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Lack of effect of meal fatty acid composition on postprandial lipid, glucose and insulin responses in men and women aged 50–65 years consuming their habitual diets

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The aim of the study was to determine the effect of consuming meals with different fatty acid compositions on the postprandial changes over 6 h in plasma triacylglycerol, NEFA, total cholesterol, glucose and insulin concentrations in middle-aged men and women. Men (*n* 11; 58 (5) years) and women (*n* 11; 56 (4) years) consumed four test meals with a similar macronutrient energy content in random order: a reference meal based on the habitual pattern of fatty acid intake in the UK, a meal with an increased (155 %) linoleic acid (LA) to α-linolenic acid (αLNA) ratio (high LA:αLNA), a meal with increased (23 %) MUFA content (high MUFA) and a meal with increased (583 %) EPA and DHA content (high EPA + DHA). The high-LA:αLNA and high-EPA + DHA meals selectively increased the ratio of LA to αLNA (men 341 %; women 310 %) and the EPA + DHA (men 414 %; women 438 %) concentration in plasma triacylglycerol. The high-MUFA meal did not alter the change in MUFA content of the plasma. Plasma triacylglycerol, NEFA, glucose and insulin, but not total cholesterol, concentrations changed significantly after each meal. There was no significant effect of meal fatty acid composition or gender on maximum change in concentration, time to maximum concentration or area under the curve of any of the metabolites measured in the blood. These results suggest that differences in meal fatty acid composition exert little or no effect on postprandial changes in plasma lipids, glucose and insulin concentrations.

Postprandial lipaemia: Gender: PUFA

The magnitude of the change in blood triacylglycerol (TAG) concentration postprandially is an independent risk factor for CVD (Patsch *et al.* 1993; Karpe, 1997) and risk of myocardial infarction (Stampfer *et al.* 1996). These associations reflect both an impairment of HDL-cholesterol metabolism by excessive transfer of TAG from TAG-rich lipoproteins, specifically chylomicrons and VLDL, and by the formation of small dense chylomicron remnant particles and LDL3, which are able to cross the vascular endothelium and so contribute to atherogenesis (Sethi *et al.* 1993; Patsch, 1994; Williams, 1997). Recent studies also suggest that the consumption of a fatty meal promotes the secretion of proinflammatory cytokines, which impair vascular reactivity (reviewed in Burdge & Calder, 2005).

The magnitude of the postprandial increase in TAG-rich lipoproteins is modified by physiological and dietary factors. Postprandial lipaemia is greater in men than premenopausal women (Cohn *et al.* 1988), although this advantage is lost in women after the menopause, which suggests that oestrogen may play a central role in limiting the postprandial response in women (van Beek *et al.* 1999). The increase in blood TAG-rich lipoprotein concentration after a meal also increases with greater visceral fat mass (Couillard *et al.* 1998) and is modified by apoE genotype (Bergeron & Havel, 1996). Consuming a meal at breakfast produces a greater lipaemic response than if the same meal is consumed at lunch (Burdge *et al.* 2003). The lower response at lunch appears to be due to suppression of VLDL TAG secretion. Dietary fat and carbohydrate intake also influence postprandial lipaemia. Increasing the sucrose content of a test meal results in a raised TAG response (Mann *et al.* 1971; Cohen & Schall, 1988; Grant *et al.* 1994) and a biphasic change in plasma TAG concentration (Shishehbor *et al.* 1998). An increase in the carbohydrate content of the background diet also results in a greater postprandial TAG response (Nestel *et al.* 1970; Swanson *et al.* 1992).

Several studies have investigated the effect of modifying the fatty acid content of the background diet on postprandial lipaemia following a standardised test meal (reviewed in Williams, 1997). Although there are differences in study design, the general experimental approach was to increase the dietary intake of unsaturated fatty acids compared with a reference diet with a predominately saturated fatty acid (SFA) content for several weeks and then to measure the postprandial response to a standard test meal. Increasing the intake of EPA (20 : 5*n*-3) and DHA acid (22 : 6*n*-3) in the background decreased the magnitude of the postprandial TAG response (Harris & Connor, 1980; Harris *et al*. 1988; Weintraub *et al.* 1988; Brown & Roberts, 1991; Harris & Windsor, 1991; Williams *et al.* 1992; Harris & Muzio, 1993; Finnegan *et al.* 2003), although others did not find

Abbreviations: αLNA, α-linolenic acid; LA, linoleic acid; SFA, saturated fatty acid; TAG, triacylglycerol.
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this (Lovegrove *et al.* 1997). Increasing the intake of α-linolenic acid (αLNA; 18 : 3*n*-3) did not alter the magnitude of the postprandial TAG response (Finnegan *et al.* 2003). An increased consumption of linoleic acid (LA; 18 : 2*n*-6; Demacker *et al.* 1991) or MUFA (Lichtenstein *et al.* 1993) also limited the increase in TAG concentration after a meal in some studies, but not others (Lovegrove *et al.* 1997).

The effects of altering the fatty acid content of a test meal on the magnitude of postprandial lipaemia are more variable than those reported following modification of the fatty acid content of the background diet. This may reflect, at least in part, differences in experimental design (Williams, 1997). Some studies report a significant decrease in postprandial TAG response compared with reference meals enriched in SFA following the consumption of a meal enriched in EPA + DHA (Zampelas *et al.* 1994*b*), whereas others did not report a significant effect (Harris *et al.* 1988; Weintraub *et al.* 1988; Zampelas *et al.* 1995). Increasing the MUFA or LA content of the test meal also had no effect on postprandial lipaemia compared with reference meals enriched in SFA (Demacker *et al.* 1991; de Bruin *et al.* 1993; Zampelas *et al.* 1994*a*; Jackson *et al.* 1999; Pedersen *et al.* 1999). Koutsari *et al.* (2004), however, reported a significant time–meal fatty acid composition interaction in young men, but not in young women, and that the postprandial TAG response was greater after a MUFA-enriched meal than a SFA-enriched meal. In contrast, Jackson *et al.* (2005) showed in middle-aged men that a meal enriched in *n*-6 PUFA reduced the postprandial TAG response and increased NEFA suppression compared with an SFA-enriched reference meal, although there was no effect of increasing the MUFA content of the meal on postprandial lipaemia. The *n*-6 PUFA-enriched and MUFA-enriched meals produced a lower glucose response compared with the SFA-enriched meal but did not alter the postprandial insulin response.

