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CAPILLARY COLUMN GAS CHROMATOGRAPHY–MASS SPECTROMETRY FOR THE DETERMINATION OF THE FATTY ACID COMPOSITION OF HUMAN ADIPOSE TISSUE

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SUMMARY

A procedure for determining the fatty acid composition of human adipose tissue using gas chromatography–mass spectrometry was developed. Adipose tissue was obtained from the lateral upper aspect of the right thigh by needle biopsy and prepared for analysis by lyophilisation, total lipid extraction and base-catalysed transesterification of the complexed fatty acids to form fatty acid methyl esters. Capillary column gas chromatography resolved thirty different peaks, ranging in carbon length from 12 to 24. Provisional identification of the peaks was by cochromatography with authentic standards and confirmed by gas chromatography–mass spectrometry using electron-impact ionisation. Fatty acid methyl esters were quantified in absolute amounts with respect to dry tissue weight and as a percentage of the total fat. Statistical analysis of the results from twenty healthy subjects using the two-tailed unpaired Student's *t*-test demonstrated women had significantly higher levels of myristoleic and palmitoleic acids ($p < 0.001$) and lower levels of palmitic acid ($p < 0.05$) in adipose tissue when compared with the male group. Similarly total saturated fatty acids was lower ($p < 0.05$) and total monounsaturated fatty acids was higher in women than in men.

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INTRODUCTION

The determination of the fatty acid composition of adipose tissue is of interest since variations in composition may be related to the risk of coronary heart disease [1–3]. There were few early studies, the analytical techniques available, such as determination of melting points, iodine numbers and fractional crystallisation being cumbersome.

The introduction of gas chromatography (GC) in the 1950s improved analytical capabilities, enabling more detailed studies of adipose tissue to be undertaken. Hirsch and co-workers [4,5], using packed columns, found 35–40 fatty acid peaks. The proportions of 22 different fatty acids were reported, of which 6 [myristic ($C_{14\ 0}$), palmitic ($C_{16:0}$), palmitoleic ($C_{16\ 1}$), stearic ($C_{18:0}$), oleic ($C_{18\ 1}$) and linoleic ($C_{18\ 2}$)] comprised more than 90% of the total. Subsequent studies using the same technique examined the effect of biopsy site variation [6–9], gender [10–12], diet [3,13–24] and various disease states [1,7,9,22–33] on the fatty acid composition of adipose tissue.

Results of some comparable studies have conflicted [12,27,31–33]; possible factors to explain these discrepancies include inadequate resolution [7,34] and quantification difficulties [34]. Most previous GC studies have used packed columns. The quantity of individual fatty acids derived from adipose tissue have usually been expressed as a percentage of the total rather than an absolute amount. Previous chromatographic identification of the individual fatty acid components of adipose tissue has been by comparing their retention times with those of authentic standards.

In the present study, differences in the fatty acid composition of adipose tissue has been determined in males and females using: (1) the greater efficiency of capillary column GC; (2) gas chromatography–mass spectrometry (GC–MS) for positive identification of the individual fatty acid methyl esters derived from adipose tissue; and (3) the construction of calibration tables using carbon numbers of fatty acids for which no standards could be obtained.

EXPERIMENTAL

Fatty acid methyl ester standards were obtained from Sigma (Poole, U.K.), Alltech Assoc. (Carnforth, U.K.) and Fluorochem (Glossop, U.K.). Glycerol [1-^{14}C]trioleate (specific activity 47.5 mCi/mmol) was obtained from Amersham International (Amersham, U.K.). Analar solvents were from BDH (Blaydon-on-Tyne, U.K.). Methanolic-base reagent (0.5 *M*) was obtained from Supelchem (Sawbridgeworth, U.K.).

The internal standard solution was prepared by dissolving 200 mg of tricosanoic acid methyl ester ($C_{23\ 0}$) in 100 ml of benzene. The calibration solutions were prepared by dissolving known quantities of fatty acid methyl ester stan-

dards in heptane, containing 2,6-di-*tert.*-butyl-*p*-cresol (butylated hydroxytoluene, BHT) obtained from BDH.

