Plasma cholesteryl ester fatty acid composition, insulin sensitivity, the menopause and hormone replacement therapy

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Abstract

This study was designed to determine the effect of menopause and hormone replacement therapy (HRT) on plasma cholesteryl ester fatty acid (CEFA) composition and insulin sensitivity and the relationships between these variables in perimenopausal women (aged 40–55 years) including 49 who were premenopausal and 32 who were postmenopausal. Plasma cholesteryl ester proportions of dihomogamma-linolenic acid (20:3 n-6) were correlated significantly with insulin sensitivity index ($r=-0.319$, $P=0.005$), fasting serum insulin levels ($r=0.230$, $P=0.038$), body mass index ($r=0.242$, $P=0.03$) and per cent body fat ($r=0.329$, $P=0.003$) in perimenopausal women ($n=81$). Similar associations were observed in premenopausal women. Regression analysis suggested the

relationships between 20:3 n-6 proportions and indices of insulin action may be partly mediated by levels of adiposity. In postmenopausal women, 6 months of HRT significantly ($P=0.008$) increased the ratio of arachidonic acid (20:4 n-6) to linoleic acid (18:2 n-6), which is an indicator of activity in the pathway of 20:4 n-6 synthesis, compared with placebo. These findings suggest that the type of fat in the diet indicated by plasma CEFA composition is linked to adiposity and insulin action. They also suggest that in postmenopausal women, HRT may increase the synthesis of 20:4 n-6, which is the precursor for eicosanoids with important cardiovascular functions.

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Introduction

Fatty acid composition of tissues is believed to influence their responsiveness to insulin by modifying cell membrane properties (Borkman *et al.* 1993). In healthy men, insulin sensitivity is positively associated with arachidonic acid (20:4 n-6) proportions and the ratio of 20:4 n-6 to dihomogamma-linolenic acid (20:3 n-6) in phospholipids from skeletal muscle (Borkman *et al.* 1993), the principal site of insulin-mediated uptake of glucose from the blood (De Fronzo *et al.* 1981). The ratio of 20:4 n-6 to 20:3 n-6 is an index of activity of Δ^5 -desaturase, an enzyme that inserts a double bond at the ninth carbon from the fatty acid carboxyl terminal. The fatty acid composition of serum cholesteryl esters, which to a degree reflects the composition in cells (Boberg *et al.* 1985) and skeletal muscle (Vessby *et al.* 1994b), is also related to insulin sensitivity. Serum cholesteryl ester proportions of palmitic (16:0), palmitoleic (16:1 n-7) and 20:3 n-6 acids are inversely related and proportions of linoleic acid (18:2 n-6) are positively related to insulin sensitivity in elderly men (Vessby *et al.* 1994b). Few studies have examined fatty acid

composition in plasma cholesteryl esters in relation to insulin sensitivity in women.

There is evidence that hormonal factors may influence fatty acid metabolism in women. Hormone replacement therapy (HRT) reduces proportions of polyunsaturated fatty acids in platelet membranes in postmenopausal women (Raganath *et al.* 1996). However, the influence of HRT on plasma cholesteryl ester fatty acid (CEFA) composition and its relationship with insulin sensitivity has not been studied.

We therefore studied the relationships between plasma CEFA composition and insulin sensitivity in pre- and postmenopausal women and the effect of oral HRT with 17 β -oestradiol (a naturally occurring oestrogen) and norethisterone in those who were postmenopausal.

Subjects and Methods

Subjects

Eighty-one perimenopausal women aged 40–55 years were enrolled from women in the Dunedin population

who responded to an advertisement in the newspaper. A narrow age range was chosen to minimise the effect of age on measured variables. Inclusion criteria were (i) non-smoker, (ii) no history of hypertension, ischaemic heart disease, renal disease or diabetes and (iii) receiving no medications including oral contraceptives, and for postmenopausal women, no previous exposure to HRT. Postmenopausal women were identified using standard criteria including an absence of menstruation for at least 6 months and elevated follicle-stimulating hormone (FSH) levels (>35 U/l) consistent with postmenopausal status. All subjects received a full medical review prior to participation in the study. The subjects were volunteers and gave informed consent to the study, which was approved by the Southern Regional Health Authority Ethics Committee (Otago).

