Original Article

Supplementation with *trans* fatty acid at 1% energy did not increase serum cholesterol irrespective of the obesity-related genotypes in healthy adult Japanese

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Background and Objectives: The excessive intake of *trans* fatty acids increases serum low-density lipoprotein-cholesterol and reduces high-density lipoprotein-cholesterol. We studied the effects of 1% energy *trans* fatty acid supplementation on serum lipid concentrations in healthy adult Japanese with different obesity-related gene polymorphisms. **Methods and Study Design:** A randomized, double-blind, parallel trial was conducted in 53 healthy adults. The volunteers consumed one cookie containing either 1% energy or <0.01% energy (control) of *trans* fatty acids every day for 4 weeks, and a blood sample was then obtained after overnight fasting. The single nucleotide polymorphisms of the fat mass- and obesity-associated gene rs9939609 and beta-3 adrenergic receptor rs4994 were genotyped. **Results:** The mean *trans* fatty acid intake of the control and *trans* fatty acid groups corresponded to 0.28% and 1.31 % energy, respectively. There were no significant differences in serum cholesterol (total, low-density lipoprotein and high-density lipoprotein) or triacylglycerol between the control and *trans* fatty acid groups. The responses of serum cholesterol, triacylglycerol, glucose, insulin and hemoglobinA1c were also independent of the fat mass- and obesity-associated gene and beta-3 adrenergic receptor gene variants. **Conclusions:** Our findings indicate that supplementation with 1% energy *trans* fatty acids has little effect on serum cholesterol in healthy adult Japanese, regardless of genotype of fat mass- and obesity-associated gene or beta-3 adrenergic receptor. More systematic studies, with respect to dietary *trans* fatty acid intakes above those used here, may be warranted to determine the tolerable upper level of dietary *trans* fatty acid.

Key Words: partially hydrogenated oil, trans fatty acid, serum lipid, intervention trial, single nucleotide polymorphisms

INTRODUCTION

A number of human studies in Western countries have shown that *trans* fatty acids (TFAs) at a dietary intake above 4%–6% energy (%E) increased blood LDL-cholesterol and reduced HDL-cholesterol.[1] However, there are few intervention studies on the effect of low-level TFA intakes , and no satisfactory evidence exists relevant to the tolerable upper level of TFA intake,[2,3] although a few data points were found for the allowable level of TFAs from partially hydrogenated vegetable oils at <3% energy.[4] The WHO has recommended that TFA intake should be <1% of total energy intake, but limited data are available as to the influence of TFA intake of as low as 1% on serum cholesterol. In a series of our feeding trials[5-7] there were no appreciable adverse effects of supplementation with 0.6%E (young and adult Japanese women) or 1% TFAs (young Japanese women, 18.3±0.8 years old) on serum cholesterol.

Genome-wide association studies have shown that variance in the fat mass- and obesity-associated (FTO) gene is associated with the risk of obesity in Europeans and Asians.[8,9] Significant associations of the single nucleotide polymorphism (SNP) of FTO rs9939609 with the risks of cardiovascular disease and type 2 diabetes have been observed.[8,10] In addition, high dietary saturated fat intake accentuates the obesity risk associated with the FTO gene in adults[11] and strengthens the association between the FTO gene and BMI.[12]

The beta-3 adrenergic receptor plays a significant role in the control of lipolysis and energy expenditure.[13,14] Polymorphism of the beta-3 adrenergic receptor gene is connected with increased weight gain, insulin resistance, and the development of non-insulin-dependent diabetes mellitus.[15] In Japanese, the allelic frequency of the variant reaches 20%, whereas this frequency is 4%–11% in European countries and Caucasian populations in the U.S.[15] We speculated that individuals with the obesity-related

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gene variant might be influenced more strongly by dietary TFAs, and we conducted the present study to determine the influence of 1% TFA intake in subjects with either of both of the above-mentioned gene variants.

SUBJECTS AND METHODS

Subjects

Forty-three healthy women and 14 men (age 30–69 years) were recruited in Toyama Prefecture, Japan. Two men and one woman with dyslipidemia were excluded, and one man allocated to the TFA group dropped out of the study for personal reasons. All participants were Japanese, generally healthy, and had no history of diabetes. All subjects received a complete verbal and written explanation of the study, and written informed consent was obtained from all participants.

