Eur J Nutr (2004) 43 : 313–318
DOI 10.1007/s00394-004-0479-x

ORIGINAL CONTRIBUTION

Ewa Stachowska
Barbara Dołęgowska
Dariusz Chlubek
Teresa Wesołowska
Kazimierz Ciechanowski
Piotr Gutowski
Halina Szumiłowicz
Radosław Turowski

Dietary trans fatty acids and composition of human atheromatous plaques

Summary Dietary fatty acids are incorporated into atheromatous plaques mainly in the form of cholesterol esters. Physicochemical properties of the plaque (e. g. mechanical strength) depend on its fatty acid composition. Trans isomers of unsaturated fatty acids (TFA) are known to reduce the availability of fatty acid precursors for the synthesis of anticoagulant PG_1 and PG_3 prostaglandins.

The present study was undertaken to determine the content of trans isomers in atheromatous plaques and to search for correlations between trans isomers in the plaque and adipose tissue. Atheromatous plaques were obtained from 31 patients who underwent surgery due to atherosclerotic stenosis of the abdominal aorta, iliac or femoral arteries. Fatty acids were extracted and separated as methyl esters using gas chromatography (GC) with an internal standard. Correlations were searched for with statistical methods, taking the level of significance as $p < 0.05$.

We found spatial and positional isomers of sixteen- and eighteen-carbon fatty acids in plaques and adipose tissue, with elaidic acid (C18:1 trans-9) being the most abundant. Every plaque and adipose tissue sample contained linolelaidic acid (C18:2 trans-9 trans-12) which is derived exclusively from linoleic acid, as well as conjugated dienes of linoleic acid (CLA) produced during oxidative processes. The presence of trans isomers of fatty acids in the atheromatous plaque seems to be of relevance to plaque formation. Of much concern is the detection of elaidic and linolelaidic acids which adversely affect the physiologically important metabolism of eicosanoids. The TFA pool in adipose tissue has little effect on the amount of these acids in the atheromatous plaque. Apparently, the presence of TFA in atheromatous plaques is the result of processes taking place during plaque formation and maturation.

Key words cis and trans octadecenoates – cis-trans fatty acid isomers – conjugated dienes of linoleic acid – atheromatous plaque

Received: 24 June 2003
Accepted: 26 November 2003
Published online: 27 January 2004

E. Stachowska (✉) · B. Dołęgowska · D. Chlubek
Dept. of Biochemistry & Chemistry
Pomeranian Medical University
al. Powstancow Wlkp 72
70-111 Szczecin, Poland
Tel.: +48-91/466-1515
Fax: +48-91/466-1515
E-Mail: ewast@sci.pam.szczecin.pl

T. Wesołowska
Department of Clinical Biochemistry
Pomeranian Medical University
Szczecin, Poland

K. Ciechanowski
Department of Internal Medicine
Pomeranian Medical University
Szczecin, Poland

P. Gutowski · H. Szumiłowicz · R. Turowski
Department of Vascular Surgery
Pomeranian Medical University
Szczecin, Poland

Abbreviations

BMI	body mass index
BHT	butylated hydroxytoluene
CLA	conjugated dienes of linoleic acid
IHD	ischemic heart disease
HDL	high density lipoprotein
LDL	low density lipoprotein
MUFA	monounsaturated fatty acids
PUFA	polyunsaturated fatty acids
SFA	saturated fatty acids
TLC	thin layer chromatography
TFA	trans fatty acids
TG	triacylglycerols

European Journal of Nutrition (2004) Vol. 43, Number 5

Introduction

One of the basic dietary recommendations in the management of atherosclerosis is the replacement of animal fat by plant oils and soft margarines. However, chemically hydrogenated plant oils contain varying amounts of trans isomers of fatty acids [1–3] raising concern over the therapeutic value of such diets [4–7]. Little is known about the effects of trans isomers on the formation and maturation of the plaque. Kummerow reported that under certain circumstances trans isomers increase the influx of calcium into endothelial cells, thus accelerating calcification processes in the vascular wall [8]. Other studies have shown that trans isomers adversely affect eicosanoid homeostasis. By inhibiting Δ^6-desaturase responsible for desaturation of ω6 (linoleic) and ω3 (α-linolenic) fatty acids, TFA reduce the availability of fatty acid precursors for the synthesis of anticoagulant PG_1 and PG_3 prostaglandins [9–11]. These findings provide some explanation why dietary polyunsaturated fatty acids have been implicated in plaque formation [12].