Study design

The study was in two parts. First a cross-sectional comparison between pre- and postmenopausal women was made; secondly a single blind placebo-controlled trial of HRT for 6 months in the postmenopausal group was performed. The postmenopausal women ($n=32$) were randomised to receive either HRT or placebo. The HRT consisted of 2 mg 17β -oestradiol for days 1 to 12, 2 mg 17β -oestradiol and 1 mg norethisterone for 10 days and 1 mg 17β -oestradiol for 6 days (Trisequens; Novo Nordisk, Copenhagen, Denmark). Clinical and biochemical measurements were made in all women at baseline and again at 3 and 6 months following the start of HRT therapy. Factors measured included anthropometric data including per cent total body fat (BF) using dual energy X-ray absorptiometry (Taylor *et al.* 1998), insulin sensitivity (measured by the hyperinsulinaemic, isoglycaemic clamp), fasting blood glucose, serum fasting insulin (FIns) and plasma oestradiol, FSH, lipids, high density lipoprotein cholesterol (HDL-C) and CEFA composition. All studies took place at the same time of the day and on day 6 of the woman's cycle (premenopausal women) and on day 6 of the HRT cycle (oestrogen-only phase) in the postmenopausal group. The women were asked to maintain their current lifestyle with no changes in their dietary or exercise habits. This was reviewed at each study visit.

Hyperinsulinaemic isoglycaemic clamp

Each woman reported to the research laboratory at 0830 h following an overnight fast. Clinical measurements were recorded and fasting bloods obtained. Insulin sensitivity was measured using a 2 h hyperinsulinaemic, isoglycaemic clamp (De Fronzo *et al.* 1979) using the modified manual technique of primed continuous insulin infusion (Black *et al.* 1982, Ponchner *et al.* 1984, Walker *et al.* 1995). Hyperinsulinaemia was established in subjects by a 10 min priming insulin infusion (Actrapid HM, Novo Nordisk) at

rates determined by body weight and height. A constant infusion of insulin (40 mU/m^2 per min) was then established and maintained for 110 min (Black *et al.* 1982). Arterialised venous blood was obtained by the heated-hand method (Abumrad *et al.* 1981) and was assayed for glucose every 10 min using a bench-top glucose analyser (YSI 1500 Sidekick glucose analyser, Yellow Springs, OH, USA). Glucose infusion rates were adjusted to maintain baseline glucose levels using a negative feedback algorithm calculated on a microcomputer (Walker *et al.* 1995). Serum insulin was measured at baseline, 60 min, 90 min and 120 min and an average insulin level during the clamp was calculated from values at the last three time points. Insulin-mediated glucose uptake values were calculated from 10 min glucose concentrations over the final 60 min of the clamp study. The insulin sensitivity index (ISI) was determined by dividing the average insulin-mediated glucose uptake value by the mean serum insulin concentration over the final 60 min.

Laboratory methods

Blood was taken in the morning after an overnight fast into tubes containing EDTA or into plain tubes. Plasma was immediately separated by low-speed centrifugation at 4°C . Glucose was measured by the glucose oxidase method and insulin was determined by radioimmunoabsorbent assay (Diagnostic Products Inc., Los Angeles, CA, USA). Plasma oestradiol, FSH and luteinising hormone were measured by immunofluorescence assays (National Hormone Assay Service, NZ). HDL-C was measured in the supernatant after precipitation of apolipoprotein B-containing lipoproteins with dextran sulphate and magnesium chloride (Warnick *et al.* 1982). Cholesterol and triglycerides (TG) were measured in plasma and plasma fractions using enzymatic kits and calibrators from Boehringer Mannheim (Mannheim, Germany). Plasma CEFA composition was measured essentially as described previously (Vessby *et al.* 1994a). Methanol (2.5 ml) was added to plasma (0.5 ml) followed by 5 ml chloroform containing 0.005% butylated hydroxytoluene (BHT) and 7.5 ml 0.2 mol/l sodium dihydroxyphosphate. The mixture was mixed thoroughly and left at 4°C for 1–4 days. The chloroform phase was isolated then evaporated to dryness under nitrogen and the cholesteryl esters were separated by thin layer chromatography with hexane/diethyl ether/glacial acetic acid (85/15/2.5 v/v/v). The cholesteryl ester fraction was scraped off the plate and was eluted with chloroform containing 0.005% BHT. The solvent was evaporated under nitrogen and 2 ml boron trifluoride/methanol (14% w/v) and toluene (1 ml) were added. The tube was sealed and heated for 90 min in a boiling water bath. Water (1.5 ml) was added and the methyl esters were extracted with 3 ml hexane containing BHT (4.4 mg/l). The hexane phase was isolated and the solvent was evaporated under nitrogen. The methyl esters