To date, most studies that have investigated the effect of altered meal fatty acid composition on the change in TAG concentration after a meal have focused on young men (Harris *et al.* 1988; Weintraub *et al.* 1988; Demacker *et al.* 1991; de Bruin *et al.* 1993; Zampelas *et al.* 1994*a*,*b*, 1995; Jackson *et al.* 1999; Pedersen *et al.* 1999) or young men and women (Koutsari *et al.* 2004), an exception being that of Jackson *et al.* (2005). Furthermore, none of the preceding studies has determined the extent to which the test meal alters the fatty acid composition of the plasma TAG pool. Failure to change significantly the fatty acid composition of circulating TAG after a meal is one possible explanation for the lack of effect of an altered meal fatty acid content on postprandial lipaemia. In the present study, we have investigated the effect of consuming meals enriched in LA, MUFA or EPA + DHA on the postprandial change in TAG, NEFA, total cholesterol, glucose and insulin responses in middle-aged men and in postmenopausal middle-aged women who were not using hormone-replacement therapy.

Subjects and methods

Subjects

The subjects were healthy men (*n* 11) and women (*n* 11) aged 50–65 years, who had a BMI of between 20 and 30 kg/m^2, a fasting TAG concentration of less than 2 mmol/l and a total cholesterol of less than 8 mmol/l (Table 1) measured at a pre-study health screen. The women were postmenopausal and were not using hormone-replacement therapy. Volunteers who consumed restricted diets (for example, vegetarians and vegans), consumed more than two portions of oily fish per week, habitually took vegetable or fish oil or vitamin dietary supplements, had diagnosed type 2 diabetes mellitus or smoked tobacco were excluded from the study. The study received ethical approval from the South and West Hampshire Local Research Ethics Committee, and volunteers gave written informed consent.

Postprandial study

The design of the postprandial study was based upon Burdge *et al.* (2003). Each participant took part in four postprandial studies, with an interval of at least 14 d between each study. Subjects consumed their habitual diet throughout the study. On the day preceding the postprandial study, subjects were asked not to consume alcohol and to refrain from strenuous exercise. They were asked to consume their evening meal by 20.00 hours, but could consume water freely, and to fast until the postprandial study commenced. Subjects arrived at the Wellcome Trust Clinical Research Facility, Southampton General Hospital, Southampton, UK at approximately 07.30 hours.

A cannula was inserted into a forearm vein and a baseline blood sample (12 ml) drawn. Part of the sample (10 ml) was placed into a tube containing heparin sulphate, and the

Table 1. Subject characteristics
(Mean values and standard deviations)

	Men (*n* 11)		Women (*n* 11)		
	Mean	SD	Mean	SD	Student's *t* test (*P*)
Age (years)	58	5	56	4	NS
BMI (kg/m^2)	25·8	3·3	25·6	3·6	NS
Fat mass (%)	24·5	2·5	34·5	10·7	0·02
Triacylglycerol (mmol/l)	1·3	0·4	1·3	0·5	NS
Total cholesterol (mmol/l)	4·7	0·7	5·8	1·0	0·006
Glucose (mmol/l)	5·0	0·4	4·9	0·5	NS
Insulin (mU/l)	4·6	2·3	5·8	3·7	NS

Age, BMI and fasting blood metabolite concentrations were measured at an initial health screen before commencing the study, and the proportion of body fat was measured during the study. Values for men and women were compared using the Student's unpaired *t* test. For details of subjects and procedures, see this page.

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remainder was placed into a tube and allowed to clot. Participants were given one of four test meals in random order at approximately 08.00 hours. These were a reference meal based on the pattern of fatty acids in the UK diet, a meal with an increased ratio of LA to αLNA (high LA:αLNA), a meal with increased MUFA content (high MUFA) and a meal with increased EPA + DHA content (high EPA + DHA). The details of the macronutrient and fatty acid compositions of the individual test meals are summarised in Table 2.

The fat and protein component of the meals was administered in an emulsion (Burdge *et al.* 2003) that was composed of double cream, a blend of safflower, olive, linseed and fish oils, Nesquik milkshake power, casein and carbohydrate. The total volume was made up to 160 ml with water. The carbohydrate component of the meal was given as toast with jam or marmalade. The fatty acid composition of the each meal was determined by GC (see later). Subjects consumed the test meal during a period of approximately 15 min. Blood samples (12 ml) were collected at 0·5, 1, 1·5, 2, 2·5, 3, 4, 5 and 6 h after consumption of the test meal began. Participants remained resting throughout the study and were allowed free access to water. The proportion of body fat was determined by bioelectrical impedance using a Bodystat device (Bodystat Ltd, Douglas, Isle of Man, UK).

Measurement of metabolites in blood

Plasma was separated from cells by centrifugation and frozen. TAG, NEFA, glucose and total cholesterol concentrations were measured using a Konelab 20 autoanalyser (Labmedics Limited, Salford Quays, Manchester, UK). Reagents were from Labmedics Limited and Alpha Laboratories (Eastleigh, Hampshire, UK). Within-assay CV were TAG 1·9 %, NEFA 2·0 %, total cholesterol 1·9 % and glucose 1·1 %. Between-assay CV were TAG 2·9 %, NEFA 4·4 %, total cholesterol 2·7 % and glucose 3·4 %. Plasma insulin concentration was measured by automated immunoassay (within-assay CV 2·4 %, between-assay CV 5·9 %) by the Endocrinology Unit, Department of Chemical Pathology, Southampton General Hospital, Southampton, UK.