Aim of the study

On these grounds, it seemed interesting to determine the extent to which trans isomers of dietary fatty acids present in hydrogenated plant oils accumulate in the plaque and adipose tissue and whether correlations can be disclosed between the content of trans fatty acids in the adipose tissue and atheromatous plaque.

Methods

Subjects

The study group comprised thirty-one patients, Caucasian whites (31 males), aged 45 to 75 years, operated due to atherosclerotic stenosis of the abdominal aorta, iliac or femoral arteries (peripheral artery disease). All patients reported regular consumption of chemically processed plant oils. Baseline mean body mass index (BMI) was calculated as kg/m^2 (mean ± SD). Ten patients were smokers, eight were ex-smokers and three never smoked tobacco. An average standard of living was found for all patients and there were no cases of malnutrition. Individuals older than 75 years and patients with a history of hypertension or diabetes were excluded from the study. Patients were fully informed as to the study objectives and benefits and provided written consent prior to enrolment. The study protocol complied with ethical standards laid down in the Declaration of Helsinki and was approved by the Committee on Human Research at the Pomeranian Medical University.

Blood sampling and biochemical determinations

Venous blood for lipid and lipoprotein analyses was collected into tubes (without anticoagulant) after an overnight fast. Serum was obtained by centrifugation at 4 °C, 2500 RPM for 10 min. Total cholesterol and triacyloglycerols were assayed with enzymatic test kits [13, 14]. HDL-cholesterol was measured after precipitation with sodium magnesium phosphotungstate [15]. LDL-cholesterol was calculated from total cholesterol, triacyloglycerols and HDL-cholesterol concentrations using Fredewald's formula [16].

Tissue sampling

Only advanced atheromatous plaques that contained macroscopic gruel on incision were collected for examination. Typically, gruel from 3–6 lesions per subject was chosen. Atheroma wet weights were typically 5–25 mg. The atheromatous gruel was scooped out with the blunt side of the scalpel blade. A small sample (< 20 mg) of abdominal subcutaneous adipose tissue was obtained from each patient. Samples were sent to the laboratory within 1 hour of collection and were stored at –80 °C under nitrogen until assayed.

Extraction of lipids

The samples (plaque and adipose tissue) were thawed at room temperature (under nitrogen), weighed on aluminum foil degreased with chloroform and fragmented using a metal homogenizer immersed in liquid nitrogen. Care was taken to minimize exposure of samples to air.

Total lipids were extracted using 2:1 (v/v) chloroform/methanol containing 0.01 % (w/v) butylated hydroxytoluene as antioxidant [17], saponified in 2 % KOH and methylated with 20 % BF_3 in methanol for 15 min at 65 °C. Fatty acid methyl esters were extracted with hexane and concentrated under nitrogen. Fatty acid methyl esters were identified by comparison of their retention times with pure standards (Sigma-Aldrich Chemie GmbH, Germany). Fractions containing cis and trans C18:1 methyl esters (n = 15) were first isolated by thin-layer chromatography ($AgNO_3$-TLC) on pre-coated Merck 60 F_{254} silica gel plates (10x10 cm, Merck, Germany), impregnated with $AgNO_3$ by immersing in 10 % (w/v) $AgNO_3$ in acetonitrile, dried and activated at 110 °C for 1 hour. The plates were developed in chloroform containing 0.75 % ethanol and visualized under UV light after spraying with a 0.2 % (w/v) ethanolic solution of 2',7'-dichlorofluorescein. The separated bands were scraped from the plate, extracted with 1:1 (v/v) hexane:chloroform and concentrated under nitrogen for GC analysis [18].

A Perkin-Elmer gas chromatograph (model 8500) equipped with a flame ionization detector, split-splitless injector, and 105 m x 0.25 mm capillary column coated with RTX 2330 (Resteck Co, Bellefonte, PA, USA) was used to analyze in duplicate the plaque methyl esters. The oven temperature was held at 165 °C for 38 min, raised to 235 °C at 8 °C/min, and held at 235 °C for 20 min [19]. Fatty acids were computer identified in a temperature-programmed run (Chromed, Medson, Poland) and an electronic integrator was used to measure peak areas. All biochemical reagents were purchased from Sigma (Sigma-Aldrich Chemie GmbH, Germany).

