Biosci. Biotechnol. Biochem., **76** (9), 1627–1632, 2012

Trans Fatty Acid Intake and Serum Cholesterol Levels in Young Japanese Women

Hiroyuki TAKEUCHI,[1,†] Eriko ITO,[1] Tetsuhisa TOMIOKA,[1] Eiichi TABUCHI,[1] Ken-ichi FUHSHUKU,[2] and Yasuhisa ASANO[2]

[1]*Department of Food and Nutrition, Toyama College, 444 Gan-kaiji, Toyama 930-0193, Japan*
[2]*Biotechnology Research Center and Department of Biotechnology, Toyama Prefectural University, 5180 Kurokawa Imizu, Toyama 939-0398, Japan*

Received February 14, 2012; Accepted June 14, 2012; Online Publication, September 7, 2012
[doi:10.1271/bbb.120105]

There are very limited data concerning the influence of low-level *trans* fatty acid (TFA) intake on blood lipid levels. In this study, correlation of total and diene TFA intake with serum cholesterol levels was studied in young Japanese women. The mean intakes of total and diene TFAs were 0.36% and 0.05% of energy, respectively. There was a significant correlation between total fat intake and TFA intake. TFA intake was significantly correlated with erythrocyte TFA content. Total TFA intake was not correlated with total, LDL- or HDL-cholesterol levels. No correlatuon was found between diene TFA intake and cholesterol level. Total and diene TFA intake were not correlated with hemoglobin A1c or C-reactive protein levels. These results suggest that the average TFA intake of young Japanese women does not adversely affect serum cholesterol levels.

Key words: *trans* fatty acids; cholesterol; erythrocyte; fat; young Japanese women

Trans fatty acids (TFAs) are unsaturated fatty acids with at least one non-conjugated double bond in the *trans* configuration. They come mostly from the industrial hydrogenation of vegetable oils, and they are found commonly in manufactured products such as margarines, cakes, pastries, and breads.[1)] TFAs are also generated by the natural bacterial hydrogenation in the rumen of polygastric animals such as cattle, sheep, and goats. Hence, TFAs from ruminants are found in dairy products derived from animal milk and meat.

Excessive TFA intake, more than 4 to 6% of total energy intake, increases blood LDL-cholesterol and reduces HDL-cholesterol.[2–4)] A number of epidemiologic studies conducted in Western countries have provided undisputed evidence that the consumption of excessive TFAs from industrial sources increases the risk of cardiovascular disease.[5–7)] The influence of excessive TFAs on blood lipid levels and cardiovascular disease has been well established,[8)] but satisfactory evidence regarding the tolerable upper level of TFA intake does not exist.[9)] Average TFA intake in Japan is estimated to be approximately 0.3 to 0.9% of total energy.[10,11)] There are very limited data on the influence of low-level TFA intake, *i.e.*, intake of less than 1% of total energy, on blood lipid levels. Hence it is difficult to determine how TFA influences serum cholesterol levels in the Japanese population at present.[9)] In this study, the correlation of TFA intake with serum cholesterol levels in Japanese women was investigated.

Various kinds of TFA are defined on the basis of numbers of carbon and double bonds, and the position of the *trans* double bond. Lemaitre *et al.*[12)] reported that *trans* isomers of oleic acid in erythrocytes, a biomarker of TFA intake, were not correlated with risk of primary cardiac arrest, whereas higher levels of *trans* isomers of linoleic acid were correlated with a 3-fold increase in risk. Second purpose of this study was to determine whether diene TFA intake is associated with serum cholesterol levels in young Japanese women.

Materials and Methods

Subjects. Volunteers were found among from students in the Department of Food and Nutrition of Toyama College. All of the subjects were healthy and did not take any medicine for dyslipidemia or diabetes. A total of 137 students were recruited, but two subjects did not complete their dietary records, and we could not obtain blood samples from two subjects for personal reasons. Hence data for 133 subjects were analyzed. Table 1 shows the characteristics of the subjects.

