [e]	73 (61 to 88)	−9 (−38 to 0)	82 (69 to 98)	2 (−25 to 19)	−11 (−31 to 15)%	.37
p-NEFA (μmol/L)	626 (550 to 702)	−100 ± 31	673 (599 to 748)	−25 ± 55	−47 (−153 to 60)	.38
OGTT-derived parameters						
$ISI_{composite}$ [e,f]	5.8 (5.0 to 6.8)	−0.5 (−2.0 to 0.5)	6.0 (5.2 to 7.0)	−0.1 (−0.7 to 0.5)	−4 (−23 to 20)%	.72
B_{total} [f]	2.7 (2.3 to 3.1)	0.2 ± 0.2	2.4 (2.0 to 2.8)	−0.4 ± 0.2	0.3 (−0.3 to 0.8)	.36
Disposition index [e,f]	14.2 (11.8 to 17.1)	−0.7 (−3.0 to 3.0)	12.5 (10.4 to 14.9)	−1.1 (−2.7 to 1.2)	14 (−12 to 47)%	.31
MCRi (L/min) [e]	2.8 (2.5 to 3.0)	−0.5 ± 0.3	2.6 (2.3 to 2.8)	−0.2 ± 0.1	0.2 (−0.2 to 0.6)	.35
$NEFA_{mean,\ 90\text{-}120\ min}$ (μmol/L) [e]	41 (31 to 56)	10 (−15 to 26)	41 (30 to 55)	3 (−18 to 7)	2 (−34 to 56) %	.94
$\Delta NEFA/insulin_{iAUC}$ (%·L/[min pmol]) [e]	3.2 (2.7 to 3.9)	−0.2 (−1.2 to 0.4)	3.4 (2.8 to 4.1)	0.1 (−0.7 to 0.5)	−5 (−27 to 23) %	.68
$Glycerol_{mean,\ 90\text{-}120\ min}$ (pmol/L)	29 (23 to 36)	6.1 ± 4.0	24 (17 to 31)	−3.8 ± 4.5	5.5 (−4.5 to 15.4)	.27
$\Delta Glycerol/insulin_{iAUC}$ (%·L/[min pmol]) [g]	2.4 (1.8 to 2.9)	−1.0 ± 0.4	2.9 (2.4 to 3.4)	0.2 ± 0.2	−0.5 (−1.2 to 0.3)	.38
Intramuscular lipids						
Intramyocellular lipids (%) [e,h]	1.8 (1.6 to 2.1)	0.0 (−0.9 to 1.1)	1.6 (1.4 to 1.9)	−0.1 (−0.4 to 0.6)	12 (−11 to 42)%	.30
Extramyocellular lipids (%) [e,h]	4.7 (4.0 to 5.6)	−0.2 (−0.8 to 1.0)	4.2 (3.5 to 5.0)	−0.6 (−1.3 to 0.4)	12 (−11 to 42)%	.33
Total myocellular lipids (%) [e,h]	6.7 (5.8 to 7.7)	0.5 (−1.0 to 1.3)	6.1 (5.3 to 7.0)	−0.9 (−1.4 to 0.5)	10 (−10 to 35)%	.33

[a] Values are least squares means (95% confidence interval).
[b] Values are means ± SEM or medians (interquartile range) for skewed values; completers only.
[c] Mean baseline-adjusted difference (or relative difference) between diet groups at week 16 (TFA – CTR).
[d] *P* value is for differences between diet groups at week 16 with week 0 as a covariate, by ANCOVA.
[e] ANCOVA performed on log10-transformed values.
[f] n = 23 in the TFA group.
[g] n = 22 in the TFA group.
[h] Assessed by magnetic resonance spectroscopy of psoas major; n = 23 in the TFA group and n = 22 in the control group due to a few erroneous scans.

4. Discussion

The present study investigated if the positive association between intake of industrially produced TFA and risk of type 2 diabetes mellitus observed in the Nurses' Health Study [1] might be explained by adverse effects on insulin sensitivity and β-cell function. We found that, in metabolically vulnerable postmenopausal women, a high intake of TFA from partially hydrogenated soybean oil for 16 weeks had no measurable effect on any of the examined diabetes-related end points compared with intake of a control oil where TFA was replaced by mainly oleic but also palmitic and linoleic acid.

We did not find an effect of TFA exposure on insulin sensitivity in this study, despite high self-reported compliance with the intervention diet (98% of bread rolls consumed) and a significant enrichment with TFA isomers in red blood cell membranes of all subjects in the TFA group vs none in the CTR group, as reported previously [14]. Furthermore, the fact that the TFA group experienced a significant 34% increase in the plasma LDL/HDL cholesterol ratio [14] is indicative of good compliance. Dietary TFA has consistently been shown to increase this ratio compared with all other fatty acid classes [23].