Statistical analysis

Statistical analyses were performed with Statistica 5.1 software (Stat Soft Inc., Tulusa, USA). Correlation analyses were performed using Spearman's rank correlation test with regard to Bonferroni correction. All data in the tables are presented as mean ± SD.

Results

Age, BMI and lipid values in serum of 31 patients who participated in the study are shown in Table 1. Table 2 shows the mean content of fatty acids expressed as percent of total amount of fatty acids and standard deviation (SD) for each of the 32 fatty acids in plaques and adipose tissue from the patients.

The content of fatty acids in adipose tissue vs. atheromatous plaque was as follows: SFA 30.61 % vs. 29.82 %; MUFA 51.56 % vs. 34.82 %; n-6 series PUFA 14.89 % vs. 22.22 %; n-3 series PUFA 1.37 % vs. 1.15 %. The following fatty acids predominated in the adipose tissue: SFA – palmitic 21.29 %; MUFA – oleic 40.57 %; n-6 PUFA – linoleic 14.16 %; n-3 PUFA – linolenic 0.94 %. Predom-

Table 1 Patient data (mean ± S. D.) (n = 31)

Age (years)	61 ± 9.3
BMI (kg/m^2)	27.8 ± 2.4
Total Plasma Cholesterol (mM)	5.01 ± 0.5
HDL Plasma Cholesterol (mM)	1.2 ± 0.2
LDL Plasma Cholesterol (mM)	3.2 ± 0.6
Plasma Triacyloglycerol (mM)	1.4 ± 0.4

Table 2 Fatty acids content (nonisomeric and cis isomers) in atheromatous plaques and adipose tissue (%) (n = 31). Detection limit was 0.01 % of the total area. Unidentified peaks accounted for 1 % of the total area

	Length of fatty acids carbohydrate chain	Percentages of fatty acids in adipose tissue Mean ± SD	Percentages of fatty acids in atheromatous plaque Mean ± SD
Saturated (SFA)	12:0	0.36 ± 0.16	1.17 ± 0.32
	14:0	2.1 ± 0.95	1.32 ± 0.32
	15:0	0.51 ± 0.11	0.52 ± 0.15
	16:0	21.29 ± 1.01	20.64 ± 2.26
	17:0	0.30 ± 0.06	0.15 ± 0.04
	18:0	5.81 ± 1.29	5.61 ± 1.31
	20:0	0.24 ± 0.16	0.41 ± 0.16
	Total (SFA)	**30.61 ± 0.53**	**29.82 ± 0.65**
Monounsaturated (MUFA)	9c-14:1	0.27 ± 0.04	0.20 ± 0.06
	7c-16:1	0.93 ± 0.19	0.07 ± 0.01
	9c-16:1	7.57 ± 1.79	2.83 ± 0.72
	9c-18:1	40.57 ± 1.01	29.85 ± 4.45
	11c-18:1	0.83 ± 0.23	0.52 ± 0.26
	12c-18:1	0.80 ± 0.27	0.70 ± 0.25
	11c-20:1	0.59 ± 0.12	0.65 ± 0.19
	Total (MUFA)	**51.56 ± 0.52**	**34.82 ± 0.84**
Polyunsaturated (PUFA) n-6	18:2-n-6	14.16 ± 1.03	15.87 ± 2.00
	20:2 n-6	0.18 ± 0.04	0.46 ± 0.12
	20:3 n-6	0.15 ± 0.01	0.93 ± 0.17
	20:4 n-6	0.40 ± 0.05	4.96 ± 0.07
	Total (PUFA n-6)	**14.89 ± 0.28**	**22.22 ± 0.59**
Polyunsaturated (PUFA) n-3	18:3 n-3	0.94 ± 0.1	0.76 ± 0.28
	20:3 n-3	0.04 ± 0.01	0.04 ± 0.01
	20:5 n-3	0.07 ± 0.01	0.04 ± 0.01
	22:5 n-3	0.18 ± 0.04	0.20 ± 0.06
	22:6 n-3	0.14 ± 0.02	0.11 ± 0.01
	Total (PUFA n-3)	**1.37 ± 0.03**	**1.15 ± 0.07**

inating fatty acids in the atheromatous plaque were: SFA – palmitic 20.64 %; MUFA – oleic 29.85 %; n-6 PUFA – linoleic 18.56 %; n-3 PUFA – linolenic 0.76 %.