We divided the subjects randomly into two groups: the control (n=27) and TFA (n=26) groups. Table 1 shows the characteristics of the subjects. Nine of the control and 11 of the TFA subjects had the FTO gene variant with one homozygote each. In both groups, beta-3 adrenergic receptor gene variant was observed in 10 subjects with one homozygote each. The gene variants were thus evenly distributed in the two groups.

The mean TFA intake from the participant's usual diets was the same for the two groups, approx. 0.35%E, ranging from 0.01% to 0.79%E. There was no significant difference in cholesterol intake between the two groups. The protocol of this study was approved by the Ethical Review Board of Toyama College (No. H27-6), and the study was performed in accordance with the Helsinki Declaration.

Study design

A randomized, double-blind, parallel trial was conducted to assess the effects of the intake of 1%E as TFAs added to the subjects' usual diets on serum cholesterol for 4 weeks. In addition to their regular diets, the subjects were instructed to consume one cookie a day that contained either high oleic sunflower oil (the control group) or partially hydrogenated rapeseed oil (the TFA group) throughout the experimental period. At the end of the experiment, blood was collected after overnight fasting (>11 hours) and the serum was harvested. The dietary survey was conducted in the third week.

The high oleic sunflower oil (Showa Sangyo, Tokyo) contained 82.5 g of C18:1 cis (oleic acid) and 0.1 g of TFAs per 100 g of fatty acids. The partially hydrogenated rapeseed oil (Yokozeki Oil and Fat Industries, Ibaraki, Japan) contained 44.5 g of C18:1 cis and 36.5 g of TFAs (Table 2). Both types of cookie contained the same levels of energy, protein, fat and carbohydrate (105 kcal, 1.0 g, 5.7 g, and 12.3 g per cookie). The TFA cookie contained 2.0 g of TFAs, which corresponded to a 1%E intake of total energy.

Analysis of blood samples

Each subject's serum total cholesterol, LDL-cholesterol, HDL-cholesterol, triacylglycerol, glucose, insulin and hemoglobin A1c were analyzed as described.[5] Briefly, serum lipids and glucose were measured by enzymatic methods, and hemoglobin A1c was measured by means of automated clinical chemistry analyzers (TBA-200FR, Hitachi, Tokyo, and HLC-723G8, Tosoh, Tokyo). Serum insulin was measured by a turbidimetric immunoassay. Lipids in erythrocytes were extracted by the method of Folch et al,[16] and the TFA content was measured by gas chromatography (GC-2010, Shimadzu, Kyoto, Japan) with a capillary column (TC-70, 0.25mm × 60 m, GL-Science, Tokyo) after methylation with boron trifluoride.[5] Fatty acid methyl ester standards (a 37-component FAME mix, *trans*-9-elaidic and *trans*-11-vaccenic acid methyl esters, and a mixture of linoleic and linolenic acid methyl ester isomers) were purchased from Sigma-Aldrich Japan

Table 1. Characteristics of the subjects (n=53)

	Control	TFA
No. of subjects (male/female)	27 (6/21)	26 (5/21)
Age, years	40.0±11.8	40.7±11.4
Male	47.2±13.0	46.4±9.8
Female	37.9±10.9	39.4±11.5
Height, cm	162±6	162±7
Male	169±4	169±3
Female	159±5	160±7
Body weight, kg	56.9±8.8	56.0±8.8
Male	66.3±5.3	64.7±11.5
Female	54.3±7.6	53.9±6.8
BMI, kg/m^2	21.8±3.0	21.4±3.0
Male	23.2±2.2	22.6±3.6
Female	21.4±3.1	21.2±2.9
No. of subjects with gene variant		
FTO gene (hetero and homo)	9	11
FTO gene (homo)	1	1
Beta-3 adrenergic receptor gene (hetero and homo)	10	10
Beta-3 adrenergic receptor gene (homo)	1	1
TFA intake, %E	0.349±0.161	0.346±0.132
Cholesterol intake, mg/day	296±97	293±118

FTO gene: fat mass- and obesity-associated gene; TFA: *trans* fatty acid.
Values are mean±SD.
No significant differences were observed in any of the parameters between the two groups.

(Tokyo).