Analysis of fatty acid composition by GC

The fatty acid composition of the test meals and of the plasma samples was measured by GC essentially as described elsewhere (Burdge *et al.* 2000). For the test meals, all of the meal components were placed in a food blender and homogenised. An aliquot (0·8 ml) was then extracted with chloroform and methanol (Folch *et al.* 1957), and fatty acid methyl esters were prepared as described later. Plasma samples (0·8 ml), collected at baseline and at the maximum TAG concentration following the meal, were extracted with chloroform and methanol (Folch *et al.* 1957). TAG was isolated from the total lipid extracts by solid-phase extraction using 100 mg aminopropylsilical cartridges (BondElut; Varian, Oxford, UK; Burdge *et al.* 2000). Fatty acid methyl esters were prepared from the total lipid extracts of the test meals and from plasma TAG by incubation with methanol containing 2 % (v/v) H_2SO_4 at 50°C for 2 h (Burdge *et al.* 2000). Fatty acid methyl

Table 2. Test meal composition

	Test meal			
	Reference	High LA:αLNA	High MUFA	High EPA + DHA
Total fat (g)	55·1	53·4	47·7	55·9
Total carbohydrate (g)	130·0	130·0	130·0	130·0
Total protein (g)	12·0	12·0	12·0	12·0
Total energy (kJ)	4·3	4·2	4·0	4·3
Major sources of fatty acids				
Cod-liver oil (ml)	0·9	0·9	0·9	8·5
Safflower oil (ml)	8·8	8·7	5·1	8·8
Double cream (ml)	47·6	47·6	47·0	50·0
Linseed oil (ml)	1·8	0·2	1·0	1·8
Olive oil (ml)	6·9	6·8	11·0	5·8
Fatty acid composition (%)				
Lauric acid	1·8	2·0	1·8	1·6
Myristic acid	6·3	6·4	6·1	6·6
Palmitic acid	21·5	21·8	21·6	21·4
Stearic acid	8·4	8·5	8·3	7·9
Total saturated fatty acid	38·0	38·6	37·6	37·5
Palmitoleic acid	1·5	1·5	1·5	2·6
Oleic acid	34·0	34·8	42·0	32·4
Total MUFA	35·5	36·3	43·5	34·9
LA	22·1	22·9	15·5	19·7
αLNA	3·7	1·5	2·6	3·4
EPA	0·3	0·3	0·3	2·1
Docosapentaenoic acid (*n*-3)	0·1	0·1	0·1	0·4
DHA	0·3	0·3	0·3	2·0
EPA + DHA	0·6	0·6	0·6	4·1
Total PUFA	26·5	25·1	18·8	27·6
LA:αLNA	6·0	15·3	6·0	6·0

αLNA, α-linolenic acid; LA, linoleic acid.
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esters were resolved using a 6890 GC (Agilent, Cheadle, Cheshire, UK) equipped with a 30 m × 0·25 μm × 0·25 mm BPX-70 fused silica capillary column (SGE, Milton Keynes, UK) and flame ionisation detection. The concentrations of individual fatty acids were determined by measuring the peak area using ChemStation software (Agilent), and each fatty acid is expressed here as a proportion of the total. For plasma samples, the concentration of each fatty acid was calculated from the proportion of total fatty acids and the total plasma TAG concentration.

Statistical analysis

Statistical comparisons of the changes in concentration of individual metabolites in the blood over time between test meals and between men and women were carried out by two-way or one-way ANOVA with repeated measures and with gender as a between-subject factor as appropriate. The Student's paired *t* test was used for some comparisons.

Results

Subject characteristics

There were no significant differences between men and women in terms of age or BMI, or in fasting TAG, glucose and insulin concentrations (Table 1). The proportion of body fat was 41 % greater and the fasting total cholesterol concentration 23 % greater in women than men.

Macronutrient and fatty acid composition of the test meals

The macronutrient and fatty acid contents of the test meals are summarised in Table 2. The total carbohydrate, total protein and total energy contents were comparable between the four test meals. The reference meal, high-LA:αLNA, and high-EPA + DHA meals contained similar amounts of total fat, whereas the high-MUFA meal contained 7·4 g less fat than the reference meal. The test meals showed specific differences in fatty acid composition. The high-LA:αLNA meal contained 59 % less αLNA, which provided a 155 % greater ratio of LA:αLNA than the reference meal (Table 2). The high-MUFA meal contained 24 % more oleic acid, which resulted in 23 % more total MUFA than the reference meal. The high-EPA + DHA meal contained 567 % more EPA, 300 % more docosapentaenoic acid and 233 % more DHA, which resulted in a 583 % increase in combined EPA + DHA content compared with the reference meal. All other fatty acids were present in similar amounts in the four test meals.

The effect of consuming the test meals on plasma triacylglycerol fatty acid composition

The fatty acid composition of the plasma TAG is summarised in Fig. 1. Palmitic acid, oleic acid and LA were the major fatty acids in men and women at baseline (Fig. 1(A) and (B)). There were no significant differences in the concentrations of individual fatty acids at baseline between the test meals or between men and women for each test meal (Fig. 1(A) and (B)). Consumption of the reference meal significantly increased the concentrations of myristic acid, palmitic acid, stearic acid, palmitoleic acid, oleic acid, LA and αLNA in men (93 %, 40 %, 60 %, 28 %, 38 %, 65 and 95 %, respectively) and women (88 %, 48 %, 82 %, 24 %, 47 %, 93 % and 115 %, respectively) compared with baseline (Fig. 1(C)). There were no significant differences between men and women in the effect of consuming the reference meal on the change in concentration of the individual fatty acids.

The differences in the concentration of individual fatty acids in plasma TAG at the maximum total TAG concentration are summarised in Table 3. The response to each test meal is compared statistically with the magnitude of change in each fatty acid as a result of consuming the reference test meal. Consuming the high-LA:αLNA meal resulted in a selective decrease (men 66 %, women 87 %) in the increment in αLNA concentration compared with the reference meal, and an increase in the ratio of LA:αLNA of 341 % in men and 310 % in women. There was no significant difference in the change in concentrations of any fatty acid following the high-MUFA meal compared with those achieved with the reference meal alone. Consuming the high-EPA + DHA meal increased the change in EPA concentration by 325 % in men and women, and in DHA concentration by 317 % in men and 817 % in women, which resulted in an increase in the change in total EPA + DHA concentration of 414 % in men and 438 % in women. There were no significant differences in the change in concentrations of any other fatty acids measured compared with the response to the reference meal, or between men and women.