We found less n-6 series PUFA than reported previously [20] and a lower content of these fatty acids in adipose tissue (14.89 %) than plaques (22.22 %; Table 2). Interestingly, plaque content of arachidonic acid (C20:4, n-6), one of the metabolically most active members of n-6 PUFA, was increased.

The content of trans fatty acids in adipose tissue and in plaques (given as parts per thousand; Table 3) was similar to that reported by Hudgins et al. [19]. The main trans isomers present in the fatty acid pool of margarine and animal fat are vaccenic (11 t C18:1) and elaidic (9 t C18:1) acids. Much smaller quantities of trans isomers of linoleic and α-linolenic polyunsaturated fatty acids have been reported [18]. Our findings are similar (Table 3), with trans isomers of oleic acid predominating and with lesser amounts of linoleic acid species (9 t 12 t; 9 c 12 t; 9 t 12 c) originating from partially hydrogenated plant oils and milk fat (linoleic acid in plant oils is 9 c 12 c C18:2).

It is worth noting that atheromatous plaques have a great content of conjugated dienes of linoleic acid (particularly 9 c 11 t) (Table 3), probably due to specific processes taking place in the plaque.

The content of trans isomers in the plaque seems quite unrelated to the content of their counterparts in adipose tissue (Table 4). Only one statistically significant correlation was noted between the content of elaidic acid in adipose tissue and a cis isomer (11 c C18:1) in plaques. The coefficient of correlation with regard a Bonferroni correction carried out p = 0.01 (Table 4).

Table 3 Trans fatty acid given as parts per thousand in adipose tissue and atheromatous plaques (n = 31). Detection limit was 0.01 % of the total area. Unidentified peaks accounted for 1 % of the total area

	Content of trans fatty acids in adipose tissue (parts per thousand) Mean ± SD	Content of trans fatty acids in atheromatous plaque (parts per thousand) Mean ± SD
Monounsaturated (trans)		
9t 16:1	7±1	3±1
8t 18:1	14±6	9±4
9t 18:1	69±34	26±9
10t 18:1	67±26	23±6
11t 18:1	59±1	15±5
Total MUFA trans	**216±17**	**76±6**
Polyunsaturated (trans)		
9t12t 18:2	1±0.6	8±1
9c12t 18:2	14±1	12±3
9t 12c 18:2	8±1	6±2
CLA	38±14	21±6
Total PUFA trans	**61±4**	**47±2**

Table 4 Spearman's rank correlation coefficients R for fatty acids in atheromatous plaques and adipose tissue * n = 31, p = 0.01

Plaque/Adipose Tissue	Spearman's R
*cis*11 C18:1/trans 9 C18:1	0.49*

Discussion

In spite of extensive studies, controversy remains as to the involvement of dietary trans isomers of fatty acids in the etiology of ischemic heart disease (IHD) [21–23]. Several reports [5, 7] have shown that the present level of consumption of trans isomers in Europe is unrelated to the risk of IHD, notwithstanding the possibility that risk might be associated with higher consumption levels. On the other hand, population studies in the USA have unequivocally implicated trans isomers in the etiology of IHD [24].

Trans isomers are not synthesized in humans. Thus, the whole pool of trans fatty acids in the adipose tissue is derived exclusively from the diet and as a consequence, levels of these acids closely reflect dietary habits of the individual [12]. We have demonstrated that trans isomers of fatty acids accumulate in human adipose tissue and atheromatous plaques (Table 3). One of our aims was to determine the importance of adipose tissue as a source of TFA for the atheromatous plaque by analogy to the finding of Felton [12] that essential fatty acids (EFA) from the diet are intensely exchanged between the adipose tissue and plaque (which is relatively devoid of metabolic activity). Felton showed that the adipose tissue supplies fatty acids that are metabolized by the plaque. In this connection, we wanted to know whether adipose tissue TFA (derived exclusively from the diet) can be incorporated into the atheromatous plaque.