Dietary survey

A dietary survey was conducted for three consecutive days (one holiday and two weekdays) during the third week of the 4-week diet period, with the use of a written dietary record kept by each subject along with a photographic record with a scale card (7.5×5 cm^2).[17] Leftovers were also recorded in the dietary and photographic records. The subjects were instructed to submit the labels of all processed foods that they consumed. The daily intakes of energy, nutrients and TFAs were calculated by dietitians using commercially available nutrient calculation software (Excel Eiyo-Kun ver. 6.0, Kenpakusha, Tokyo) and the data from the Basal Report of the Evaluation of TFAs in Food.[18]

Genetic analysis

The genotyping analysis was carried out by the Laboratory of EBS Inc. (Hiroshima, Japan). Genomic DNA was extracted from the mucous membrane in the subject's oral cavity using the KAPA MG kit (Kapa Biosystems, Boston, MA, USA) according to the manufacturer's instructions. The SNPs of FTO rs9939609 and beta-3 adrenergic receptor rs4994 were genotyped by a duplex PCR with confronting two-pair primers.[19]

Statistical analysis

The results are expressed as means±SD. The significance of differences between the two diet groups was determined by unpaired *t*-test. The difference between the genotypes, i.e., the data of the subjects with and without variants of FTO gene and beta-3 adrenergic receptor gene, was determined by unpaired *t*-test. *P*-values <0.05 were considered significant. Statistical calculations were performed using SPSS 13.0 J software (SPSS Japan, Tokyo).

RESULTS

Nutrient intake

The results of the subjects' energy and lipid intakes during the experimental period are shown in Table 3. There were no significant differences in energy or fat intakes between the control and TFA groups. The intakes of saturated, monounsaturated, n-6 polyunsaturated and n-3 polyunsaturated fatty acids were also the same between the two diet groups. The TFA intake was significantly higher in the TFA group than in the control group, and the mean TFA intake of the control and TFA groups corresponded to 0.28%E and 1.31%E, respectively. There was no significant difference in cholesterol intake between the control and TFA groups.

Serum lipids, glucose, insulin, and hemoglobinA1c

There were no significant differences in the baseline levels of serum cholesterol (total, LDL and HDL) or triacylglycerol between the control and TFA groups (Table 4). After the 4-week experimental period, again no significant differences were observed in the serum cholesterol or triacylglycerol levels between the two groups. Thus, the serum LDL-cholesterol were not increased and the HDL-cholesterol was e not decreased even after the daily consumption of TFAs. The baseline and post-treatment values, before and after the intervention, of glucose, insulin, and hemoglobinA1c were all the same between the control and TFA groups. No significant differences were observed in the percentage changes from before to after the trial between the two groups (data not shown). In the women, there were no significant differences in the serum cholesterol between the two groups after the 4-week experimental period (data not shown). No significant differences were also observed in the serum cholesterol between the two groups in the men.

Table 2. Major fatty acid composition of experimental oils

	High oleic sunflower oil (Control)	Partially hydrogenated rapeseed oil (TFA)
	g/100 g fatty acids	
C16:0†	3.4	4.6
C18:0	2.6	11.4
C18:1 *trans*	0.1	34.5
C18:1 *cis*	82.5	44.5
C18:2 *trans*	ND	2.0
C18:2 *cis*	9.3	0.6
C18:3 *trans*	ND	ND
C18:3 *cis*	0.5	0.8
Others	1.6	1.6
Total TFAs	0.1	36.5

ND: not detected; TFA: *trans* fatty acid.
†No. of carbon atoms:number of double bonds.

Table 3. Intake of energy and lipids during experimental period

	Control (n=27)	TFA (n=26)
Energy, kcal/day	1807±300	1888±338
Fat, %E	30.9±4.7	29.4±3.7
Fatty acid, %E		
Saturated	7.66±1.95	8.11±1.68
Monounsaturated	11.9±2.1	11.5±1.5
n-6 polyunsaturated	5.24±1.43	4.74±0.95
n-3 polyunsaturated	1.00±0.42	0.96±0.29
TFA, %E	0.28±0.17	1.31±0.31**
Cholesterol, mg/day	264±100	302±124

TFA: *trans* fatty acid.
Values are mean±SD.
Significantly different from the control group, $^{**}p<0.01$.

Fatty acid contents of erythrocytes

The fatty acid compositions of the subjects' erythrocytes after 1%E TFA supplementation for 4 weeks are shown in Table 5. The contents of C18:1 *trans* (*trans*-octadecenoic acids) were <1.0 g/100 g fatty acids in both groups, but were significantly higher after the consumption of the TFA diet compared to the control diet, at 0.5 vs. 0.9 g/100g, $p<0.01$. No significant difference was observed in C18:2 *trans* (*trans*-octadecadienoic acid) contents between the two diet groups. C18:3 *trans* (*trans*-octadecatrienoic acid) was not detected in either group.