The effect of consuming the test meals on the postprandial triacylglycerol, NEFA, total cholesterol, glucose and insulin responses

There were no significant differences in the concentrations of plasma TAG, NEFA, total cholesterol, glucose and insulin at baseline between test meals or between men and women before consuming each test meal (Table 4). The changes in concentration of plasma TAG, NEFA, glucose and insulin are summarised in Figs 2–5. The results of analysis of the postprandial change in TAG, NEFA glucose and insulin concentrations by 2-way ANOVA showed a significant effect of time (Figs 2–5), but not of meal fatty acid composition or gender. There was no significant time–meal interaction. There was no effect of time after the meal, fatty acid composition of the meal or gender on the total cholesterol concentration after the meal. The results of the analysis of the total cholesterol concentration after each meal will not be discussed further.

There was no significant difference between meals or between men and women in the incremental area under the time *v.* concentration curve, the time to reach maximum or minimum concentration or the maximum or minimum concentration for plasma TAG, NEFA, glucose or insulin (Table 3).

Discussion

The results of this study show that, in middle-aged individuals, modifying the fatty acid composition of a meal had no significant effect on the magnitude of the postprandial plasma lipid, glucose or insulin response. In addition, these results indicate that it cannot be assumed that differences in the fatty acid

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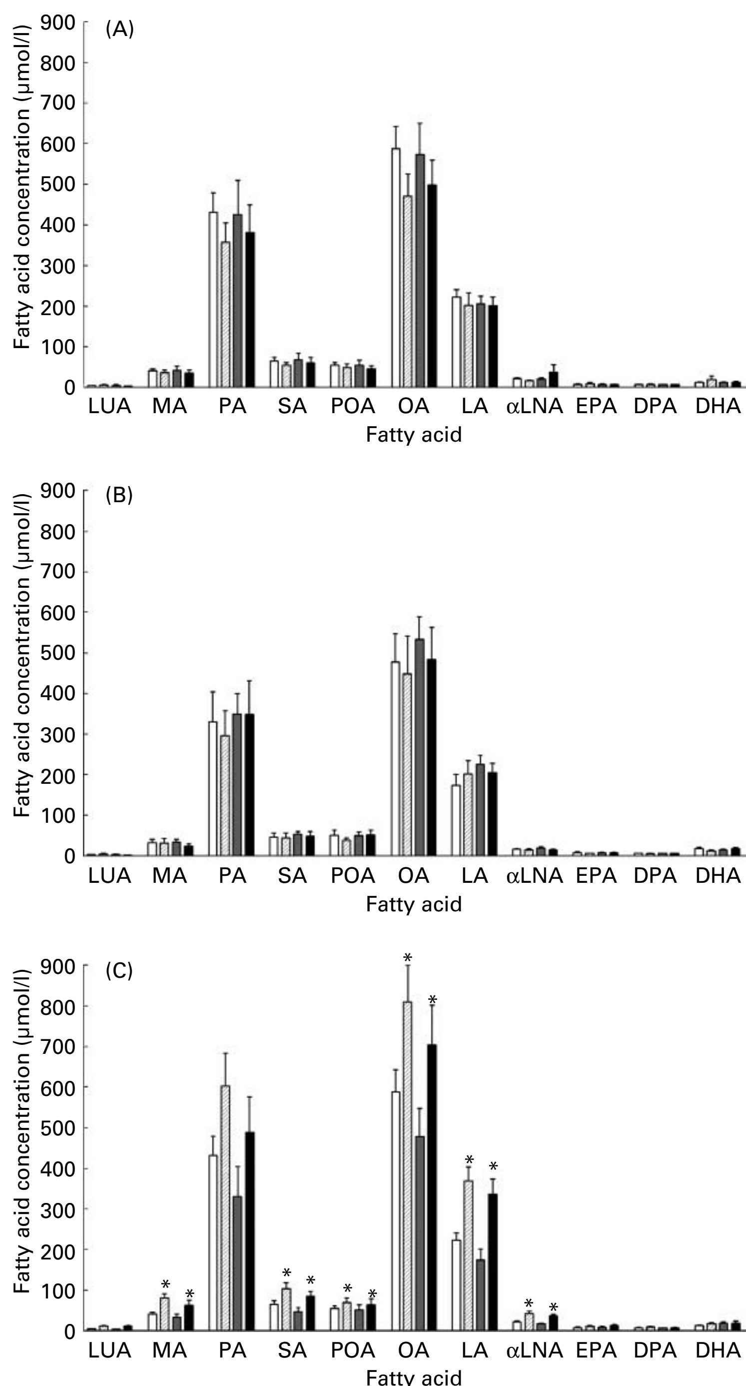

Fig. 1. Fatty acid composition of plasma triacylglycerol in (A) men (*n* 11) and (B) women (*n* 11) at baseline for each test meal. Values are means with their standard errors. Comparison of the concentration of each fatty acid between meals and between genders was by one-way repeated-measures ANOVA with gender as a between subject factor. *Post hoc* analysis was carried out using Bonferroni's test. □, Reference; ▨, high LA:αLNA; ■, high MUFA; ■, high EPA+DHA; (C) Change in the concentration of individual fatty acids in plasma triacylglycerol following the reference test meal in men and women. Statistical comparisons were carried out by one-way ANOVA with Bonferroni's *post hoc* analysis. Mean values were significantly different: $^*P<0{\cdot}05$. □, Men baseline; ▨, Men postprandial; ■, Women baseline; ■, Women postprandial; αLNA, α-linolenic acid; DPA, docosapentaenoic acid (*n*-3); LA, linoleic acid; LUA, lauric acid; MA, myristic acid; OA, oleic acid; PA, palmitic acid; POA, palmitoleic acid; SA, stearic acid. For details of subjects and procedures, see p. 490.

composition of meals will be reflected in the postprandial plasma TAG fatty acid composition.