No correlation between the content of trans fatty acids in the adipose tissue and plaque was found by us (Table 4), other than the correlation between 11c C18:1 in the plaque and 9t C18:1 in the adipose tissue. It can thus be inferred that the adipose tissue contributes little to the content of TFA in the plaque (Table 4), as opposed to the diet which appears to be the chief source of plaque TFA. Moreover, trans isomers of fatty acids are a readily accessible source of energy [25, 26]. In this context one should remember that plaque TFA are essentially in the form of cholesterol esters and that esterification of circulating cholesterol takes place in the HLD_2 lipoprotein fraction. Fatty acids used in this process are derived from triacyloglycerol-rich lipoprotein fractions, i. e. chylomicrons produced during intestinal resorption of fat and VLDL continuously synthesized by the liver from endogenous fatty acids (e. g. released by lipolysis in adipocytes). Lack of correlation between TFA content in plaque and adipose tissue probably indicates that TFA

for cholesterol esterification in HDL_2 are chiefly derived from chylomicrons. The rate of this process would depend on fat resorption. Cholesterol esters are delivered to the atheroma by LDL, having been transferred to this lipoprotein class with the mediation of CETP. It has been shown that TFA enhance CETP activity, resulting in higher levels of cholesterol in LDL [23, 27, 28].

The majority of trans isomers are taken up from chemically processed plant oils, with a minor contribution by animal fat [29]. As shown in Table 3, atheromatous plaques contain trans isomers of fatty acids, with elaidic acid and conjugated dienes of linoleic acid being the major species in plaques and adipose tissue alike. Their sources differ, insofar as elaidic acid is exclusively exogenous, while CLA are partly of dietary origin (animal fat) and partly a by-product of free radical reactions [25, 29]. The double bonds in conjugated dienes of linoleic acid are separated by a single bond and are usually present at positions 9 c and 11 t or 10 c and 12 t. The spatial arrangement of CLA is cis-cis, trans-trans, cis-trans or trans-cis. CLA have protective properties against atherosclerosis and tumorigenesis and their levels seem to reflect the rate of peroxidation [25, 29]. Reports from in vivo and in vitro studies have confirmed their antimutagenic and anticancerogenic action, as well as antioxidative properties which are reflected in the protection of the cellular membrane against free radicals. It has been hypothesized that these compounds are oxidized inside cells to highly cytotoxic radicals. Studies in mice have shown that 9 c 11 t C18:2 is the biologically most active diene of the CLA group and is preferentially incorporated into phospholipids of cell membranes. Significant quantities of this CLA species have been found by us in atheromatous plaques (Tables 3). We could not demonstrate any correlation between both CLA species (9 c 11 t and 10 c 12 t) in the plaque or adipose tissue. The finding of large quantities of CLA in plaques and absence of correlations suggest that plaque CLA are derived partly from the diet and probably from free radical oxidation of linoleic acid [25]. Even though oxidation of linoleic acid to CLA has previously been postulated by Dormandi [30], satisfactory evidence in favor of this process remains to be presented. Some researchers believe that vaccenic acid (11 t C18:1) is transformed in humans to 9 c 11 t CLA [30, 31]. Unexpectedly, we did not observe any correlation between 9 c 11 t CLA and its metabolic precursor – vaccenic acid (11 t C:18:1). This result can be explained by relatively low levels of vaccenic acid in the plaque or by the limited number of plaques examined by us [25].

Once it is accepted that trans isomers cannot be synthesized "de novo" in humans, the major factor affecting their content in plaque (Tables 3) was the diet of the patients. In this case, the beneficial increase in the plaque content of CLA would depend on the affinity for CLA of the cholesterol–fatty acid transfer system.