Table 1 Clinical characteristics of the pre- and postmenopausal women. Values are mean $\pm$ S.D.

	Premenopausal (n=49)	Postmenopausal (n=32)	P
Age (years)	46 $\pm$ 4	52 $\pm$ 3	<0.001
Body weight (kg)	66.2 $\pm$ 9.1	72.5 $\pm$ 11.9	0.03
BMI (kg/m ²)	24.5 $\pm$ 3.3	27.2 $\pm$ 4.4	0.002
%BF	34.7 $\pm$ 7.5	40.4 $\pm$ 7.0	0.001
Plasma oestradiol (pmol/l)	484 $\pm$ 395	42 $\pm$ 12	<0.001
Plasma FSH (IU/l)	13.2 $\pm$ 11.3	74.8 $\pm$ 20.6	<0.001
Fasting insulin (μ mol/l)	8 $\pm$ 4	11 $\pm$ 6	0.07
Fasting glucose (mmol/l)	4.57 $\pm$ 0.58	4.77 $\pm$ 0.59	0.06
M value (μ mol/kg $\times$ min)	9.56 $\pm$ 2.70	7.30 $\pm$ 1.90	<0.001
ISI (M value M/mU/l $\times$ 100)	5.88 $\pm$ 1.91	4.38 $\pm$ 2.33	0.005
TC (mmol/l)	5.59 $\pm$ 0.98	6.15 $\pm$ 0.90	0.003
TG (mmol/l)	1.09 $\pm$ 0.38	1.50 $\pm$ 0.90	0.002
HDL-C (mmol/l)	1.54 $\pm$ 0.33	1.47 $\pm$ 0.42	0.30

M value, insulin-mediated glucose disposal; TC, plasma total cholesterol concentration.

were redissolved in 0.5 ml dichloromethane and were then separated by gas liquid chromatography on a Chrompack CP Sil88 capillary column (50 m $\times$ 0.22 mm) in a Varian 3700 gas chromatograph. A 3 μ l sample was injected onto the column at a split ratio of 1:10. Helium (0.8 kg/m² column head pressure and flow rate 1.5 ml/min) was used as the carrier gas, the injector temperature was 260 °C and the flame ionization detector (FID) detector temperature was 280 °C. Column temperature was programmed from 165 to 210 °C at 3 °/min. Peak area was measured using a Spectra-Physics SP 4290 integrator (San Jose, CA, USA). Fatty acids were identified by reference to authentic standards (Nu Check Prep, Elysian, MN, USA). The coefficients of variation for measurement of major fatty acids were 3–7%.

Statistics

Unpaired data were compared using the Mann–Whitney U test. Analysis of covariance (ANCOVA) was used to adjust ISI in pre- and postmenopausal women for variation in body mass index (BMI) and per cent BF. Pearson's product-moment correlation coefficients and multiple linear regression analysis were used to test for relationships between variables. Two-factor ANOVA with repeated measures was used to compare the effects of HRT and placebo on log-transformed plasma CEFA composition. Two-way tests of significance were used and a *P* value of <0.05 was considered to be statistically significant.

Results

Clinical characteristics and mean ISI of the pre- and postmenopausal women are shown in Table 1. As expected, the postmenopausal women were older and had

a significantly lower insulin sensitivity and higher BMI, per cent total BF and plasma cholesterol and TG concentrations. ISI was no longer significantly different between pre- and postmenopausal women when the per cent total BF (*P*=0.12) was taken into account in ANCOVAs. Insulin sensitivity remained significantly (*P*=0.03) different between these groups of women when BMI replaced per cent BF in this ANCOVA.