Serum lipids, glucose, insulin and hemoglobinA1c in the subjects with FTO and beta-3 adrenergic receptor gene variants

Among the 20 participants with an FTO gene variant, the

Table 4. Serum cholesterol, triacylglycerol, glucose, insulin and hemoglobin A1c levels after 4 weeks of dietary intervention

		Dietary group		
		Control (n=27)	TFA (n=26)	*p*
Total cholesterol	Baseline, mg/100 mL	187±33	196±29	0.283
	After treatment, mg/100 mL	189±31	202±29	0.124
LDL-cholesterol	Baseline, mg/100 mL	110±26	120±29	0.214
	After treatment, mg/100 mL	110±27	122±28	0.116
HDL-cholesterol	Baseline, mg/100 mL	64.4±18.7	64.2±12.1	0.975
	After treatment, mg/100 mL	65.2±16.2	64.2±13.1	0.808
LDL-C:HDL-C ratio	Baseline	1.83±0.63	1.97±0.78	0.470
	After treatment	1.78±0.58	2.03±0.78	0.189
Triacylglycerol	Baseline, mg/100 mL	87.0±53.9	86.5±52.3	0.973
	After treatment, mg/100 mL	86.9±44.5	96.5±65.5	0.535
Glucose	Baseline, mg/100 mL	87.2±7.5	86.5±6.6	0.741
	After treatment, mg/100 mL	88.3±7.3	87.1±8.9	0.588
Insulin	Baseline, μU /100 mL	5.18±2.35	5.24±3.53	0.938
	After treatment, μU /100 mL	6.64±2.34	6.40±4.07	0.795
Hemoglobin A1c	Baseline, %	5.46±0.19	5.43±0.34	0.710
	After treatment, %	5.45±0.23	5.48±0.30	0.735

TFA: *trans* fatty acid; LDL-C: HDL-C ratio: LDL-cholesterol: HDL-cholesterol ratio.
Values are mean±SD.
No significant differences were observed in any of the parameters between the two groups.

post-treatment cholesterol, triacylglycerol, glucose, insulin, and hemoglobinA1c values were not significantly different between the control and TFA groups after the 4-week treatment (Table 6). The same trend was observed in the 20 subjects with beta-3 adrenergic receptor gene variants.

The percentage changes of cholesterol, triacylglycerol, glucose, insulin, and hemoglobinA1cafter TFA supplementation in the TFA diet group without or with FTO and beta-3 adrenergic receptor gene variants are shown in Table 7. No significant differences in the percentage changes were observed in any serum parameters between the participants with or without the two gene variants in the TFA diet group.

DISCUSSION

We examined the influence of a low (1%E) dietary TFA intake on serum LDL- and HDL-cholesterol in healthy adult Japanese women and men. After 1%E TFA supplementation for 4 weeks, no significant changes in cholesterol values were observed, similar to a prior intervention in young women.[7] In the present study, the supplementation with 1%E TFAs did not show a significant effect on serum lipid concentrations in the subjects irrespective of FTO or beta-3 adrenergic receptor gene variants. No significant difference was observed in the percentage change of serum cholesterol between the participants with or without the two gene variants in the TFA diet group. The supposition that the effect of dietary TFAs on serum lipids could be strengthened in people with the two obesity gene variants was therefore not confirmed.

Thus, the results of the present study indicate that supplementation with 1%E TFAs has little effect on cholesterol values in healthy adult Japanese, regardless of the genotype of FTO or beta-3 adrenergic receptor. To the best of our knowledge, there have been no reports about the effect of dietary TFAs on serum cholesterol in sub jects with FTO or beta-3 adrenergic receptor gene variants. However, it is plausible that saturated fatty acids and TFAs may differently affect lipid metabolism.[11,12]

TFAs are present in various foods, and it is not easy to reduce the intake of TFAs to zero even the use of partially hydrogenation oils and fats was completely eliminated. Therefore, it is at present necessary to clarify the allowable intake at which TFAs do not exert adverse effects on serum lipids, in particular the LDL- and HDL-cholesterol and thus coronary heart disease. However, the evidence supporting a tolerable intake is limited. To clarify the safe level of TFA intake, intervention studies are necessary in which the dose-response to TFA intake is measured systematically. In most developed countries, however, the average current consumption of TFAs seems to be below that allowable.[20]