We found a higher content of fatty acids in the adipose tissue than plaque, except for n-6 PUFA (Table 2) and their prominent representative – arachidonic acid (C20:4). It has well been established that arachidonic acid is derived from two sources: diet and de novo synthesis in tissues. Apparently, de novo synthesis of C20:4 contributed to the higher content of this acid in atheromatous plaques and provided the substrate for numerous inflammatory processes taking place in these structures. In this context, the presence in plaques of PUFA derived from linoleic acid (9 t 12 t, 9 c 12 t, 9 t 12 c C18:2) is an interesting finding, considering their potent antagonistic action on the metabolism of arachidonic acid [19]. Trans isomers of linoleic acid detected by us in plaques and adipose tissue are known for their adverse effects on cellular metabolic reactions. The 9 t 12 t isomer inhibits the conversion of linoleic (9 c 12 c C18:2) to arachidonic acid, thus affecting the rate of synthesis of eicosanoids. Another isomer, 9 c 12 t C18:2, inhibits Δ^6-desaturase and subsequently suppresses the biosynthesis of polyunsaturated fatty acids [19].

It is known from the literature that trans isomers of mono- and polyunsaturated fatty acids antagonize the metabolism of essential polyunsaturated fatty acids (PUFA), leading to their deficiency in the cell [26, 32]. For example, positional isomers of C18:1 are specific inhibitors of elongation and desaturation of some cis-unsaturated fatty acids [9]. This fact may be of importance in plaque growth, particularly with regard to the role of PUFA as a substrate for the synthesis of hemostatically active prostanoids (thromboxanes and prostacyclins) [15]. Trans isomers seem to displace the respective cis isomers from metabolic pathways, thereby disturbing the balance between anticoagulant prostacyclins and procoagulant thromboxanes [11]. Evidence for such action comes from rat studies showing that 9 t 12 t C18:2 isomer displaces its 9 c 12 c C18:2 counterpart in plaques, inhibiting the conversion of linoleic acid and decreasing prostaglandin production [28].

Acknowledgments Source of funding: This study was supported by research grant 130–649 from the Pomeranian Medical University, Szczecin, Poland.

Consent and permission: Patients were fully informed as to the study objectives and benefits and provided written consent prior to enrolment. The study protocol complied with ethical standards laid down in the Declaration of Helsinki and was approved by the Committee on Human Research at the Pomeranian Medical University.