Optiphase "Safe" was obtained from Fisons, Scientific Equipment Division (Loughborough, U.K.); N,O-bis(trimethylsilyl)acetamide and pyridine were obtained from Pierce & Warriner (U.K.) (Chester, U.K.); trimethylchlorosilane was from BDH. 3% OV-1 Chromosorb W-HP (80–100 mesh) was obtained from Alltech Assoc./Applied Science (Carnforth, U.K.).

Apparatus

A Hewlett Packard Series 5790A gas chromatograph with a flame ionisation detector and heated injection ports and detectors was used (Hewlett Packard, Wokingham, U.K.). An SP2330 wall-coated open tubular (WCOT) fused-silica capillary column (30 m×0.24 mm I.D.; film thickness 0.20 μm) was obtained from Supelchem. Filters to remove oxygen and moisture from the carrier gas (nitrogen) were used. MS data were obtained using a Finnigan MAT 1020 automated GC–MS system (Finnigan MAT, San Jose, CA, U.S.A.). An LKB-Wallac 'Rackbeta' scintillation counter was used for measuring the radioactivity of glycerol [1-^{14}C]trioleate.

Methods

Twenty healthy volunteers (ten males and ten females) were recruited from hospital, medical or laboratory staff. All were Caucasian. Blood levels of glucose, glycosylated haemoglobin, cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, urate, thyroid, renal and liver function tests were determined. The body mass index was calculated from weight and height measurements. A simple dietary history was obtained. The subjects gave written consent. The study was approved by the Newcastle Health Authority Ethical Committee.

Samples of adipose tissue were aspirated using a closed biopsy technique from the upper lateral aspect of the right thigh and stored in the dark under nitrogen at −20°C until analysed. Stored fat was stable for at least twelve months. Antioxidants were not necessary.

Total lipids were extracted from adipose tissue by a modification of a previously reported procedure [35]. Approximately 10–20 mg adipose tissue was used. Glycerol [1-^{14}C]trioleate in toluene (100 μl) was added to assess subsequent recovery of lipid during extraction. After lyophilisation the dry weight of the tissue was obtained, and the lipids were extracted with 3×3 ml of chloroform–methanol (2:1, v/v). The extracts were pooled and mixed with 2.25 ml of 0.12 mol/l potassium chloride solution. The upper phase was discarded and the lower phase shaken with 1.25 ml of methanol–water (1:1, v/v). After discarding the upper phase, the lipid extract was evaporated to dryness at 37°C under nitrogen. The lipids were then redissolved in 1 ml internal standard solution. A 100-μl aliquot was removed for measurement of radioactive triglyceride recovered during extraction.

Fatty acids were derivatised by base-catalysed transesterification. To 1 ml lipid solution, 1 ml methanolic-base reagent was added and heated at 80°C for 20 min. After cooling, distilled water (3 ml) and diethyl ether (3 ml) were added. The upper organic phase was washed with 2 ml water and recentrifuged. The organic phase was transferred to a stoppered glass tube and dried with a small quantity of anhydrous sodium sulphate. Fatty acid methyl ester solution (1.5 ml) was transferred into a small glass vial and evaporated to dryness under nitrogen. The residue was redissolved in a suitable volume (50–200 μl) of heptane prior to injection into the GC system.

The gas chromatograph was used in the split injection mode. A split ratio of 25:1 was used. The injector mixing chamber was packed with 3% OV-1 Chromosorb W-HP (80–100 mesh), previously deactivated to prevent adsorption effects by immersion in a mixture of 1 ml N,O-bis(trimethylsilyl)acetamide, 1 ml trimethylchlorosilane and 1 ml pyridine, for 15 min. Results using this method of injection were compared with those obtained by on-column injection to establish if there was selective absorption on the injector.