Many intervention studies have shown that excess TFA

Table 5. Major fatty acid contents of erythrocytes after 4 weeks of dietary intervention

	Dietary group	
	Control (n=27)	TFA (n=26)
	g/100 g fatty acids	
C16:0†	23.3±1.0	23.1±0.8
C18:0	13.8±1.4	13.7±0.8
C18:1 *trans*	0.5±0.3	0.9±0.4**
C18:1 *cis*	18.4±1.2	18.2±2.0
C18:2 *trans*	0.2±0.1	0.2±0.1
C18:2 *cis*	14.6±1.3	15.0±1.5
C18:3 *trans*	ND	ND
C18:3 *cis*	0.4±0.1	0.4±0.1
C20:4	12.6±1.1	12.6±1.1
C24:0	2.1±0.5	2.2±0.5
C22:5	1.9±0.4	1.9±0.2
C22:6	6.7±0.9	6.5±1.0
Others	5.5±0.5	5.3±0.7

ND: not detected; TFA: *trans* fatty acid.
Values are mean±SD, n=27 and 26.
†No. of carbon atoms: number of double bonds.
Significantly different from the control group, $^{**}p<0.01$.

Table 6. Serum cholesterol, triacylglycerol glucose, insulin and hemoglobin A1c levels after 4 weeks of dietary intervention in the subjects with fat mass- and obesity-associated (FTO) or beta-3 adrenergic receptor gene variants

		With FTO gene variant			With beta-3 adrenergic receptor gene variant		
		Dietary group			Dietary group		
		Control (n=9)	TFA (n=11)	*p*	Control (n=10)	TFA (n=10)	*p*
Total cholesterol	Baseline, mg/100 mL	189±24	199±30	0.403	189±29	195±28	0.662
	After treatment, mg/100 mL	192±24	198±19	0.525	197±31	199±29	0.854
LDL-cholesterol	Baseline, mg/100 mL	109±14	121±28	0.231	117±25	119±31	0.850
	After treatment, mg/100 mL	109±23	119±21	0.317	121±29	119±30	0.834
HDL-cholesterol	Baseline, mg/100 mL	66.5±23.3	65.1±14.5	0.882	55.5±10.5	64.0±12.3	0.113
	After treatment, mg/100 mL	67.8±20.5	63.9±16.0	0.645	59.6±10.5	65.9±12.8	0.246
Triacylglycerol	Baseline, mg/100 mL	85±41	104±73	0.478	103±63	79±59	0.384
	After treatment, mg/100 mL	88±38	115±74	0.645	102±48	87±51	0.497
Glucose	Baseline, mg/100 mL	86.7±9.7	87.0±5.4	0.941	88.0±6.7	85.3±5.5	0.338
	After treatment, mg/100 mL	87.5±8.2	89.2±10.5	0.676	90.2±7.0	86.8±11.2	0.427
Insulin	Baseline, μU/100 mL	4.77±1.77	6.32±4.28	0.288	4.92±1.74	5.76±4.82	0.611
	After treatment, μU/100 mL	6.54±2.37	7.17±5.83	0.746	7.17±2.84	7.19±6.03	0.993
Hemoglobin A1c	Baseline, %	5.41±0.22	5.57±0.40	0.276	5.46±0.16	5.52±0.25	0.533
	After treatment, %	5.38±0.28	5.54±0.26	0.198	5.44±0.23	5.56±0.16	0.193

TFA: *trans* fatty acid.
Values are mean±SD.
No significant differences were observed in any of the parameters between the two groups.

Table 7. Percentage changes† of serum cholesterol, triacylglycerol glucose, insulin and hemoglobin A1c levels after 1%E *trans* fatty acid (TFA) supplementation for 4 weeks in subjects without or with fat mass- and obesity-associated (FTO) or beta-3 adrenergic receptor gene variants

	TFA diet group			TFA diet group		
	FTO gene			Beta-3 adrenergic receptor gene		
	Without variant (n=15)	With variant (n=11)	*p*	Without variant (n=16)	With variant (n=10)	*p*
Total cholesterol	5.2±7.3	0.3±7.8	0.128	4.1±8.5	2.6±6.5	0.629
LDL-cholesterol	5.3±13.1	-0.1±12.0	0.311	5.8±15.2	-0.5±6.3	0.226
HDL-cholesterol	1.2±10.4	-2.2±8.0	0.398	-2.0±9.1	3.3±10.0	0.172
Triacylglycerol	10.6±33.1	26.2±42.6	0.310	12.7±36.8	21.2±37.7	0.578
Glucose	-0.3±5.2	2.5±9.2	0.328	0.1±5.2	1.6±9.1	0.599
Insulin	46.3±62.1	20.5±52.6	0.300	73.7±127.6	33.5±60.9	0.363
Hemoglobin A1c	1.4±1.7	-0.2±3.4	0.113	0.9±2.8	0.8±2.1	0.945