References

1. Slover HT, Thompson JR, Davis CS, Merola GV (1985) Lipids in margarines and margarine-like foods. J Am Oil Chem Soc 62:775–786
2. Zock PL, Katan MB (1997) Butter, margarine and serum lipoproteins. Atherosclerosis 131:7–16
3. Judd JT, Baer DJ, Clevidence BA, Muesing RA, Chen SC, Weststrate JA, Meijer GW, Wittes J, Lichtenstein AH, Villela-Bach M, Schaefer EJ (1998) Effects of margarine compared with those of butter on blood lipid profiles related to cardiovascular disease risk factors in normolipemic adults fed controlled diets. Am J Clin Nutr 68:768–777
4. Willett WC, Stampfer MJ, Manson JE, Colditz GA, Speizer FE, Rosner BA, Sampsson LA, Hennekens CHH (1993) Intake of trans fatty acids and risk of coronary heart disease among women. Lancet 34:581–585
5. Aro A, Kardinaal AFM, Salminen I, Kark JD, Riemersma RA, Delgado-Rodriguez M, Gomez-Aracena J, Huttuenen JK, Kohlemer L, Martin BC, Martin-Moreno JM, Mazaev VP, Ringstad J, Thamm M, van't Veer P, Kok FJ (1995) Adipose tissue isomeric trans fatty acids and risk of myocardial infarction in nine countries: the Euramic study. Lancet 345:273–278
6. Ascheiro A, Hennekens CHH, Burning JE, Master C, Stampfer MJ, Willett WC (1994) Trans fatty acids intake and risk of myocardial infarction. Circulation 89:94–101
7. van der Vijver LP, Kardinaal AF, Couet C, Aro A, Kafatos A, Steingrimsdottir L, Amorim Cruz JA, Moreiras O, Becker W, van Amelsvoort JMM, Vidal-Jessel S, Salaminen I, Moschandreas J, Sigfusson N, Martins I, Carbajal A, Ytterfors A, van Poppel G (2000) Association between trans fatty acid and cardiovascular risk factors in Europe: the Transfair study. Eur J Clin Nutr 54:126–135
8. Kummerow FA, Zhou Q, Mahfouz MM (1999) Effect of trans fatty acids on calcium influx into human arterial endothelial cells. Am J Clin Nutr 70: 832–838
9. Lawson LD, Hill EG, Holman RT (1983) Suppression of arachidonic acid in lipids of rat tissues by dietary mixed isomeric cis and trans octadecenoates. J Nutr 113:1827–1835
10. Cook H, Emken EA (1990) Geometric and positional fatty acid isomers interact differently with desaturation and elongation of linoleic and linolenic acids in cultured glioma cells. Biochem Cell Biol 68:653–660
11. Jones D (1993) Trans fatty acids and dieting. Lancet 341:1093
12. Felton CV, Crook D, Davies MJ, Oliver MF (1994) Dietary polyunsaturated fatty acids and composition of human aortic plaques. Lancet 344:1195–1196
13. Allain CC, Poon LS, Chang GSC, Richmond W, Fu PC (1974) Enzymatic determination of total serum cholesterol. Clin Chem 20:470–475
14. Bucolo G, David H (1973) Quantitative determination of serum triglycerides by use of enzymes. Clin Chem 19: 476–482
15. Grove TH (1979) Effect of reagent pH on determination of high-density lipoprotein cholesterol by precipitation with sodium phosphotungstate-magnesium. Clin Chem 25:560–564
16. Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of preparative ultracentrifuge. Clin Chem 18: 499–502
17. Folch J, Lees M, Stanley Sloane V (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509
18. Ulberth F, Henninger M (1992) Simplified method for the determination of trans monoenes in edible fats by TLG-GLC. JAOCS 69:829–831
19. Hudgins LC, Hirsch J, Emken EA (1991) Correlation of isomeric fatty acids in human adipose tissue with clinical risk factors for cardiovascular disease. Am J Clin Nutr 53:474–482
20. Felton CV, Crook D, Davies MJ, Oliver MF (1993) Lipid and fatty acid distribution in nonulcerated and ulcerated human aortic plaques. J Vasc Med Biol 4: 228–234
21. Katz AM (2002) Trans-fatty acids and sudden cardiac death. Circulation 105: 669–671
22. Lemaitre RN, King IB, Raghunathan TE, Pearce RM, Weinmann S, Knopp RH, Copass MK, Cobb LA, Siscovick DS (2002) Cell membrane trans fatty acid and the risk of primary cardiac arrest. Circulation 105:697–701
23. Katan MB (2000) Trans fatty acids and plasma lipoproteins. Nutr Rev 58: 188–191
24. Sebedio JL, Vermunt S H, Chardigny JM, Beaufrere B, Mensink RP, Armstrong RA, Christie WW, Niemela J, Henon G, Riemersma RA (2000) The effect of dietary trans alpha-linolenic acid on plasma lipids and platelet fatty acid composition: the TransLinE study. Eur J Clin Nutr 54:104–113
25. Bartnikowska E (2000) Can CLA be regarded as a nutraceutical? Pol J Food Nutr Sci 35:55–59
26. Gurr MI (1990) Trans fatty acids and health – an update. Lipid Technology 2:105–107
27. Abbey M, Nestel PJ (1994) Plasma cholesterol ester transfer protein activity is increased when trans-elaidic acid is substituted for cis-oleic acid in the diet. Atherosclerosis 106:99–107
28. Katan MB, Zock PL, Mensik RP (1995) Trans fatty acids and their effect on lipoprotein in humans. Ann Rev Nutr 15:473–793
29. Pariza MW, Park Y, Cook ME (2000) Mechanisms of action of conjugated linoleic acid: evidence and speculation. Proc Soc Exp Biol Med 223:8–13
30. Dormandi TL, Wickens DG (1987) The experimental and clinical pathology of diene conjugation. Chem Phys Lipids 45:353–364
31. Adlof RO, Duval S, Emken EA (2000) Biosynthesis of conjugated linoleic acid in humans. Lipids 35:131–135
32. Kinsella JE, Bruckner G, Mai J, Shimp J (1981) Metabolism of trans fatty acids with emphasis on the effect of trans, trans-octadecadienoate on lipid composition, essential fatty acid and prostaglandins: an overview. Am J Clin Nutr 43:2307–2318