Following injection, the oven temperature was maintained at 170°C for 5 min, then programmed to increase at a rate of 5°C/min to a final temperature of 220°C for 5 min. The gas pressures which gave optimal performance of the flame ionisation detector were: hydrogen, 140 KPa; air, 165 KPa, auxiliary (nitrogen make-up gas), 140 KPa. Gas flow-rates were: hydrogen, 40 ml/min; air, 200 ml/min; column flow-rate, 2 ml/min; auxilliary flow-rate, 24 ml/min; split flow-rate, 48 ml/min; septum purge flow-rate, 3 ml/min. A 1-μl injection of test material was made.

An estimate of the smallest quantity of fatty acid methyl esters that could be detected was assessed by analysing dilutions of standards (1 mg/ml) C_{14}–C_{24}. A peak was deemed to be measurable if its height was at least twice that of the baseline noise. The detector response to fatty acid methyl esters of different chain-length was determined by repeated analysis of mixtures containing known proportions of standard fatty acid methyl esters. The detector response was expressed as an area per mol of fatty acid methyl ester relative to the molar response of palmitic acid methyl ester ($C_{16\ 0}$). The linearity of the detector response was investigated by injecting oleic acid over the concentration range 0–125 mg/ml.

The mass spectrometer was used in the electron-impact mode. The ion source temperature was maintained at 80°C, multiplier voltage was 2.0 kV, emission current was 0.55 mA and electron energy was 70 eV. The initial oven temperature was 90°C. Following a 0.5-μl injection, the temperature was programmed to increase at a rate of 6°C/min to a final temperature of 220°C for 2 min. Helium was used as the carrier gas with a flow-rate of 2 ml/min.

The fatty acid methyl esters derived from adipose tissue were provisionally identified by cochromatographing with authentic commercial fatty acid methyl ester standards. Positive identification was by GC–MS. Quantification of fatty

TABLE I

COMPARISON OF DETECTOR RESPONSE FOR FATTY ACID METHYL ESTER (FAME) STANDARDS ($n=6$) USING ON-COLUMN AND SPLIT MODE INJECTION

C.V. = coefficient of variation.

FAME	On-column		Split mode		
	Mean area (relative to palmitic acid)	C.V. (%)	Mean area (relative to palmitic acid)	C.V. (%)	Recovery compared to on-column (%)
$C_{14\ 0}$	0.885	3.4	0.850	0.5	96.0
$C_{14\ 1}$	0.877	1.7	0.882	0.6	100.6
$C_{15\ 0}$	0.934	1.8	0.926	0.2	99.1
$C_{17\ 0}$	1.082	1.6	1.053	0.4	97.3
$C_{18\ 0}$	1.162	1.9	1.104	0.8	95.0
$C_{18\ 0}$	1.181	2.0	1.090	1.5	92.3
$C_{18\ 2}$	1.144	2.3	1.136	2.6	99.3
$C_{18\ 3n3}$	1.110	2.8	1.094	2.0	98.6
$C_{19\ 0}$	1.209	3.8	1.107	3.7	91.6
$C_{20\ 0}$	1.225	4.1	1.230	1.0	100.4
$C_{20\ 1}$	1.272	5.9	1.203	0.8	94.6
$C_{22\ 0}$	1.354	5.4	1.338	4.2	99.1

TABLE II

DETECTOR RESPONSE FOR FATTY ACID METHYL ESTER (FAME) STANDARDS

FAME	Mean area (per mol relative to palmitic acid methyl ester)	FAME/palmitic acid methyl ester molecular weight ratio
$C_{12\ 0}$	0.692	0.793
$C_{14\ 0}$	0.827	0.896
$C_{14\ 1}$	0.808	0.889
$C_{15\ 0}$	0.926	0.948
$C_{17\ 0}$	1.060	1.052
$C_{18\ 0}$	1.110	1.106
$C_{18\ 1n9}$	1.090	1.097
$C_{18\ 2n6}$	1.100	1.089
$C_{18\ 3n6}$	1.041	1.082
$C_{18\ 3n3}$	1.065	1.082
$C_{19\ 0}$	1.226	1.156
$C_{20\ 0}$	1.220	1.207
$C_{20\ 1n9}$	1.280	1.200
$C_{22\ 0}$	1.332	1.311