The protocol of the study was approved by the Ethics Review Board of Toyama College, and the study was performed in accordance with the Helsinki Declaration. Written informed consent was obtained from all participants.

Dietary survey. Dietary surveys were conducted in May 2009 ($n = 33$), 2010 ($n = 31$), and 2011 ($n = 69$). Each dietary survey was conducted for 3 consecutive days (one holiday and two weekdays) using a written dietary record and a photographic record with a scale card ($7.5 \times 5\,cm^2$). Leftovers were also recorded in the dietary record and the photographic record. Subjects were asked to submit the labels of processed foods that they ate. Daily intake of energy, nutrients, and TFAs was calculated by dietitians using commercially available nutrient calculation software (Excel Eiyo-Kun ver. 4.0, Kenpakusha, Tokyo) and the data from the Basal Report on the Evaluation of TFAs in Food.[13)] Industrial TFA intake was calculated as the sum of TFA intake from breads, margarines, fats, and oils excluding butter, confectioneries, and mayonnaise. Natural TFA intake was determined as the sum of TFA intake from meats, milks, and butter.

Blood analysis. Blood was sampled between 8:30 and 10:00 AM after overnight fasting. Total cholesterol, LDL-cholesterol, HDL-

† To whom correspondence should be addressed. Fax: +81-76-436-0133; E-mail: takeuchi@toyama-c.ac.jp
Abbreviations: TFA, *trans* fatty acid

cholesterol, triacylglycerol, lipoprotein (a), glucose, insulin, hemoglobin A1c, and C-reactive protein levels were analyzed following to a previous report.[14)]

Lipids in the erythrocytes were extracted by the method of Folch.[15)] The TFA contents of the erythrocytes were measured by a gas chromatography mass system (GC-2010 and GCMS-QP2010 Plus, Shimadzu, Kyoto, Japan) with a capillary column (TC-70, 0.25 mm × 60 m, GL-Science, Tokyo) after methylation with boron trifluoride.[14)] Standards of fatty acid methyl esters (a 37-component FAME mix, *trans*-9-elaidic and *trans*-11-vaccenic acid methyl ester, and a linoleic and linolenic acid methyl ester isomer mixture) were purchased from Sigma-Aldrich Japan (Tokyo).

Statistical analyses. Results were expressed as means ± SD. Pearson correlation analyses were used to evaluate relationships among variables. p values of less than 0.05 were considered statistically significant. Statistical calculations were performed with IBM SPSS Statistics 20 for Windows (IBM Japan, Tokyo).

Table 1. Characteristics of Subjects[1]

	Women (n = 133)
Age, years	18.9 ± 1.8
Height, cm	158 ± 5
Body weight, kg	53.7 ± 7.9
Body mass index, kg/m^2	21.0 ± 2.9
Energy intake, kcal/d	1677 ± 345
Protein intake, % of energy	15.1 ± 2.3
Fat intake, % of energy	30.5 ± 5.2
Carbohydrates intake, % of energy	54.4 ± 6.0
Fatty acid intake, % of energy	
Saturated	9.2 ± 2.3
Monounsaturated	10.9 ± 2.4
n-6 polyunsaturated	5.1 ± 1.4
n-3 polyunsaturated	1.0 ± 0.4
Cholesterol intake, mg/d	372 ± 155

[1]Values are mean ± SD.

Results

Intake of total, monoene, diene, and triene TFAs

The mean intake of total TFA was 0.67 g/d (Table 2). The foods that contributed most to TFA intake were vegetable fats and oils, followed by beef cattle and margarines. The SD of total TFA intake from margarines was more than twice the mean value, and the total TFA intake ranged from 0 to 0.95 g/d. The SDs of cakes, buns, pastries, and biscuits were also more than twice the mean values.