We assessed insulin sensitivity by use of the OGTT-derived $ISI_{composite}$. This index of whole-body insulin sensitivity has been shown to correlate well with the rate of insulin-mediated glucose disposal during the euglycemic insulin clamp [16], although small changes in insulin sensitivity may be detected more accurately by use of the criterion standard glucose clamp [24]. An advantage of the OGTT is that it reflects physiological stimulation of insulin secretion. In addition, the few studies that have suggested an insulin-raising effect of TFA have used meal tests [10,25,9], suggesting that the enteroinsular axis may be of relevance.

However, it is a limitation of this study that sample size calculations were not based on insulin sensitivity. The relative mean difference between diets in $ISI_{composite}$ was −4% (95% confidence interval, −23 to 20) after the intervention (Table 2), indicating that we would not have been able to detect TFA-induced changes in $ISI_{composite}$ smaller than 20%. However, the glucose, insulin, and C-peptide responses to a glucose challenge were practically unaltered in both diet groups after the 16 weeks of intervention (Fig. 1), strongly suggesting that a larger sample size would not have led to opposing conclusions.

It is possible that the higher intake of palmitic acid in the CTR group may have attenuated putative differences between diet groups, as palmitic acid has been shown to induce insulin resistance in muscle tissue [26]. Yet, no within-group change was observed in either diet group. Furthermore, TFA feeding

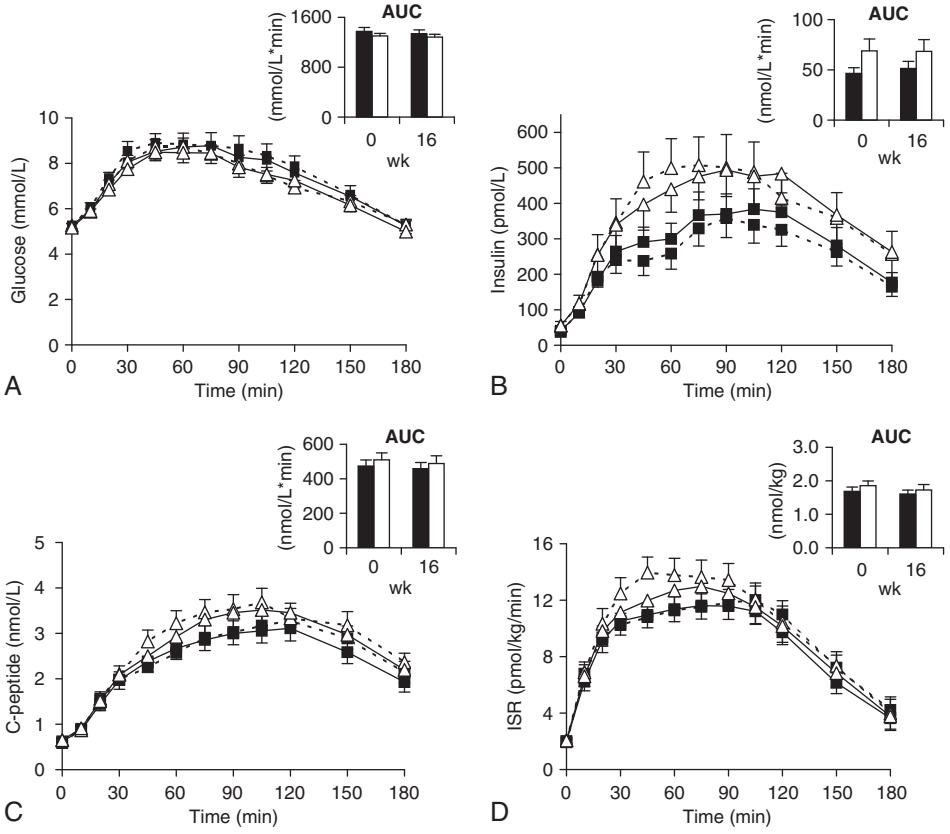

Fig. 1 – Glucose and insulin metabolism during OGTT. Mean (± SEM) glucose (A), insulin (B), and C-peptide (C) concentrations and ISRs (D) during the 3-hour OGTT performed at baseline (week 0; dotted lines) and after the dietary intervention (week 16; solid lines) in subjects who consumed 15.7 g/d *trans*-fatty acids (TFA; n = 24; black squares) and a control oil (CTR; n = 25; white triangles). The corresponding AUCs are also depicted (black bars = TFA; white bars = CTR). No diet × time interaction was observed for glucose (*P* = .81), insulin (*P* = 1.00), C-peptide (*P* = .98), or ISR (*P* = .90) by repeated-measures analysis; nor were the AUCs significantly different in baseline-adjusted analyses (*P* > .39). The insulin response was slightly higher in the control group at baseline (significant diet × time interaction in repeated-measures analysis [*P* < .001], with no significant difference between diets at individual time points after Bonferroni correction); however, the AUC value was not statistically different from the TFA group (*P* = .11 by unpaired *t* test). There were no significant baseline differences between the 2 groups for the other variables.

has been found to adversely affect insulin sensitivity even when compared with palmitic acid in rodents [27,28]. Moreover, the evidence for a detrimental effect of saturated fatty acids on insulin sensitivity in humans is not strong; 2 large multicenter studies, the Reading, Imperial, Surrey, Cambridge, and Kings trial and LIPGENE (n = 548 and 417, respectively), recently failed to detect an effect of diets rich in saturated fat on insulin sensitivity, assessed by the intravenous glucose tolerance tests [29,30].