TABLE III

RELATIVE RESPONSE RATIOS OF FATTY ACID METHYL ESTER (FAME) STANDARDS TO THE INTERNAL STANDARD TRICOSANOIC ACID METHYL ESTER ($C_{23\ 0}$)

FAME	Relative response ratio[a]
$C_{12\ 0}$	1.087
$C_{14\ 0}$	0.988
$C_{14\ 1}$	1.027
$C_{15\ 0}$	0.943
$C_{16\ 0}$	0.929
$C_{16\ 1}$	0.968
$C_{17\ 0}$	0.943
$C_{18\ 0}$	0.969
$C_{18\ 1n9}$	0.947
$C_{18\ 2n6}$	0.927
$C_{18\ 3n6}$	1.200
$C_{18\ 3n3}$	0.956
$C_{19\ 0}$	0.973
$C_{20\ 0}$	0.980
$C_{20\ 1}$	0.940
$C_{20\ 2}$	0.901
$C_{20\ 3}$	0.920
$C_{20\ 4}$	0.914
$C_{20\ 5}$	1.147
$C_{22\ 0}$	0.970
$C_{22\ 1}$	0.986
$C_{24\ 0}$	0.993
$C_{24\ 1}$	1.001

[a]Relative response ratio = (area of internal standard × weight of fatty acid methyl ester in standard solution) / (area of fatty acid methyl ester × weight of internal standard in standard solution).

acid methyl esters was achieved by internal standardisation ($C_{23\ 0}$) using peak-area integration. Fatty acid methyl esters for which no standard was available were quantified using calibration tables of relative response ratios constructed according to carbon number (using GC–MS). Results of the fatty acid composition of adipose tissue were expressed with respect to the dry tissue weight, in addition to the percentage of the total fat.

Statistical analysis of the results was by the two-tailed unpaired Student's *t*-test.

RESULTS

The sensitivity of the analytical method allowed quantification of all fatty acids measured at 1 μg/ml. Recovery, using a packed injection port in the split

mode, approached 100%, this method being more precise than on-column injection (Table I). The detector response, expressed as an area per mol fatty acid methyl ester relative to the molar response of $C_{16\ 0}$ increased as the chain-length of the fatty acid increased (Table II). When expressed on a weight basis compared to the internal standard ($C_{23\ 0}$) response ratios approached 1 (Table III). The relative response of oleic acid to internal standard was linear between 0 and 125 mg/ml, a concentration range greater than that found in adipose tissue.

All twenty subjects studied had normal renal, hepatic and thyroid function tests, and fasting cholesterol and triglyceride levels of <6.5 mmol/l and <1.8 mmol/l, respectively. The mean ($\pm$S.E.M.) age in the male group was 30 ± 1