The mean intake of monoene TFAs was 0.45 g/d (0.24% of total energy), accounting for 67% of total TFAs. The food that contributed most to monoene TFA intake was beef cattle (0.060 ± 0.092 g/d), followed by margarines (0.056 ± 0.148 g/d). In contrast to total TFA intake, the monoene TFA intake from vegetable fats and oils was only 0.03 g/d, accounting for only 7% of monoene TFA intake. The mean intake of diene TFA was 0.10 g/d (0.05% of energy). The foods that contributed most to diene TFA intake were vegetable fats and oils, which accounted for 40% of diene TFA intake. The mean intake of triene TFA was 0.11 g/d (0.06% of total energy). The major contributors to triene TFA intake were vegetable fats and oils and mayonnaise.

Intake of total, industrial, and natural TFAs

The mean intake of total TFAs was 0.36% of total energy, and it ranged from 0.06 to 1.03% (Table 3). Only one woman took in TFAs in an amount greater than 1% of total energy. In the women, the intake of breads, butter, and margarines was 3 times or more the mean value. The mean intake of industrial TFAs was 0.22% of total energy, accounting for 60% of total TFA intake. The mean intake of natural TFAs was 0.14% of total energy. The relationship between total fat intake

Table 2. Intake of Total, Monoene, Diene, and Triene *Trans* Fatty Acids (TFAs) from Various Food Groups[1]

Food group	TFA intake (g/d)			
	Total	Monoene	Diene	Triene
Cereals				
Breads	0.05 ± 0.04	0.03 ± 0.04	0.01 ± 0.01	—[2]
Meats				
Cattle	0.07 ± 0.11	0.06 ± 0.09	0.01 ± 0.02	—
Milks				
Liquid milks	0.05 ± 0.07	0.04 ± 0.05	0.01 ± 0.01	—
Cheeses	0.04 ± 0.06	0.03 ± 0.05	0.01 ± 0.01	—
Yogurt and lactic acid bacteria beverages	0.01 ± 0.01	0.01 ± 0.01	—	—
Others	0.04 ± 0.08	0.04 ± 0.07	—	—
Fats and oils				
Butter	0.04 ± 0.06	0.03 ± 0.05	0.01 ± 0.01	—
Margarines	0.07 ± 0.19	0.06 ± 0.15	0.01 ± 0.01	—
Vegetable fats and oil	0.13 ± 0.09	0.03 ± 0.02	0.04 ± 0.03	0.07 ± 0.05
Confectioneries				
Cakes, buns and pastries	0.06 ± 0.12	0.05 ± 0.10	0.01 ± 0.01	—
Biscuits	0.04 ± 0.11	0.04 ± 0.10	—	—
Others	0.02 ± 0.03	0.01 ± 0.02	—	—
Seasonings and spices				
Mayonnaise	0.04 ± 0.06	—	—	0.03 ± 0.05
Total	0.67 ± 0.37	0.45 ± 0.29	0.10 ± 0.05	0.11 ± 0.08

[1]Values are mean ± SD, n = 133.
[2]Less than 0.01 g

Table 3. Intake of Total, Industrial, and Natural *Trans* Fatty Acids (TFAs)[1]

	TFA intake (% of energy)	%
Total TFAs	0.36 ± 0.17 (0.06–1.03)	100
Industrial TFAs[2]	0.22 ± 0.15 (0.03–0.80)	60
Natural TFAs[3]	0.14 ± 0.10 (0–0.43)	40

[1]Values are mean ± SD (minimum–maximum), n = 133.
[2]Industrial TFA intake was determined as the sum of TFA intake from breads, margarines, fats, and oils, except for butter, confectioneries and mayonnaise.
[3]Natural TFA intake was determined as the sum of TFA intake from meats, milk, and butter.