Table 2 summarises plasma CEFA composition in pre- and postmenopausal women. There were few differences in the composition between the two groups of women. Proportions of 16:0 were significantly lower in postmenopausal women. The ratio of 20:4 n-6 to 20:3 n-6 was not significantly different between pre- and postmenopausal women.

Table 3 summarises correlation coefficients between plasma CEFA composition and indices of insulin

Table 2 Plasma CEFA composition (per cent weight) in pre- and postmenopausal women. Values are mean $\pm$ S.D.

	Premenopausal (n=49)	Postmenopausal (n=32)	P
Fatty acid			
16:0	13.0 $\pm$ 2.0	12.5 $\pm$ 2.0	0.03
16:1 n-7	3.3 $\pm$ 1.5	3.5 $\pm$ 1.6	0.23
18:0	4.0 $\pm$ 1.3	3.9 $\pm$ 1.4	0.86
18:1 n-9	25.1 $\pm$ 3.0	24.8 $\pm$ 3.7	0.27
18:2 n-6	45.8 $\pm$ 5.2	46.2 $\pm$ 5.6	0.23
18:3 n-6	0.8 $\pm$ 0.3	0.8 $\pm$ 0.3	0.93
18:3 n-3	2.5 $\pm$ 1.9	2.0 $\pm$ 1.7	0.49
20:3 n-6	0.7 $\pm$ 0.2	0.8 $\pm$ 0.3	0.07
20:4 n-6	4.3 $\pm$ 1.3	4.7 $\pm$ 1.6	0.07
20:5 n-3	0.8 $\pm$ 0.4	1.0 $\pm$ 0.8	0.51

16:0, palmitic acid; 16:1 n-7, palmitoleic acid; 18:0, stearic acid; 18:1 n-9, oleic acid; 18:2 n-6, linoleic acid; 18:3 n-6, gamma-linolenic acid; 18:3 n-3, alpha-linolenic acid; 20:3 n-6, dihomo-gamma-linolenic acid; 20:4 n-6, arachidonic acid; 20:5 n-3, eicosapentaenoic acid.

Table 3 Correlations between plasma CEFA composition (per cent weight), indices of insulin action and adiposity in perimenopausal women (n=81)

	M value (μmol/kg × min)	ISI (M/mU/l × 100)	FIns (μmol/l)	BMI (kg/m ²)	%BF
Fatty acid					
16:0	−0.231*	−0.167	0.262*	0.271*	0.188
16:1 n-7	−0.221	−0.227*	0.153	0.447‡	0.397‡
18:0	−0.033	0.007	0.060	−0.071	−0.014
18:1 n-9	−0.063	−0.005	0.168	0.133	0.131
18:2 n-6	0.174	0.142	−0.262*	−0.225*	−0.207
18:3 n-6	−0.070	−0.136	0.096	0.226*	0.242*
18:3 n-3	0.154	0.159	−0.018	−0.318†	−0.326†
20:3 n-6	−0.271*	−0.319†	0.230*	0.242*	0.329†
20:4 n-6	−0.035	−0.135	−0.037	0.053	0.103
20:5 n-6	−0.031	−0.076	0.037	0.005	0.055
BMI (kg/m ²)	−0.496‡	−0.331†	0.402‡	—	—
%BF	−0.551‡	−0.523‡	0.340†	—	—

M, insulin-mediated glucose disposal rate.
**P*<0.05; †*P*<0.01; ‡*P*<0.001.
Fatty acid abbreviations as in Table 2.

sensitivity and adiposity in perimenopausal women. Significant correlations were observed between 20:3 n-6 proportions and all indices of insulin sensitivity and adiposity. Similar correlations with ISI (*r*=−0.299, *P*=0.049) and per cent BF (*r*=−0.308, *P*=0.037) were observed in premenopausal women. Proportions of 16:1 n-7 and alpha-linolenic acid (18:3 n-3) were correlated significantly with both indices of adiposity and 16:0 and 18:2 n-6 with one index of adiposity in perimenopausal women (Table 3). Highly significant correlations between indices of adiposity and insulin sensitivity and insulin-mediated glucose disposal rate were also seen in premenopausal women. The corresponding correlations in postmenopausal women did not achieve statistical significance (data not shown). The ratio of 20:4 n-6 to 20:3 n-6 was correlated significantly with ISI (*r*=0.241, *P*=0.03), FIns (*r*=−0.285, *P*=0.012), BMI (*r*=−0.245, *P*<0.05) and per cent BF (*r*=−0.256, *P*<0.05) in perimenopausal women. Age was not correlated significantly with plasma CEFA composition. We recognise that multiple statistical testing may result in up to two correlations at *P*<0.05 in Table 3 occurring by chance.