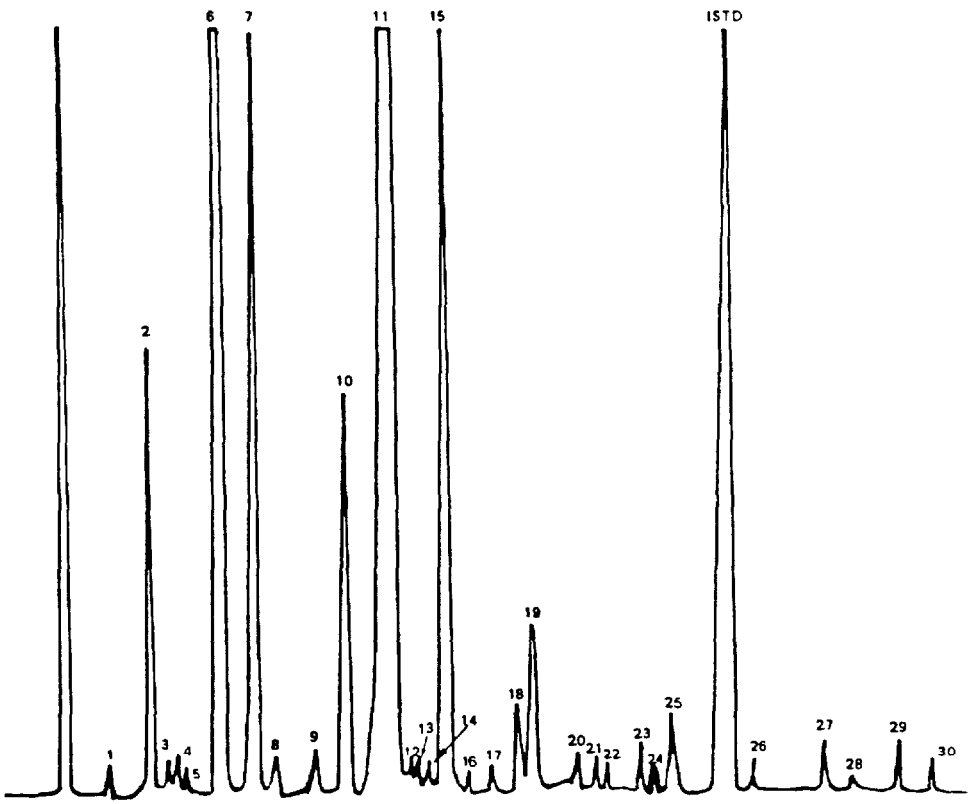

Fig. 1. Gas chromatogram of fatty acid methyl esters of human adipose tissue. Peaks: $1 = C_{12\ 0}$; $2 = C_{14\ 0}$; $3 = C_{14\ 1}$; $4 = C_{15\ 0}$; $5 = C_{16\ 0i}$; $6 = C_{16\ 0}$; $7 = C_{16\ 1}$; $8 = C_{17\ 0}$; $9 = C_{17\ 1}$; $10 = C_{18\ 0}$; $11 = C_{18\ 1}$; $12 = C_{18\ 1i}$; $13 = C_{19\ 0}$; $14 = C_{18\ 2i}$; $15 = C_{18\ 2}$; $16 = C_{18\ 3n6}$; $17 = C_{20\ 0}$; $18 = C_{18\ 3n3}$; $19 = C_{20\ 1}$; $20 = C_{20\ 1i}$; $21 = C_{20\ 2}$; $22 = C_{22\ 0}$; $23 = C_{20\ 3}$; $24 = C_{22\ 1}$; $25 = C_{20\ 4}$; $26 = C_{20\ 5}$; $27 = C_{24\ 0}$; $28 = C_{22\ 4}$; $29 = C_{22\ 5}$; $30 = C_{22\ 6}$; ISTD $= C_{23\ 0}$ (internal standard). i = isomer.

TABLE IV

COMPARISON OF PRESENT STUDY WITH OTHER RESULTS

Fatty acid	Mean percentage fatty acid composition of adipose tissue							
	Krut and Bronte-Steward [10] (buttock fat)		Antonini et al. [12] (buttock fat)		Van Staveren et al. [24] (buttock fat)	Berry et al. [39] (buttock fat)	Current study (thigh fat)	
	Male	Female	Male	Female	Female	Male	Male	Female
$C_{14\ 0}$	3.5	3.2	–	–	2.8	2.4	2.4	2.5
$C_{14\ 1}$	–	–	–	–	0.6	–	0.4	0.6[a]
$C_{16\ 0}$	23	22.1[a]	21	18[b]	18	19	19	18[b]
$C_{16\ 1}$	7.4	9.3[a]	6.3	5.2	7.7	6.5	7.0	9.1[a]
$C_{18\ 0}$	5.1	3.5[a]	3.6	3.9	3.5	3.7	3.3	2.9
$C_{18\ 1}$	45	46[a]	59	62[b]	45	46	46	49
$C_{18\ 2}$	9.8	9.6	11	10	15	16	13	12

[a] $p < 0.01$.
[b] $p < 0.05$.

years, in the female group 26 ± 1 years, and the mean (± S.E.M.) body mass index values were 24.3 ± 0.8 and 22.1 ± 0.6 kg/m^2, respectively. One of the female subjects was taking the contraceptive pill. Her results did not differ from the whole female group.