Previous studies of the effect of the fatty acid composition of a test meal on postprandial changes in plasma lipids have produced conflicting findings. One study, for example, showed that increasing the EPA + DHA content of a test meal modified the postprandial TAG response (Zampelas *et al.* 1994*b*), whereas others did not find this (Harris *et al.* 1988; Weintraub *et al.* 1988; Zampelas *et al.* 1995). In addition, there was no effect of increasing the

Table 3. Change in fatty acid composition of plasma triacylglycerol following each test meal

(Mean values with their standard errors)

	Test meal															
	Reference				High LA:αLNA				High MUFA				High EPA + DHA			
	Change in fatty acid concentration (μmol/l)															
	Men		Women		Men		Women		Men		Women		Men		Women	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Lauric acid	6·8	1·1	8·2	1·0	6·8	3·0	13·4	6·5	6·6	2·2	10·5	1·5	5·0	1·1	10·8	3·2
Myristic acid	42·4	7·3	29·1	6·7	40·0	10·6	35·7	7·3	38·1	8·2	45·7	5·2	23·4	7·3	45·8	8·6
Palmitic acid	217·5	36·1	158·1	22·6	200·3	45·5	183·4	46·9	199·0	38·8	187·2	30·9	153·8	27·4	117·7	26·8
Stearic acid	42·5	6·3	38·2	5·2	43·5	8·4	35·3	5·2	41·0	10·2	39·5	5·2	29·1	5·2	26·9	4·6
Total saturated fatty acids	311·9	48·0	233·6	26·8	290·6	63·2	267·8	62·2	284·7	53·0	282·9	40·5	211·3	29·7	201·3	30·1
Palmitoleic acid	19·9	4·3	12·3	4·5	14·1	6·8	20·9	9·8	21·7	6·6	14·0	4·1	18·2	4·1	11·9	1·8
Oleic acid	281·6	47·7	218·4	39·5	315·7	62·0	191·0	33·7	312·0	43·5	177·9	45·9	196·9	30·7	145·9	38·8
Total MUFA	301·4	49·9	238·7	41·0	329·8	61·4	195·2	34·2	333·6	41·1	261·9	54·8	215·0	34·0	157·8	34·1
LA	164·9	21·3	162·5	45·1	159·0	32·3	91·5	29·9	130·8	15·1	97·8	20·3	114·6	12·1	102·5	17·2
αLNA	23·4	3·6	19·8	2·8	7·9**	3·0	2·6**	1·6	23·9	5·9	18·1	3·8	20·3	2·4	22·8	4·6
EPA	3·6	1·1	4·0	0·8	2·7	1·0	2·3	0·7	3·5	0·8	3·2	0·6	15·3††	2·2	17·0††	1·3
Docosapentaenoic acid (*n*-3);	2·4	0·9	1·5	0·4	1·9	0·7	1·3	0·7	2·8	0·8	2·1	0·7	3·4	0·4	2·5	0·5
DHA	2·8	0·9	1·2	1·0	−0·1	2·5	2·9	1·2	4·3	1·6	1·8	1·1	12·1†	1·6	11·0†	1·3
EPA + DHA	5·6	1·6	5·2	1·7	2·6	2·6	5·2	1·3	7·9	2·0	4·9	1·6	30·3‡	2·3	28·0††	2·4
Total PUFA	200·1	26·7	189·0	44·5	171·4	34·4	99·9	31·8	170·8	18·4	121·5	24·6	163·8	16·5	155·7	20·0
LA:αLNA	7·6	0·8	9·3	2·8	33·5*	8·2	38·1*	7·0	6·8	0·9	7·1	1·8	6·3	0·7	5·7	1·6

αLNA, a-linolenic acid; LA, linoleic acid.

Using a Student's paired *t* test, values were significantly different from the change in plasma triacylglycerol concentration following the reference meal: *$P<0·05$, **$P<0·01$, †$P<0·001$, ††$P<0·0001$.

For details of subjects and procedures, see p. 490.

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Table 4. Concentrations of metabolites in the plasma before consumption of the test meals, integrated area under the time–concentration response curve, time to maximum change from baseline, and peak or lowest concentration following each test meal

(Mean values with their standard errors)

	Reference				High LA:αLNA				High MUFA				High EPA + DHA			
	Men (*n* 11)		Women (*n* 11)		Men (*n* 11)		Women (*n* 11)		Men (*n* 11)		Women (*n* 11)		Men (*n* 11)		Women (*n* 11)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Concentrations of metabolites at baseline																
TAG (mmol/l)	1·41	0·15	1·21	0·19	1·27	0·16	1·15	0·22	1·4	0·21	1·42	0·15	1·26	0·18	1·24	0·14
NEFA (μmol/l)	522	82	596	130	492	61	571	54	440	47	411	39	504	53	562	57
Total cholesterol (mmol/l)	4·83	0·32	5·80	0·32	4·55	0·38	5·63	0·21	4·68	0·39	5·63	0·35	4·60	0·33	5·75	0·27
Glucose (mmol/l)	4·75	0·18	4·76	0·26	4·70	0·22	4·65	0·25	4·69	0·19	4·71	0·10	4·46	0·22	4·81	0·31
Insulin (mU/l)	4·57	0·70	7·19	1·10	6·43	1·34	9·09	2·51	5·74	1·21	7·07	0·85	5·23	1·11	7·29	0·72
Area under the time–concentration curve																
TAG (mmol/l × 6 h)	2·64	0·51	2·15	0·41	2·73	0·46	1·9	0·35	2·81	0·39	2·61	0·40	2·11	0·29	1·45	0·24
NEFA (μmol/l × 6 h)	4933	479	4627	355	4970	293	4374	244	4402	409	4761	350	4988	245	4429	313
Glucose (mmol/l × 6 h)	8·90	1·76	8·41	0·83	7·67	1·20	6·32	1·00	9·02	1·07	6·28	1·04	8·01	0·89	7·50	1·44
Insulin (mU/l × 6 h)	185·5	33·0	219·9	42·7	181·4	38·2	199·1	26·4	165·6	26·3	162·6	15·7	154·7	23·9	196·7	50·0
Time to peak TAG, glucose and insulin concentration or lowest NEFA concentration following each test meal (h)																
TAG	4	0·2	3	0·2	3	0·3	2·5	0·2	4	0·3	4	0·3	3	0·3	3	0·3
NEFA	2	0·3	3	0·5	2	0·3	2	0·4	2	0·3	2	0·2	3	0·5	3	0·6
Glucose	3	0·3	3	0·3	2	0·2	3	0·4	2	0·3	3	0·5	3	0·5	3	0·6
Insulin	2	0·4	2	0·3	2	0·3	2	0·2	2	0·2	2	0·2	2	0·4	2	0·3
Peak TAG, glucose and insulin concentration or lowest NEFA concentration following each test meal																
TAG (mmol/l)	2·2	0·3	1·9	0·3	2·1	0·3	1·7	0·3	2·2	0·3	2·1	0·2	1·9	0·2	1·8	0·2
NEFA (μmol/l)	277	88	172	24	220	11	197	22	219	23	180	18	227	22	153	24
Glucose (mmol/l)	7·5	0·5	7·6	0·5	7·2	0·4	7·0	0·5	7·5	0·5	7·1	0·4	7·4	0·4	7·4	0·4
Insulin (mU/l)	71	9	81	11	74	11	78	10	73	9	78	11	63	10	70	11