To examine possible effects of TFA on glucose metabolism beyond those on whole-body insulin sensitivity, we calculated additional indices derived from the OGTT data; but neither the metabolic clearance rate of insulin, MCRi, nor the disposition index was significantly affected by the dietary intervention. We also found no effect on the ability of insulin to suppress NEFA during oral glucose ingestion, which is suggested to reflect the sensitivity of adipose tissue to the antilipolytic effect of insulin. Adipose tissue insulin sensitivity is impaired in healthy subjects with muscle insulin resistance [31].

Furthermore, we hypothesized that TFA might affect glucose metabolism through induction of ectopic intramuscular lipid accumulation, as previously observed in rats [12]. Intramuscular fat (in combination with diminished mitochondrial lipid oxidation) is thought to be a major contributor to obesity-associated insulin resistance as reviewed by Corcoran et al [11]. However, TFA did not increase the deposition of intramuscular lipids in this study. In contrast, 12 weeks of supplementation with conjugated linoleic acid (1.2 wt%) significantly increased the intramuscular fat content in growing pigs' longissimus dorsi by 19% [32]. This indicates that relatively small amounts of specific fatty acids may affect muscle lipid accumulation, at least during growth; unfortunately, insulin sensitivity was not assessed in this study.

Our results are in agreement with findings from previous studies of shorter duration (≤5 weeks) in lean and overweight subjects, finding no effect of industrial TFA on pancreatic insulin secretion or insulin sensitivity [5,6,8]. We hypothesized that the failure of these studies to show an effect was

due to (1) too short study durations not allowing time for TFA to be incorporated into myocellular membrane lipids with possible influence on insulin sensitivity [13], (2) too low TFA intake levels, and (3) too young and healthy study populations. Lovejoy et al [6] found a trend toward a decrease in insulin sensitivity after TFA intake only in overweight subjects, and Vega-Lopez et al [33] reported that TFA consumption modestly increased fasting insulin levels in older and moderately hyperlipidemic subjects.

Accordingly, in moderately overweight subjects, consumption of a single meal with 10 E% TFA resulted in increased postprandial levels of insulin and C-peptide compared with a meal with *cis*-MUFA [9]; and the same picture emerged in type 2 diabetes mellitus patients after consumption of a diet very high in industrial TFA (20 E%) [10]. In the latter study, an increase of 59% in postprandial insulin response was seen after TFA compared with MUFA intake, whereas the response did not differ from that seen after consumption of saturated fatty acids.

This raises the question of whether TFA intake only will exert adverse effects on glucose metabolism at very high intake levels in subjects with a strong underlying predisposition to insulin resistance and only when compared with MUFA. Alternatively, very long exposures may be required. After 6 years on a diet with 8 E% TFA, increased postprandial insulin concentrations were found in green monkeys compared with monkeys fed MUFA [25]. However, the question has not previously been answered by studies using adequate insulin sensitivity assessment methodology. A recent study showed no changes in peripheral insulin sensitivity assessed by hyperinsulinemic euglycemic clamps after TFA consumption in overweight postmenopausal women, but both the duration (4 weeks) and the dosing of TFA (5 g/d) may have been suboptimal. Yet, in the present study, we also failed to find an effect in abdominally obese older women after a relatively long period of high TFA exposure (15 g/d) using the validated OGTT-derived $ISI_{composite}$.

Of the 49 women who completed the present study, 30 had NGT and 19 had IGT or type 2 diabetes mellitus (based on the baseline 2-hour glucose values and using the criteria proposed by The American Diabetes Association [22]). When excluding the women with NGT in a post hoc analysis, the $ISI_{composite}$ was 15% higher (95% confidence interval: −22 to 68; $P = .46$) in the TFA group compared with controls after the intervention (data not shown), leaving no indication of a more negative effect of TFA in these women. However, this analysis included only 13 and 6 women in the TFA and CTR groups, respectively.

As no detrimental effects on glucose metabolism were seen in metabolically vulnerable subjects after 16 weeks of high daily amounts of TFA in the present study, we conclude that either (1) unrealistically high intake levels and/or a longer duration of exposure may be required for insulin sensitivity to be affected by intake of industrial TFA or (2) the observational data showing associations to risk of type 2 diabetes mellitus are potentially flawed by confounding. It may indeed be difficult to separate the effects of industrial TFA intake per se from those of an unhealthy lifestyle with high fast-food consumption.

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