All subjects were of a steady body weight. There had been no dietary changes during the eighteen months preceding the study and all subjects consumed a mixed diet, including high-fibre cereals, margarine and cooking oils high in polyunsaturated fats.

A typical chromatogram of fatty acid methyl esters derived from male human adipose tissue is shown in Fig. 1. Recovery of the radioactive tracer (glycerol [1-^{14}C]-trioleate), after extraction of the adipose tissue, was more than 90%, and earlier experiments showed 99.5% transesterification of triglycerides using the described method. The coefficients of variation of the analytical technique used varied from 0.2% for $C_{18\ 3n3}$ to 1.7% for $C_{14\ 0}$.

The results of adipose tissue analysis by GC and GC–MS are shown in Table V. The total saturated fatty acid amounts were higher and the total monounsaturated fatty acid amounts were lower in the men than women. These differences were due to statistically higher $C_{16\ 0}$ and lower $C_{14\ 1}$ and $C_{16\ 1}$ fatty acid levels in men. Despite the observed differences of certain individual fatty acids between men and women, when the major saturated fatty acids were considered with their respective monounsaturated fatty acids (i.e. $C_{14:0}+C_{14\ 1}$, $C_{16\ 0}+C_{16\ 1}$, $C_{18\ 0}+C_{18\ 1}$) no gender differences were found. A comparison of results obtained in the present study with previous data is shown in Table IV.

DISCUSSION

The method described in the present report is an adaptation of procedures previously described for fatty acid methyl ester analysis [36–38] to the analysis of adipose tissue. The principle advantages of this procedure for adipose tissue analysis over those previously described are: (1) there is improved resolution of fatty acids by using capillary columns; this overcomes the difficulty that some have found in establishing the presence of arachidonic acid [7]; (2) fatty acids have been positively identified by MS; (3) fatty acids for which no commercial standards are available have been quantified using MS.

A wide linear range was necessary to ensure accurate quantitation of components which varied from traces to large amounts. A previous report has shown that the flow-rate of hydrogen is critical, sensitivity being improved by lower flow-rates (35 ml/min) and linearity being improved by higher flow-rates (45–50 ml/min [36]). By using an intermediate flow-rate (40 ml/min) linearity and sensitivity were both satisfactory for the analysis of the widely differing amounts of fatty acid found in adipose tissue.

The derivatisation procedure used was base-catalysed transesterification. This limits the analysis to neutral fats and is more rapid than saponification

followed by derivatisation with boron trifluoride–methanol [37,38]. Methoxide was removed during extraction, thus avoiding effects such as column deterioration and bleeding [38].

Results in healthy subjects showed gender differences in fatty acid composition of adipose tissue (FACAT). The total amount of monounsaturated fat was lower and the amount of saturated fat higher in males than females. This was largely accounted for by differences in myristoleic, palmitoleic and palmitic acids. These results are in broad agreement with previous studies of buttock fat (Table IV). The levels of linoleic acid found in the present and two other recent studies [23,39] were higher than in early ones [10,11] possibly reflecting recent trends to increased dietary intake of linoleate [39]. The higher percentage of oleic acid in the study of Antonini et al. [12] probably reflects greater dietary intake on the Italian subjects studied.

The reasons for the observed sex differences are not clear. Diet is known to be an important determinant of adipose tissue composition [13–24]. Field et al. [23] reported that dietary polyunsaturated/saturated (P/S) ratio was significantly related to the saturated and polyunsaturated content of stored lipids, although an earlier study [10] indicated that the diet did not account for the sex differences in depot fat composition reported. The simple dietary histories used in the present study showed no obvious sex differences, but more detailed studies in which a weighed inventory of dietary intake is included are required before firm conclusions are drawn.