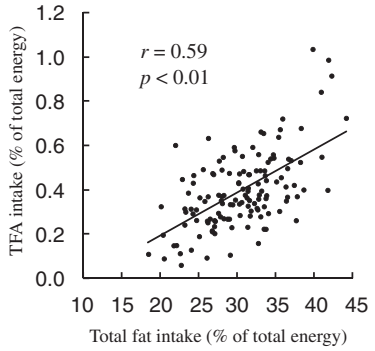

Fig. 1. Correlation of Total Fat Intake with *Trans* Fatty Acid (TFA) Intake in 133 Young Japanese Women.

and total TFA intake is shown in Fig. 1. There was a significant correlation between the total fat and the TFA intake ($p < 0.01$).

Fatty acid contents of the erythrocytes

The main fatty acid in the erythrocytes was 16:0 (palmitic acid), followed by 18:1 *cis*-9 (oleic acid) and 18:2 *cis*-6 (linoleic acid) (Table 4). The contents of 18:1 *trans*-9 (elaidic acid), 18:1 *trans*-11 (vaccenic acid), 18:2 *trans*-12, and 18:2 *trans*-9 were 0.3 g, 0.3 g, 0.1 g, and 0.2 g per 100 g of fatty acids respectively. The total TFA content of the erythrocytes was 0.9 g/100 g of fatty acids.

Correlations of TFA intake with serum and erythrocyte lipids

The correlations of TFA intake with serum levels of total, LDL-, and HDL-cholesterol and with erythrocyte TFA contents are shown in Fig. 2. TFA intake did not correlate with total cholesterol levels. No correlation was found between TFA intake and serum LDL-cholesterol or HDL-cholesterol levels. One hundred twelve volunteers were ingesting not less than 7% saturated fatty acid, the upper level of dietary reference intake for Japanese. In these volunteers, TFA intake also did not correlate with serum cholesterol levels (data not shown). Conversely, there was a significant correlation between TFA intake and erythrocyte TFA contents. No correlation was found between erythrocyte TFA content and serum cholesterol levels (data not shown). TFA intake did not correlate with serum triacylglycerol or lipoprotein (a) level (Table 5). No correlation was found between the intake of industrial and natural TFAs and serum lipid levels (data not shown).

Table 4. Fatty Acid Contents of Erythrocytes in 133 Healthy Young Japanese Women[1]

Fatty acids	(g/100 g fatty acids)
14:0[2]	0.3 ± 0.2
14:1	0.3 ± 0.2
16:0	19.0 ± 2.1
16:1	0.6 ± 0.2
18:0	13.9 ± 0.8
18:1 *trans*-9	0.3 ± 0.1
18:1 *trans*-11	0.3 ± 0.1
18:1 *cis*-9	17.2 ± 0.9
18:1 *cis*-11	2.1 ± 0.3
18:2 *cis*-9, *trans*-12	0.1 ± 0.0
18:2 *trans*-9, *cis*-12	0.2 ± 0.1
18:2 *cis*-9, *cis*-12	15.8 ± 1.5
18:3	0.3 ± 0.1
20:1	0.4 ± 0.1
20:2	0.3 ± 0.0
20:3 (n-6)	1.3 ± 0.3
20:4 (n-6)	13.8 ± 1.3
22:0	0.3 ± 0.1
20:5 (n-3)	1.2 ± 0.5
24:0	0.7 ± 0.3
22:5 (n-6)	0.3 ± 0.1
24:1	0.7 ± 0.3
22:5 (n-3)	1.9 ± 0.4
22:6 (n-3)	6.5 ± 1.2
Total 18:1 *trans*	0.6 ± 0.2
Total 18:2 *trans*	0.3 ± 0.1
Total TFA	0.9 ± 0.2

[1]Values are mean ± SD.
[2]Number of carbon atoms:number of double bonds.

Relationship between TFA intake and blood analysis

No correlations were found between TFA intake and glucose, insulin or hemoglobin A1c levels (Table 5). There was no significant correlation between TFA intake and serum C-reactive protein levels.