TFA: *trans* fatty acid.
Values are mean±SD.
†Percentage change: After treatment − Baseline / Baseline × 100.
No significant differences were observed in any of the parameters between the subjects without and with the gene variants.

intake has adverse effects on blood cholesterol and the risk of coronary heart disease.[1,21,22] On the other hand, some studies, though limited, show that low-dose TFAs do not have a significantly negative effect on serum cholesterol. For example, compared to a control diet containing 0.6%E of TFAs, the consumption of a 3.3%E TFA margarine diet for 5 weeks did not cause differences inserum LDL- or HDL-cholesterol of moderately hypercholesterolemicpeople.[23] In addition, Denke et al[24] reported that LDL-cholesterol was lower after the consumption of a margarine diet containing 1.5%E TFAs compared to a butter diet containing 0.5%E TFAs in normocholesterolemic people. As the margarine and butter diets contained 9%E and 16%E saturated fatty acids, the difference can probably be attributed to the content of saturated fatty acids in relation to TFAs. These results suggest that a small amount of dietary TFAs has little effect on serum cholesterol.

Because the consumption of saturated fatty acids was the same in both groups in the present study, the effect of saturated fatty acid can be ignored, and our results confirm that the consumption of TFAs as low as 1%E is not hazardous to our health provided, at least, the relative dietary intake of polyunsaturated fatty acids, such as linoleic acid, is satisfactory in the Japanese population.

The effect of TFAs on insulin resistance and the onset of diabetes is unclear,[25] though some cohort studies report an association between TFA intake and type 2 diabetes.[26,27] A meta-analysis of observational studies showed that TFA intake is not associated with type 2 diabetes.[28] In their meta-analysis of randomized, placebo-controlled clinical trials, Aronis et al[29] also reported that increased TFA intake does not result in changes in glucose or insulin concentrations. Our present findings demonstrated that 1%E TFA supplementation does not appear to have a

significant influence on serum glucose, insulin or hemoglobinA1c levels in healthy Japanese adults.

Several studies observed a positive correlation between TFA intake and the TFA status in erythrocytes; erythrocyte TFA is used as one of the surrogate biomarkers of TFA intake.[30] In our study, there was a detectable accumulation of TFAs in erythrocyte lipids after the supplementation of 1%E TFAs. Thus, even low TFA intakes, are deposited into tissues. However, it is likely that the intake observed in the present study is not high enough to cause disorder\ed lipid metabolism.

There are two possible limitations to this study: study duration and participant characteristics. We assessed the effect of TFA supplementation on serum lipids for 4 weeks, but longer feeding trials are needed to definitely confirm the effect of TFAs at low intakes. We must also note our limited sample size, since it is necessary to investigate a large number of subjects to detect a small change. The numbers of participants with FTO and beta-3 adrenergic receptor gene variants were only 10 and 11, respectively.

Even considering the characteristics of cholesterol metabolism in post-menopausal women due to a decline in the levels of female hormones,[31] there was no significant difference in the serum cholesterol between the two groups after the 4-week experimental period (data not shown).

A goal of our present study was to clarify the relationship between TFA and metabolic syndrome. Indeed, an association between dietary TFA and obesity has been suggested.[32] We therefore selected the two obesity-related genes deeply associated with metabolic syndrome. Several SNPs are associated with LDL- and HDL-cholesterol, such as apolipoprotein B rs693, LDL receptor rs688, and cholesteryl ester transfer protein rs180077532.[33] It is also important to examine the relationship between TFA intake and these SNPs.

Small LDL particles are considered to be strongly associated with coronary heart disease risk.[34] Although the particle size of LDL was not determined in this study, the effect of TFA supplementation in particular at the low dietary intakes as used in the present study on the particle size of LDL is unknown. In this context, the current review suggests that the LDL particle number is more influential than the particle size on cardiovascular risk.[35]

In summary, our findings showed that supplementation with 1%E TFAs had an insignificant effect on serum cholesterol in healthy adult Japanese, regardless of the genotype of the FTO or beta-3 adrenergic receptor gene. Our findings support the WHO recommendation to reduce TFAs intake to <1%E. To clarify the optimal TFA intake, dose-dependent studies that examine dietary TFAs up to 3%E are needed.