Multiple linear regression analysis with ISI as the dependent variable and 20:3 n-6 proportions and indices of adiposity as the independent variables in perimenopausal women is shown in Table 4. BMI and 20:3 n-6 proportions both contributed significantly to the variation in insulin sensitivity. In a model with per cent total BF and 20:3 n-6 proportions, only per cent BF contributed significantly to variation in insulin sensitivity. Furthermore, this model accounted for nearly twice the variance in insulin sensitivity compared with the model containing BMI.

Effect of HRT

Plasma CEFA composition in the postmenopausal women receiving HRT or placebo are shown in Table 5.

Proportions of 20:4 n-6 increased significantly during the study in the women receiving HRT (*P*=0.03, ANOVA) and this change was significantly different compared with those treated with placebo. The ratio of 20:4 n-6 to 18:2 n-6 also increased significantly compared with placebo (*P*=0.008) by an average 40% at 3 months and 30% at 6 months compared with baseline in women randomised to HRT (Fig. 1). The ratio of 20:4 n-6 to 20:3 n-6 (*P*=0.80), ISI (*P*=0.85), FIns (*P*=0.59) and fasting blood glucose concentration (*P*=0.27) did not change significantly during the study in the women receiving HRT compared with those taking placebo.

Discussion

Studies in men have indicated that proportions of 20:3 n-6 in plasma cholesteryl esters (Vessby *et al.* 1994b) and skeletal muscle phospholipids (Borkman *et al.* 1993, Pan *et al.* 1995) are associated with insulin sensitivity. Furthermore, the ratio of 20:4 n-6 to 20:3 n-6 in skeletal

Table 4 Multiple linear regression analysis of insulin sensitivity with plasma cholesteryl ester dihomo-gamma-linolenic acid 20:3 n-6 and indices of obesity as independent variables in perimenopausal women (n=81)

	β-coefficient	P
Independent variables		
20:3 n-6	−2.38	0.03
BMI	−0.16	0.016
Total model <i>r</i> ² =17.2		0.001
20:3 n-6	−1.152	0.14
%BF	−0.13	<0.001
Total model <i>r</i> ² =28.8		<0.001

Table 5 Plasma CEFA composition (per cent weight) in postmenopausal women treated with placebo or HRT. Values are mean $\pm$ S.D.

	Placebo (n=16)			HRT (n=16)			P*
	Baseline	3 months	6 months	Baseline	3 months	6 months	
Fatty acid							
16:0	11.9 $\pm$ 1.1	12.9 $\pm$ 1.2	12.8 $\pm$ 1.6	13.2 $\pm$ 2.4	13.4 $\pm$ 1.7	13.4 $\pm$ 1.6	0.73
16:1 n-7	2.7 $\pm$ 0.9	2.7 $\pm$ 1.0	2.9 $\pm$ 1.3	4.2 $\pm$ 1.8	4.5 $\pm$ 1.3	4.0 $\pm$ 1.1	0.51
18:0	4.1 $\pm$ 1.5	4.9 $\pm$ 1.7	4.3 $\pm$ 1.6	3.6 $\pm$ 1.3	3.7 $\pm$ 1.0	4.1 $\pm$ 1.5	0.58
18:1 n-9	23.7 $\pm$ 4.0	24.7 $\pm$ 3.0	22.7 $\pm$ 3.0	26.0 $\pm$ 3.0	25.4 $\pm$ 2.5	26.4 $\pm$ 3.3	0.11
18:2 n-6	48.1 $\pm$ 5.1	44.9 $\pm$ 4.6	47.5 $\pm$ 5.4	44.3 $\pm$ 5.6	42.5 $\pm$ 5.9	42.4 $\pm$ 5.2	0.17
18:3 n-6	0.8 $\pm$ 0.3	1.0 $\pm$ 0.8	0.9 $\pm$ 0.4	0.8 $\pm$ 0.3	0.7 $\pm$ 0.2	0.7 $\pm$ 0.2	0.74
18:3 n-3	2.1 $\pm$ 1.5	2.7 $\pm$ 2.4	2.1 $\pm$ 1.7	1.9 $\pm$ 2.0	2.3 $\pm$ 1.9	1.9 $\pm$ 1.3	0.58
20:3 n-6	0.8 $\pm$ 0.3	0.8 $\pm$ 0.3	0.7 $\pm$ 0.3	0.8 $\pm$ 0.3	0.8 $\pm$ 0.2	0.7 $\pm$ 0.2	0.45
20:4 n-6	5.0 $\pm$ 1.5	4.6 $\pm$ 1.8	5.3 $\pm$ 1.5	4.4 $\pm$ 1.7	5.7 $\pm$ 1.4	5.4 $\pm$ 1.1	0.05
20:5 n-3	0.9 $\pm$ 0.5	0.9 $\pm$ 0.4	1.0 $\pm$ 0.4	1.0 $\pm$ 1.0	1.0 $\pm$ 0.5	1.0 $\pm$ 0.3	0.97