Values are mean, with their standard errors, concentrations of metabolites in the blood at baseline before consumption of each of the test meals, or incremental area under the time–concentration curve (except for NEFA, for which an arbitrary baseline of −1000 μmol/l was used), time to peak and magnitude of TAG, glucose and insulin concentration or minimum NEFA concentration. Statistical analysis was by one-way ANOVA with gender as a between-subjects factor, with Bonferroni's *post hoc* analysis.

αLNA, α-linolenic acid; LA, linoleic acid; TAG, triacylglycerol.

For details of subjects and procedures, see p. 490.

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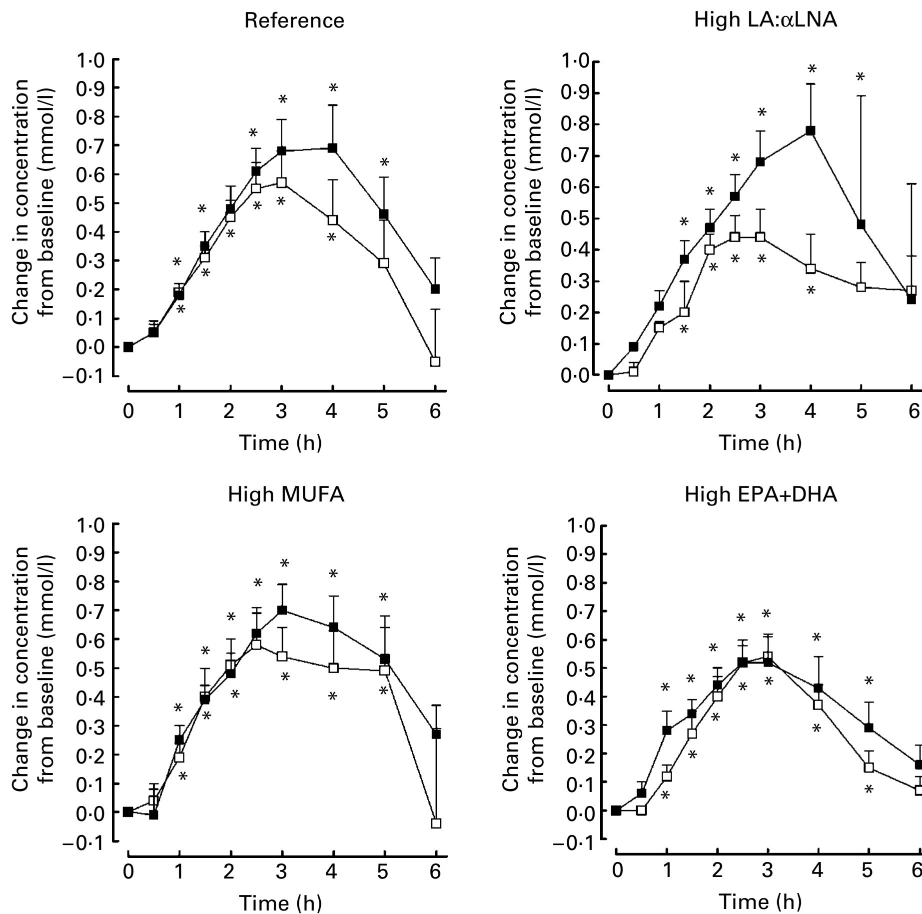

Fig. 2. Change in plasma triacylglycerol concentration following each of the test meals in men (■; *n* 11) and women (□; *n* 11). Values are mean changes from baseline with their standard errors. Statistical comparisons were by two-way ANOVA with repeated measures and with gender as a between-subjects factor. *Post hoc* comparisons between each time point and baseline were carried out using Dunnett's test. αLNA, α-linolenic acid; LA, linoleic acid. Mean values were significantly different from baseline: $^*P < 0{\cdot}05$. For details of subjects and procedures, see p. 490.

MUFA or LA content of a test meal on postprandial lipaemia (Demacker *et al.* 1991; de Bruin *et al.* 1993; Zampelas *et al.* 1994*a*; Jackson *et al.* 1999; Pedersen *et al.* 1999).

One consideration for interpreting these results is the extent to which differences in the fatty acid composition of test meals were reflected in changes in the composition of plasma lipid pools. Our data suggest that specific changes to the fatty acid composition of a test meal are not always reflected in the composition of plasma TAG. In particular, increasing the total MUFA content of the meal by about one quarter did not alter the MUFA content of plasma TAG, although altering the LA:αLNA ratio and EPA + DHA content did change the concentration of these fatty acids in the postprandial TAG pool. As previous studies did not report the fatty acid composition of plasma TAG after the meal, it is difficult to conclude whether the negative outcomes were due to an inability to change the fatty acid composition of plasma lipid pools or whether the fatty acid composition of the meal had no effect on postprandial lipaemia.