Relationship between diene TFA intake and blood analysis

There were no significant correlations between diene TFA intake or serum total, LDL-, and HDL-cholesterol levels (Table 6). No correlations were found between TFA intake and glucose, insulin or hemoglobin A1c levels. TFA intake was not correlated with C-reactive protein levels.

Discussion

One of the purposes in this study was to investigate the TFA intake of young Japanese women by means of a dietary survey using a written dietary records. It has been pointed out that TFA intake in young women might be higher than the overall mean TFA intake in the Japanese population, because young women tend to favor fast foods and snack foods containing hydrogenated oils.[16] The subjects' TFA intake was 0.36% of total energy on average. Contrary to expectations, it was found that the TFA intake of young Japanese women is similar to the overall mean in Japan. Kawabata *et al.*[17] estimated the TFA intake of Japanese university students by direct measurement in Tokyo area and Okinawa. A single-day meal was reproduced to measure TFA contents by gas chromatography. The median values

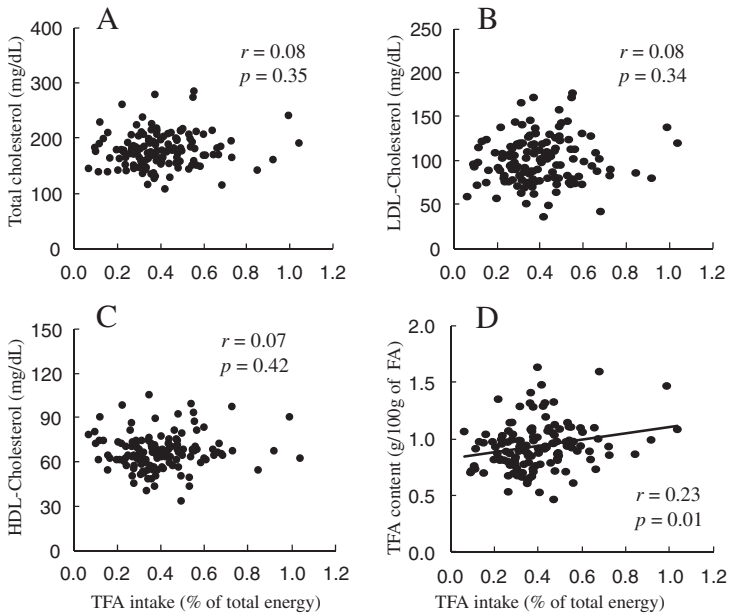

Fig. 2. Correlation of *Trans* Fatty Acid (TFA) Intake with Serum Total Cholesterol (A), LDL-Cholesterol (B), HDL-Cholesterol (C), and Erythrocyte TFA Contents (D) in 133 Young Japanese Women.

Table 5. Relationship between *Trans* Fatty Acid Intake and Results of Blood Analysis (n = 133)

	r^1	*p*
Triacylglycerol	0.05	0.54
Lipoprotein (a)	0.13	0.15
Glucose	0.04	0.63
Insulin	0.13	0.11
Hemoglobin A1c	0.14	0.11
C-reactive protein	0.03	0.76

[1]Correlation coefficient

Table 6. Relationship between C18:2 *Trans* Fatty Acid Intake and Results of Blood Analysis (n = 133)

	r^1	*p*
Total cholesterol	0.04	0.67
LDL-Cholesterol	0.07	0.40
HDL-Cholesterol	0.01	0.89
Triacylglycerol	0.02	0.87
Lipoprotein (a)	0.13	0.15
Glucose	0.09	0.29
Insulin	0.11	0.23
Hemoglobin A1c	0.08	0.37
C-reactive protein	0.04	0.62