It is also possible that metabolic and hormonal factors may contribute to gender differences [10,12]. The turnover of depot fat in both animals and man is higher in females than in males [40–45]. In vitro lipid synthesis is greater in female than male rat adipose tissue and is associated with an increase in [1-^{14}C]glucose oxidation rate which is abolished by treating the male rats with stilboestrol [40]. This suggests that gender differences in fat metabolism may occur due to hormone induced differences in adipose tissue turn-over rates. However, it is unclear if differences in the rates of metabolism will be accompanied by differences in fatty acid composition. In the present study only one female subject was taking the contraceptive pill and this, therefore, does not appear to account for the gender differences.

Regional differences in human fat cell metabolism have been observed and reflect adipocyte size [46]; furthermore differences in adipose tissue distribution between males and females occur [47]. This may contribute to the observed gender differences in fatty acid composition of buttock and thigh adipose tissue and the discrepancy between these data and the findings of McLaren et al. [6] who found no gender differences in fatty acid composition of adipose tissue.

Men have a higher risk of coronary heart disease than women, and our findings show that young men have higher amounts of total saturated fatty acids in adipose tissue than young women. Regardless of the cause, the observed

TABLE V

AVERAGE FATTY ACID COMPOSITION OF ADIPOSE TISSUE IN HEALTHY YOUNG MEN AND WOMEN

Fatty acid[a]	Males		Females	
	Percentage FAMEs of total (mean ± S.E.M.)	Amount FAMEs (mean ± S.E.M.) (mg/g)	Percentage FAMEs of total (mean ± S.E.M.)	Amount FAMEs (mean ± S.E.M.) (mg/g)
$C_{12\ 0}$	0.30 ± 0.03	2.7 ± 0.3	0.4 ± 0.06	3.6 ± 0.4
$C_{14\ 0}$	2.4 ± 0.10	24.0 ± 1.3	2.5 ± 0.1	24.8 ± 1.4
$C_{14\ 1}$	0.4 ± 0.03	3.9 ± 0.3	0.6 ± 0.05[b]	6.2 ± 0.5[b]
$C_{15\ 0}$	0.3 ± 0.04	2.9 ± 0.4	0.2 ± 0.05	2.3 ± 0.6
$C_{16\ 0i}$	0.1 ± 0.01	0.8 ± 0.01	0.1 ± 0.01	1.0 ± 0.1
$C_{16\ 0}$	19 ± 0.40	199 ± 4.0	17.8 ± 0.6[c]	178 ± 5 7[c]
$C_{16\ 1}$	7.0 ± 0.30	69.7 ± 4.0	9.1 ± 0.4[b]	91.4 ± 3.6[b]
$C_{17\ 0}$	0.2 ± 0.05	1.7 ± 0.4	0.1 ± 0.05	1.1 ± 0.5
$C_{17\ 1}$	0.4 ± 0.03	3.7 ± 0.3	0.5 ± 0.04	4.5 ± 0.4
$C_{18\ 0}$	3.3 ± 0.30	32.6 ± 3.0	2.9 ± 0.2	28.8 ± 2.0
$C_{18\ 1}$	48 ± 0.50	476 ± 5.1	49 ± 0.8	489 ± 7.6
$C_{18\ 1i}$	0.1 ± 0.09	1.0 ± 0.9	0.05 ± 0.02	0.5 ± 0.1
$C_{19\ 0}$	0.2 ± 0.01	1.5 ± 0.1	0.2 ± 0.02	1.6 ± 0.2
$C_{18\ 2i}$	0.1 ± 0.02	0.5 ± 0.1	0.1 ± 0.02	0.8 ± 0.2
$C_{18\ 2}$	13.4 ± 0.60	134 ± 5.8	12.1 ± 0.5	121 ± 5.3
$C_{20\ 0}$	0.3 ± 0.02	2.5 ± 0.2	0.2 ± 0.03	2.2 ± 0.3
$C_{18\ 3n6}$	0.003 ± 0.002	0.03 ± 0.01	–	–
$C_{18\ 3n3}$	0.9 ± 0.06	8.7 ± 0.7	1.0 ± 0 03	9.6 ± 0.4
$C_{20\ 1}$	1.8 ± 0.1	17.6 ± 0.9	2.0 ± 0.06	19.5 ± 0.6
$C_{20\ 1i}$	0.05 ± 0.02	0.5 ± 0.2	0.09 ± 0.03	0.9 ± 0.03
$C_{20\ 2}$	0.2 ± 0.01	2.0 ± 0.1	0.2 ± 0.01	2.0 ± 0.1
$C_{20\ 3}$	0.1 ± 0.02	1 2 ± 0.2	0.1 ± 0.01	1.3 ± 0.2
$C_{20\ 4}$	0.4 ± 0.02	3.5 ± 0.3	0.3 ± 0.02	3.1 ± 0.2
$C_{20\ 5}$	0.02 ± 0.02	0.2 ± 0 1	0.03 ± 0.02	0.3 ± 0.2
$C_{22\ 0}$	0.04 ± 0.02	0.4 ± 0.2	0.02 ± 0.02	0.2 ± 0.01
$C_{22\ 1}$	0.1 ± 0.03	0.7 ± 0.3	0.1 ± 0.03	0.6 ± 0.3
$C_{22\ 4}$	0.1 ± 0.01	1.1 ± 0.1	0.1 ± 0.01	0.9 ± 0.1
$C_{22\ 5}$	0.2 ± 0.01	1.8 ± 0.1	0.2 ± 0.02	1.7 ± 0.2
$C_{22\ 6}$	0.1 ± 0.01	1.4 ± 0.3	0.1 ± 0.03	1.4 ± 0.3
$C_{24\ 0}$	0.03 ± 0.01	0.3 ± 0.1	0.02 ± 0.01	0.2 ± 0.1
SFA	27 ± 0.6	265 ± 5.9	24 ± 0.7[d]	242 ± 7.4[d]
MUFA	57 ± 0.7	573 ± 6.6	61 ± 1.0[c]	611 ± 10.1[c]
PUFA	16 ± 0.16	155 ± 6.1	14 ± 0.6	143 ± 5.6