Our present results show that increasing the LA:αLNA ratio in the test meals by about 30 % or the EPA + DHA content by more than 300 % produced significant selective changes in the fatty acid composition of plasma TAG compared with a meal based on the pattern of fatty acids consumed habitually in the UK, but this failed to alter the magnitude of the postprandial TAG, NEFA, glucose or insulin responses, the peak concentration (or minimum concentration for NEFA), and the time taken to reach the maximum response (or in the case of NEFA the minimum concentration). In addition, there were no differences between men and postmenopausal women in any of these parameters. Overall, these data suggest that even substantial modifications to the PUFA composition of a meal do not modify the physiological response to the meal in middle-aged men and women. These data agree with the majority of previous studies on the effects of altering the EPA + DHA, LA or MUFA content of test meals in young men (Harris *et al.* 1988; Weintraub *et al.* 1988; Demacker *et al.* 1991; de Bruin *et al.* 1993; Zampelas *et al.* 1994*a*, 1995; Jackson *et al.* 1999; Pedersen *et al.* 1999).

However, Jackson *et al.* (2005) reported, in men of a similar age to the subjects in the study, a significant decrease in postprandial TAG response and increased NEFA suppression of increasing the *n*-6 PUFA, principally LA, content of a test meal compared with a SFA-enriched reference meal. In addition, they reported suppression of the postprandial glucose response by increasing the MUFA or *n*-6 PUFA content of the test meal. Although there were similar carbohydrate and total energy contents in the test meals used in the present

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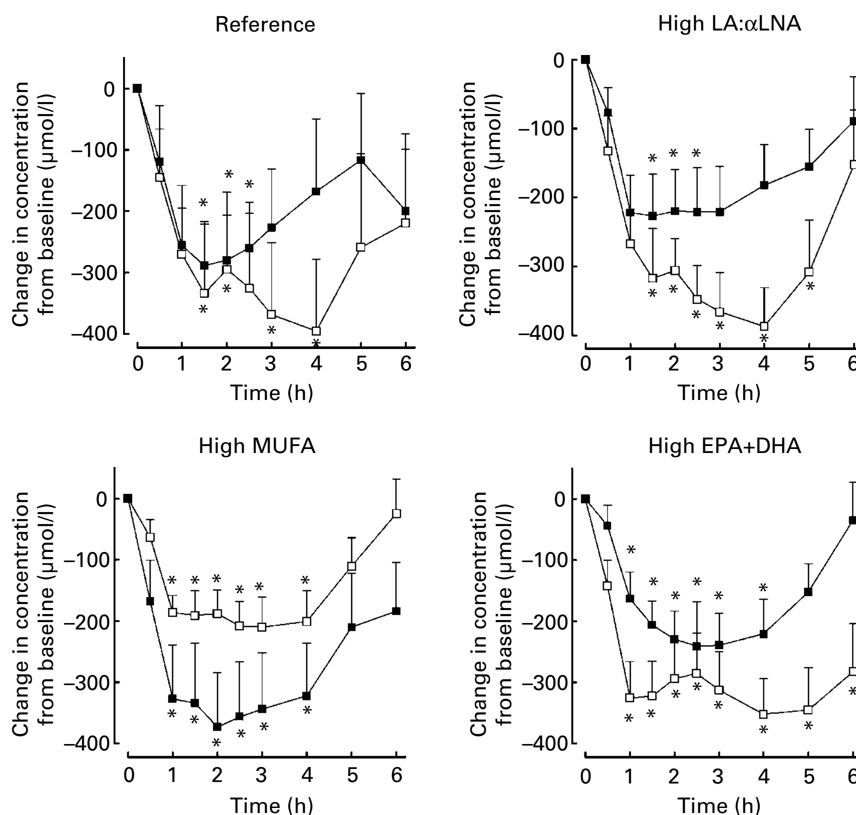

Fig. 3. Change in plasma NEFA concentration following each of the test meals in men (■; *n* 11) and women (□; *n* 11). Values are mean changes from baseline with their standard errors. Statistical comparisons were by two-way ANOVA with repeated measures and with gender as a between-subjects factor. *Post hoc* comparisons between each time point and baseline were carried out using Dunnett's test. αLNA, α-linolenic acid; LA, linoleic acid. Mean values were significantly different from baseline: *$P < 0.05$. For details of subjects and procedures, see p. 490.

study and in those reported by Jackson *et al.* (2005), there were important differences in fatty acid composition. For example, the reference meal used by Jackson *et al.* (2005) was SFA-enriched, whereas that used in the present study contained approximately 15 % less total SFA, a similar amount of MUFA and about twice as much total PUFA. In addition, as the oils used in the study by Jackson *et al.* (2005) were derived entirely from vegetable sources, it is unlikely that there would have been any long-chain *n*-3 PUFA, specifically EPA and DHA, present, whereas these fatty acids made up 0·6 % of the fatty acids in the reference test meal used in the present study. Meals enriched in SFA tend to increase the magnitude of postprandial lipasaemia (Williams, 1997). It is possible that Jackson *et al.* observed a lower lipaemic response when the meal was enriched in LA at a level 50 % greater than in the present study partly because the response to the SFA-enriched meal is likely to have been greater than with the meal containing less SFA and a greater total PUFA content that was used in the present study. This is supported by the almost two-fold greater peak TAG response reported by Jackson *et al.* (2005) compared with that reported here in men. We suggest that, because the pattern of fatty acids in the reference meal used in this study resembled the fatty aid composition of the habitual UK diet (Henderson *et al.* 2003) more closely than that reported by Jackson *et al.* (2005), the present data are of greater relevance to free-living individuals consuming their habitual diets.