[1]Correlation coefficient

were 0.29% of total energy in the Tokyo area and 0.35% in Okinawa. The median value in the present study was 0.37% of total energy. The small difference between the results of their study and those of ours might have been due in part to region. On the other hand, Yamada *et al.*[11] investigated female students' TFA intake using a self-administered diet history questionnaire that they developed, and reported that mean TFA intake was 0.90% of total energy, more than twice the level found in our study. The reason for this difference is not clear. One of the causes was perhaps that the method of the dietary survey was different. The dietary survey method with a written dietary record is relatively precise, but nutrient calculation requires a great deal of work and time. Therefore, food frequency and diet history questionnaires tend to be used in large-scale investigations.[18]

In 2003, the World Health Organization recommended that TFA intake should account for less than 1% of total energy intake to prevent diet-related chronic diseases.[19] One volunteer had a TFA intake that accounted for more than 1% of total energy in the present study. Four volunteers ingested TFAs accounting for more than 0.8% of total energy, and their total fat intake ranged from 39.6% to 42.3% of total energy. The TFA intake of those volunteers with a total fat intake ranging from 20–30% of total energy was 0.32% of total energy on average, and 0.63% at maximum. A significant correlation was found between the energy percentage accounted for by TFA intake and the total fat intake percentage. Kawabata *et al.*[20] reported a similar relationship between TFA intake (g) and total fat intake (g). Avoiding excess ingestion of total fat might be effective in preventing excess ingestion of TFAs. It is probably very rare for TFA intake to exceed 1% of total energy when the total fat intake is less than 30% of total energy.

In this study, we found no significant correlation between TFA intake and serum cholesterol levels. Yamada *et al.*[11] reported that total, LDL-, and HDL-cholesterol levels were not correlated with TFA intake in young Japanese women who ingested 0.9% of total energy (1.7 g/d) in the form of TFAs on average. Pietinen *et al.*[21] also reported that total and HDL-cholesterol levels were not correlated with TFA intake in Finnish men who ingested a median of 2.0 g/d (0.6% of energy) of TFAs. However, Mozaffarian *et al.*[22] found a significant correlation between TFA intake and HDL-cholesterol level in adult women who ingested a median of 2.7 g/d (1.3% of energy) of TFAs. Bolton-Smith *et al.*[23] reported that there was a significant correlation between TFA intake and total cholesterol levels in Scottish women who ingested an average of 6.4 g/d of TFAs. The reason a different outcome was

obtained from these studies is uncertain, but it might be related the TFA intake levels of the study populations. The results obtained from our studies suggest that serum cholesterol concentrations might be not correlated with TFA intake in a population that ingests very low levels of TFAs, *i.e.*, less than 1% of total energy on average. Recently, we reported that supplementation with TFA at of 0.6% of total energy did not influence serum cholesterol levels in a healthy young Japanese.[14)]

Erythrocyte TFA content is utilized as a biomarker of TFA intake,[24)] and some cohort studies have found positive correlations between erythrocyte TFA levels and the risk of coronary heart disease.[25,26)] In the present study, there was a comparatively weak but significant correlation between TFA intake and erythrocyte TFA content ($r = 0.23$, $p = 0.01$). Sun *et al.*[24)] found a positive correlation of erythrocyte TFA content with TFA intake in adult women who ingested an average of 1.5% of total energy in the form of TFAs. Our results in this study suggest that erythrocyte TFA contents are correlated TFA intake even in cases of low intake.

The correlations of TFA intake with triacylglycerol, lipoprotein (a), C-reactive protein, and the parameters of glucose metabolism in the blood were assessed in the present study. TFA intake was not correlated with these levels or the parameters of glucose metabolism. There are several reports that the intake of excessive TFAs increases triacylglycerol,[27)] lipoprotein (a),[28)] and CRP levels in the blood.[29)] Some epidemiological and interventional studies have indicated that excess TFA intake is related to the onset of type 2 diabetes mellitus.[30)] On the other hand, there are some reports that dietary TFAs are not related to these parameters in the blood.[31)] The results of this study suggest that a very low level of dietary TFAs does not affect these parameters adversely, although further study is needed.