*Data were log transformed before ANOVA.
Fatty acid abbreviations as in Table 2.

muscle phospholipids, an index of Δ^5 -desaturase activity, is also closely related to insulin sensitivity (Borkman *et al.* 1993, Pan *et al.* 1995). Our data indicate that plasma cholesteryl ester 20:3 n-6 proportions and the ratio of 20:4 n-6 to 20:3 n-6 are also associated with insulin sensitivity in perimenopausal women. In addition, treatment of postmenopausal women with oral HRT markedly increases the product:precursor ratio of 20:4 n-6 to 18:2 n-6, an indicator of metabolic activity in the pathway of 20:4 n-6 synthesis.

Plasma cholesteryl esters are not directly involved in insulin action and glucose disposal in the body. Consequently, other factors must mediate associations

between plasma CEFA composition and insulin sensitivity. In the present study, the association between plasma CEFA 20:3 n-6 and insulin sensitivity in perimenopausal women appeared to be at least partly mediated by adiposity, a major determinant of insulin sensitivity. However, it is possible that high plasma CEFA 20:3 n-6 is not merely an epiphenomenon of the effects of obesity on insulin sensitivity in these women. Plasma CEFA composition reflects the type of fat in the diet (Vessby *et al.* 1980, Glatz *et al.* 1987), which appears to be a determinant of both adiposity and insulin sensitivity. Lassere *et al.* (1985) have reported that a low intake of the polyunsaturate 18:2 n-6 increases levels of 20:3 n-6 in serum lipids and a high intake reduces these levels in healthy women. A diet low in polyunsaturated fatty acids also seems to favour increased adiposity and weight gain (Jones & Schoeller 1988, Pan & Storlien 1993, Pan *et al.* 1994). Associations between indices of adiposity and 16:0, 16:1 n-7 and 20:3 n-6 (positive) and 18:2 n-6 (inverse) in our data are in keeping with low polyunsaturated fatty acid intake in women who are overweight. Lastly, there is increasing evidence to suggest that diets rich in saturated fatty acids and poor in polyunsaturates increase insulin resistance (Storlien *et al.* 1987, 1991, Matsui *et al.* 1997).

Plasma cholesteryl ester content of polyunsaturated fatty acids also may reflect content of the corresponding fatty acids in skeletal muscle (Vessby *et al.* 1994b), a major site of glucose disposal in the body. It has been postulated that the fatty acid composition of skeletal muscle membranes may be a focal point at which several factors (e.g. fatty acid desaturation, dietary lipid profile, adiposity and genes) may act to influence insulin sensitivity (Pan *et al.* 1995). Levels of 20:3 n-6 and its desaturation by Δ^5 -desaturase appear to be important in the obesity–insulin sensitivity relationship. Proportions of 20:3 n-6 in skeletal muscle phospholipids and more particularly Δ^5 -desaturase activity

Figure 1 The mean ($\pm$ S.E.M.) 20:4 n-6 to 18:2 n-6 ratio in postmenopausal women receiving HRT (n=16) or placebo (n=16) for 6 months in a randomised, placebo-controlled trial. Two-factor, repeated-measures ANOVA was used to compare the effects of HRT and placebo on the ratio.

are associated with both adiposity and insulin sensitivity in men (Pan *et al.* 1995). Whether these relationships are also found in perimenopausal women and underlie the associations between insulin sensitivity, adiposity and plasma cholesteryl ester 20:3 n-6 and the ratio of 20:4 n-6 to 20:3 n-6 in the present study remains to be determined.