[a]SFA = total saturated fatty acid methyl esters (FAMEs) ($C_{12\ 0}+C_{14\ 0}+C_{15\ 0}+C_{16\ 0}+C_{17\ 0}+C_{18\ 0}+C_{19\ 0}+C_{20\ 0}+C_{22\ 0}+C_{24\ 0}$). MUFA = total monounsaturated fatty acid methyl esters ($C_{14\ 1}+C_{16\ 1}+C_{17\ 1}+C_{18\ 1}+C_{18\ 1i}+C_{20\ 1}+C_{20\ 1i}+C_{22\ 1}$). PUFA = total polyunsaturated fatty acid methyl esters ($C_{18\ 2i}+C_{18\ 2}+C_{18\ 3n6}+C_{18\ 3n3}+C_{20\ 2}+C_{20\ 3}+C_{20\ 4}+C_{20\ 5}+C_{22\ 4}+C_{22\ 5}+C_{22\ 6}$). i = isomer.
[b]$p < 0.001$.
[c]$p < 0.01$.
[d]$p < 0.05$.

gender differences may reflect a protective role in the female group. Male population studies suggest that a low proportion of linoleic acid ($C_{18.2n6}$) in adipose tissue may be associated with an increased risk of coronary heart disease [1–3]. Furthermore in a four-centre study, the highest risk group had the highest proportion of total saturated fatty acids in adipose tissue [3].

The possibility of a relationship between the findings in this study and the known gender difference in the prevalence of coronary heart disease requires further investigation, including more detailed assessments of diet and hormonal status.

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