Many types of TFA exist due to variations of the numbers of double bonds and of the position of the *trans* double bond. Biohydogenation in the rumen produces monoene TFAs predominantly, and diene and triene TFAs are present in refined vegetable oils.[13)] In the present study, monoene TFAs ingested in meat and dairy products made up about half of total monoene TFA intake. The main sources of diene and triene TFAs were vegetable fats and oils. Lemaitre *et al.*[12)] reported that the intake of diene TFAs, in contrast with monoene TFAs, is correlated with a larger increase in the risk of coronary heart disease. There was no significant correlation between diene TFA intake and risk factors for atherosclerosis in our study. The results of the present study indicate that the diene TFA intake is less than a quarter of monene TFA intake in young Japanese women, and that the level of diene TFA intake (0.05% of energy) does not have a negative effect on serum lipid levels.

The mean intake of triene TFA was higher than that of diene TFA in this study, but no triene TFA in erythrocytes was detected, whereas diene TFA content of the erythrocytes was 0.3 g/100 g of fatty acids. It has found that accumulation of the erythrocytes might differ between diene and triene TFAs. The reason for this is not clear. DeLany *et al.*[32)] found that α-linolenic acid (18:3) is highly oxidized as compared to linoleic acid (18:2) in humans. In our study the α-linolenic content of the erythrocytes was less than 2% of linolenic acid. The difference in oxidation between diene and triene TFAs might be related to different accumulation in the erythrocytes.

In summary, the correlation of total and diene TFA intake with serum cholesterol levels were investigated in young Japanese women. The average intake of total TFAs was 0.36% of total energy, and was less than the upper limit of intake (1% of energy) recommended by the World Health Organization. The women who ingested about 1% of energy in the form of TFAs ate a very high-fat diet for Japanese individuals. The total TFA intake was correlated with erythrocyte TFA content, but not with serum cholesterol levels. The average intake of diene TFAs was less than 0.1% of total energy, and there were no significant correlations between diene TFA intake and serum cholesterol levels. These results suggest that the average TFA intake in young Japanese women does not adversely affect their serum cholesterol levels.

Acknowledgments

This study was supported in part by a research grant from the Research Foundation of the First Bank of Toyama, and the Kieikai Research Foundation in 2011. We thank Ms. Kanako Hirose, Ms. Mamami Yamaki, Mr. Yutaka Shirokawa, and Mr. Taiki Kutsuwada for assistance in performing nutrient calculations. We also thank Ms. Chiharu Fujimaki and Ms. Yuko Takeuchi for technical assistance.

References

1) Mossoba MM, Moss J, and Kramer JK, *J. AOAC Int.*, **92**, 1284–1300 (2009).
2) Mensink R and Katan MB, *N. Engl. J. Med.*, **323**, 439–445 (1990).
3) Aro A, Jauhiainen M, Partanen R, Salminen I, and Mutanen M, *Am. J. Clin. Nutr.*, **65**, 1419–1426 (1997).
4) Judd JT, Baer DJ, Clevidence BA, Kris-Etherton P, Muesing RA, and Iwane M, *Lipids*, **37**, 123–131 (2002).
5) Willett WC, Stampfer MJ, Manson JE, Colditz GA, Speizer FE, Rosner BA, Sampson LA, and Hennekens CH, *Lancet*, **341**, 581–585 (1993).
6) Ascherio A, Hennekens CH, Buring JE, Master C, Stampfer MJ, and Willett WC, *Circulation*, **89**, 94–101 (1994).
7) Oomen CM, Ocké MC, Feskens EJ, van Erp-Baart MA, Kok FJ, and Kromhout D, *Lancet*, **357**, 746–751 (2001).
8) Uauy R, Aro A, Clark R, Ghafoorunissa R, L'Abbé M, Mozaffarian D, Skeaff M, Stender S, and Tavella, *Eur. J. Clin. Nutr.*, **63**, S68–S75 (2009).
9) Ministry of Health, Labour and Welfare, "Dietary Reference Intakes for Japanese, 2010," Daiichi Shuppan, Tokyo, pp. 91–103 (2009).
10) Sugano M, *Oleosciene*, **8**, 121–126 (2008).
11) Yamada M, Sasaki S, Murakami K, Takahashi Y, and Uenishi K, *Asia Pac. J. Clin. Nutr.*, **18**, 359–371 (2009).
12) Lemaitre RN, King IB, Raghunathan TE, Pearce RM, Weinmann S, Knopp RH, Copass MK, Cobb LA, and Siscovick DS, *Circulation*, **105**, 697–701 (2002).
13) Foof Safty Commission in Cabinet Office, Government of Japan, "Basal Report of Evaluation of *Trans* Fatty Acids in Food (in Japanese)," Tokyo, pp. 1–45 (2007).
14) Takeuchi H, Yamaki M, Hirose K, Hienae C, Tabuchi E, and Sugano M, *Biosci. Biotechnol. Biochem.*, **75**, 2243–2245 (2011).
15) Folch J, Lees M, and Sloane Stanley GH, *J. Biol. Chem.*, **226**, 497–509 (1957).
16) Maruyama T, *Oleoscience*, **8**, 107–114 (2008).