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AUTHOR DISCLOSURES

The authors declare no conflict of interest.

REFERENCES

1. Hunter JE. Dietary trans fatty acids: review of recent human studies and food industry responses. Lipids. 2006;41:967-92.
2. Trumbo PR, Shimakawa T. Tolerable upper intake levels for trans fat, saturated fat, and cholesterol. Nutr Rev. 2011;69: 270-8. doi: 10.1111/j.1753-4887.2011.00389.x.
3. Ascherio A, Katan MB, Zock PL, Stampfer MJ, Willett WC. Trans fatty acids and coronary heart disease. N Engl J Med. 1999;340:1994-8.
4. Liska DJ, Cook CM, Wang DD, Gaine PC, Baer DJ. Trans fatty acids and cholesterol levels: an evidence map of available science. Food ChemToxicol. 2016:98:269-81. doi: 10.1016/j.fct.2016.07.002.
5. Takeuchi H, Yamaki M, Hirose K, Hienae C, Tabuchi E, Sugano M. Effect of a 0.6% energy trans fatty acid intake on serum cholesterol concentrations in healthy young Japanese subjects. Biosci Biotechnol Biochem. 2011;75:2243-5. doi: 10.1271/bbb.110420.5.
6. Takeuchi H, Nishimura Y, Ohmori A, Tabuchi E. Little Effect of supplementation with 0.6% energy trans fatty acids on serum cholesterol levels in adult Japanese women. J Nutr Sci Vitaminol. 2015;61:422-5. doi: 10.3177/jnsv.61.422.
7. Takeuchi H, Kutsuwada T, Shirokawa Y, Harada S, Sugano M. Supplementation with 1% energy trans fatty acids had little effect on serum cholesterol levels in healthy young Japanese women. Biosci Biotechnol Biochem. 2013;77: 1219-22. doi: 10.1271/bbb.120983.
8. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science. 2007;316:889-94.
9. Li H, Kilpeläinen TO, Liu C, Zhu J, Liu Y, Hu C et al. Association of genetic variation in FTO with risk of obesity and type 2 diabetes with data from 96,551 East and South Asians. Diabetologia. 2012;55:981-95. doi: 10.1007/s00125-011-2370-7.
10. Liu C, Mou S, Pan C. The FTO gene rs9939609 polymorphism predicts risk of cardiovascular disease: a systematic review and meta-analysis. PLoS One. 2013;8: e71901. doi: 10.1371/journal.pone.0071901.
11. Phillips CM, Kesse-Guyot E, McManus R, Hercberg S, Lairon D, Planells R, Roche HM. High dietary saturated fat intake accentuates obesity risk associated with the fat mass and obesity-associated gene in adults. J Nutr. 2012;142:824-31. doi: 10.3945/jn.111.153460.
12. Corella D, Arnett DK, Tucker KL, Kabagambe EK, Tsai M, Parnell LD et al. A high intake of saturated fatty acids strengthens the association between the fat mass and obesity-associated gene and BMI. J Nutr. 2011;141:2219-25. doi: 10.3945/jn.111.143826.
13. Emorine LJ, Marullo S, Briend-Sutren MM, Patey G, Tate K, Delavier-Klutchko C, Strosberg AD. Molecular characterization of the human β3-adrenergic receptor. Science. 1989;245:1118-21.
14. Nagase I, Yoshida T, Kumamoto K, Umekawa T, Sakane N, Nikami H, Kawada T, Saito M. Expression of uncoupling protein in skeletal muscle and white fat of obese mice treated with thermogenic β3-adrenergic agonist. J Clin Invest. 1996;97:2898-904.
15. Yoshida T, Sakane N. Association between beta3-adrenoreceptor polymorphism with obesity and diabetes in Japan. Intern Med. 1999;38:207-9.
16. Folch J, Lees M, Sloane-Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem. 1957;226:497-509.
17. Takeuchi H, Ito E, Tomioka T, Tabuchi E, Fuhshuku K, Asano Y. Trans fatty acid intake and serum cholesterol