There were no significant differences between men and women in any of the parameters measured. Koutsari *et al.* (2004) reported a significant interaction between postprandial TAG response and meal composition in young men and women. It appears that any such difference in the effect of meal fatty acid composition was lost in the older subjects reported in the present study. Our data also emphasise the extent to which the lower lipaemic response found in premenopausal women is lost after the menopause (Cohn *et al.* 1988; van Beek *et al.* 1999).

Taken together, the present results suggest that, in individuals who were of an age group associated with an increased risk of CVD, there were no significant acute effects of the fatty acid composition of a meal upon postprandial lipaemia or glucose or insulin responses. As increased EPA + DHA, MUFA and LA intakes in the background diet have been shown to have beneficial effects on postprandial lipaemia (Harris & Connor, 1980; Weintraub *et al.* 1988; Brown & Roberts, 1991; Demacker *et al.* 1991; Harris & Windsor, 1991; Williams *et al.* 1992; Harris & Muzio, 1993; Lichtenstein *et al.* 1993; Finnegan *et al.* 2003), the present data suggest that chronic patterns of fatty acid intake are likely to be of greater health importance than acute differences in fatty acid intake.

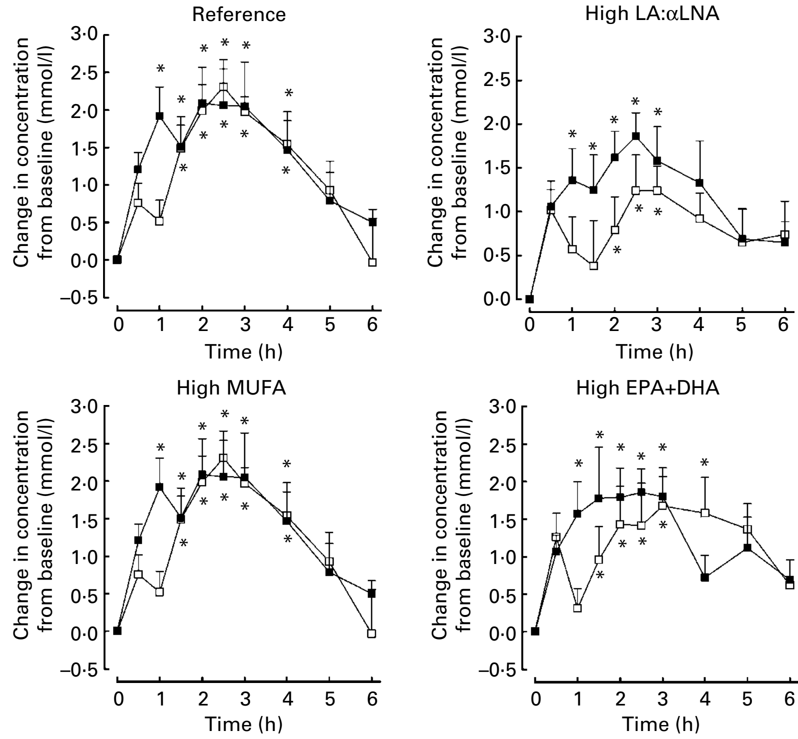

Fig. 4. Change in plasma glucose concentration following each of the test meals in men (■; *n* 11) and women (□; *n* 11). Values are mean changes from baseline with their standard errors. Statistical comparisons were by two-way ANOVA with repeated measures and with gender as a between-subjects factor. *Post hoc* comparisons between each time point and baseline were carried out using Dunnett's test. αLNA, α-linolenic acid; DHA, LA, linoleic acid. Mean values were significantly different from baseline: *$P<0.05$. For details of subjects and procedures, see p. 490.

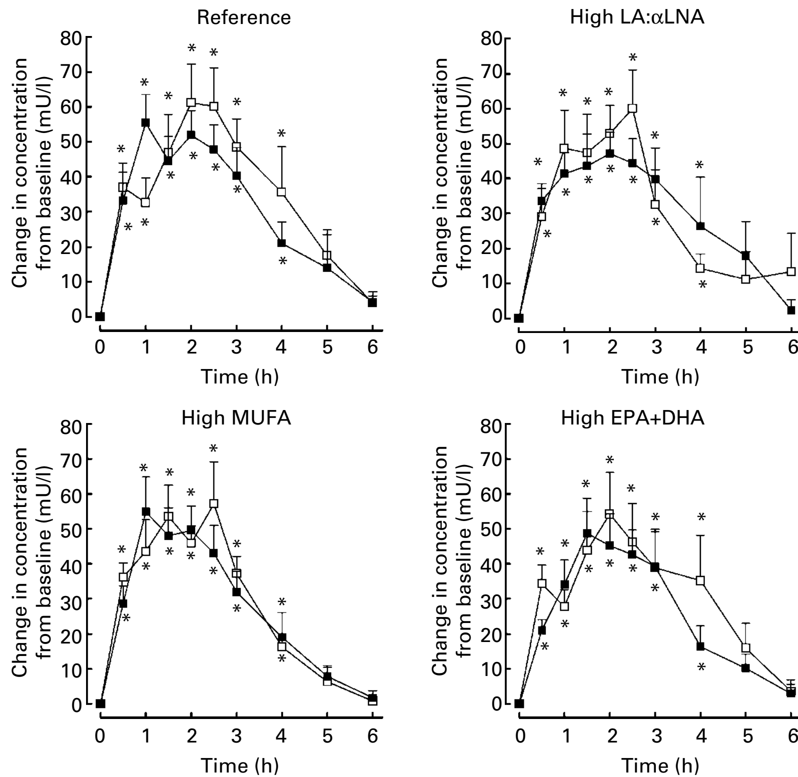

Fig. 5. Change in plasma insulin concentration following each of the test meals in men (■; *n* 11) and women (□; *n* 11). Values are mean changes from baseline with their standard errors. Statistical comparisons were by two-way ANOVA with repeated measures and with gender as a between-subjects factor. *Post hoc* comparisons between each time point and baseline were carried out using Dunnett's test. αLNA, α-linolenic acid; LA, linoleic acid. Mean values were significantly different from baseline: *$P<0.05$. For details of subjects and procedures, see p. 490.

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