The ratio of 20:4 n-6 to 20:3 n-6 in plasma cholesteryl esters predicted insulin sensitivity and adiposity less efficiently compared with 20:3 n-6 proportions in our data. It is possible that the ratio of plasma cholesteryl esters may be more sensitive to variation in dietary fatty acid intake and may relate less closely to Δ^5 -desaturase activity compared with the corresponding ratio derived from skeletal muscle phospholipid fatty acid composition. In a previous study, the ratio of 20:4 n-6 to 20:3 n-6 in plasma cholesteryl esters was unrelated to insulin sensitivity (Vessby *et al.* 1994b). Differences in diet, age, adiposity and genetic factors between study populations may contribute to the discrepant findings.

Plasma CEFA composition was not notably different suggesting that dietary fatty acid profile may be relatively similar in pre- and postmenopausal women in this study. Thus, factors other than the type of fat in the diet may be mainly responsible for the markedly higher levels of adiposity and insulin resistance in postmenopausal women. Increased adiposity measured as per cent BF appeared to account for an appreciable portion of the higher insulin resistance in these women. Lower energy expenditure during rest and leisure-time physical activity in the face of unchanged energy intake may contribute to increased adiposity associated with natural menopause (Poehlman *et al.* 1995).

In postmenopausal women randomised to HRT we did not observe any changes in insulin sensitivity and the proportions of fatty acids which were associated with insulin sensitivity. However, the ratio of 20:4 n-6 to 18:2 n-6, which is an indicator of metabolic activity in the pathway of 20:4 n-6 synthesis, increased in women receiving HRT compared with the control group (Fig. 1). Thus, it is possible that transformation of 18:2 n-6 to 20:4 n-6 by desaturase and elongase activities is stimulated by HRT. Animal studies support an increase in 20:4 n-6 synthesis from 18:2 n-6 during HRT. Levels of 20:4 n-6 increased and those of 18:2 n-6 decreased in liver lecithin of rats treated with oestradiol (Lyman *et al.* 1968).

Combined HRT decreases proportions of polyunsaturated fatty acids and mainly 20:4 n-6 in platelet membranes from postmenopausal women (Raganath *et al.* 1996). Fatty acid composition in platelets and plasma cholesteryl esters are often closely related (Boberg *et al.* 1985, Salo *et al.* 1985). However, changes in plasma CEFA composition, and particularly 20:4 n-6 levels, in postmenopausal women during HRT in the present study differed markedly from the reported HRT-induced changes in fatty acid composition in platelets (Raganath *et al.* 1996). It is possible that activation of platelets

may account for these disparate changes in fatty acid composition during HRT. Activation of platelets followed by phospholipase-stimulated release of membrane 20:4 n-6 for eicosanoid synthesis may underlie the reported decrease in platelet 20:4 n-6 content during HRT. There is evidence that platelet activation with increased thromboxane A₂ formation occurs during oral HRT (Ylikorkala *et al.* 1984, Viinikka *et al.* 1997).

In conclusion, the results of this study suggest that the dietary fatty acid profile indicated by plasma CEFA composition is linked to adiposity and insulin action in premenopausal women but does not account for increased adiposity and reduced insulin sensitivity in women following the menopause. Our data also suggest that oral HRT may increase the synthesis of 20:4 n-6, which is a substrate for the production of eicosanoids (e.g. thromboxane A₂ and prostacyclin), with important cardiovascular functions. This finding needs confirmation in a study of longer duration. Whether high plasma cholesteryl ester 20:3 n-6 levels in middle-aged women predict increased risk of developing non-insulin dependent diabetes mellitus as they do in men aged 50 (Vessby *et al.* 1994a) also remains to be determined.

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