levels in young Japanese women. Biosci Biotechnol Biochem. 2012;76:1627-32. doi: 10.1271/bbb.120105.
18. Foof Safty Commission in Cabinet Office Government of Japan. Basal report of evaluation of transfatty acids in food. Tokyo: Cabinet Office of Government of Japan; 2007. pp. 1-45.
19. Tamakoshi A, Hamajima N, Kawase H, Wakai K, Katsuda N, Saito T et al. Duplex polymerase chain reaction with confronting two-pair primers (PCR-CTPP) for genotyping alcohol dehydrogenase beta subunit (ADH2) and aldehyde dehydrogenase 2 (ALDH2). Alcohol Alcohol. 2003;38:407-10.
20. Wang Q, Afshin A, Yakoob MY, Singh GM, Rehm CD, Khatibzadeh S et al. Impact of nonoptimal intakes of saturated, polyunsaturated, and trans fat on global burdens of coronary heart disease. J Am Heart Assoc. 2016:5: e002891. doi: 10.1161/JAHA.115.002891.
21. Mensink R, Katan MB. Effect of dietary trans fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. N Engl J Med. 1990;323:439-45. doi: 10.1056/NEJM199008163230703.
22. Zock PL, Katan MB, Mensink RP. Dietary trans fatty acids and lipoprotein cholesterol. Am J ClinNutr. 1995;61:617.
23. Lichtenstein AH, Ausman LM, Jalbert SM, Schaefer EJ. Effects of different forms of dietary hydrogenated fats on serum lipoprotein cholesterol levels. N Engl J Med. 1999; 340:1933-40.
24. Denke MA, Adams-Huet B, Nguyen AT. Individual cholesterol variation in response to a margarine- or butter-based diet: A study in families. JAMA. 2000;284:2740-7.
25. Ghafoorunissa G. Role of trans fatty acids in health and challenges to their reduction in Indian foods. Asia Pac J Clin Nutr. 2008;17:212-5.
26. Salmerón J, Hu FB, Manson JE, Stampfer MJ, Colditz GA, Rimm EB, Willett WC. Dietary fat intake and risk of type 2 diabetes in women. Am J Clin Nutr. 2001;73:1019-26.
27. Meyer KA, Kushi LH, Jacobs DR Jr, Folsom AR. Dietary fat and incidence of type 2 diabetes in older Iowa women. Diabetes Care.2001;24:1528-35.
28. de Souza RJ, Mente A, Maroleanu A, Cozma AI, Ha V, Kishibe T et al. Intake of saturated and trans unsaturated fatty acids and risk of all cause mortality, cardiovascular disease, and type 2 diabetes: systematic review and meta-analysis of observational studies. BMJ. 2015;351:h3978. doi: 10.1136/bmj.h3978.
29. Aronis KN, Khan SM, Mantzoros CS. Effects of trans fatty acids on glucose homeostasis: a meta-analysis of randomized, placebo-controlled clinical trials. Am J Clin Nutr. 2012;96:1093-9. doi: 10.3945/ajcn.112.040576.
30. Sun Q, Ma J, Campos H, Hankinson SE, Hu FB. Comparison between plasma and erythrocyte fatty acid content as biomarkers of fatty acid intake in US women. Am J Clin Nutr. 2007;86:74-81.
31. Samaan SA, Crawford MH. Estrogen and cardiovascular function after menopause. J Am Coll Cardiol. 1995;26:1403-10.
32. Field AE, Willett WC, Lissner L, Colditz GA. Dietary fat and weight gain among women in the Nurses' Health Study. Obesity. 2007;15:967-76. doi: 10.1038/oby.2007.616.
33. Kathiresan S, Melander O, Anevski D, Guiducci C, Burtt NP, Roos C et al. Polymorphisms associated with cholesterol and risk of cardiovascular events. N Engl J Med. 2008;358: 1240-9. doi: 10.1056/NEJMoa0706728.
34. Austin MA, Rodriguez BL, McKnight B, McNeely MJ, Edwards KL, Curb JD, Sharp DS. Low-density lipoprotein particle size, triglycerides, and high-density lipoprotein cholesterol as risk factors for coronary heart disease in older Japanese-American men. Am J Cardiol. 2000;86:412-6.
35. Allaire J, Vors C, Couture P, Lamarche B. LDL particle number and size and cardiovascular risk: anything new under the sum? Curr Opin Lipidol. 2017:28;261-6.doi: 10.1097/MOL.0000000000000419.