17) Kawabata T, Shigemitsu S, Adachi N, Hagiwara C, Miyagi S, Shinjo S, Maruyama T, and Sugano M, *J. Nutr. Sci. Vitaminol.*, **56**, 64–70 (2010).
18) Sasaki S, "Evidence-Based Nutrition & Nutritional Epidemiology (in Japanese)," Dobunshoin, Tokyo, pp. 134–144 (2005).
19) World Health Organization, Diet, Nutrition and the Prevention of Chronic Diseases, WHO technical report series 916, pp. 54–60 (2003).
20) Kawabata T, Hyogo H, Hagiwara C, Matsuzaki S, and Shinjo S, *J. Jpn. Soc. Nutr. Food Sci.*, **61**, 161–168 (2008).
21) Pietinen P, Ascherio A, Korhonen P, Hartman AM, Willett WC, Albanes D, and Virtamo J, *Am. J. Epidemiol.*, **15**, 876–887 (1997).
22) Mozaffarian D, Pischon T, Hankinson SE, Rifai N, Joshipura K, Willett WC, and Rimm EB, *Am. J. Clin. Nutr.*, **79**, 606–612 (2004).
23) Bolton-Smith C, Woodward M, Fenton S, and Brown CA, *Eur. Heart J.*, **17**, 837–845 (1996).
24) Sun Q, Ma J, Campos H, Hankinson SE, and Hu FB, *Am. J. Clin. Nutr.*, **86**, 74–81 (2007).
25) Park Y, Lim J, Lee J, and Kim SG, *Br. J. Nutr.*, **102**, 1355–1361 (2009).
26) Sun Q, Ma J, Campos H, Hankinson SE, Manson JE, Stampfer MJ, Rexrode KM, Willett WC, and Hu FB, *Circulation*, **115**, 1858–1865 (2007).
27) Ascherio A, Katan MB, Zock PL, Stampfer MJ, and Willett WC, *N. Engl. J. Med.*, **340**, 1994–1998 (1999).
28) Mensink RP, Zock PL, Katan MB, and Hornstra G, *J. Lipid Res.*, **33**, 1493–1501 (1992).
29) Baer DJ, Judd JT, Clevidence BA, and Tracy RP, *Am. J. Clin. Nutr.*, **79**, 969–973 (2004).
30) Odegaard AO and Pereira MA, *Nutr. Rev.*, **64**, 364–372 (2006).
31) Mozaffarian D, Aro A, and Willett WC, *Eur. J. Clin. Nutr.*, **63**, S5–S21 (2009).
32) DeLany JP, Windhauser MM, Champagne CM, and Bray GA, *Am. J. Clin. Nutr.*, **72**, 905–911 (2000).