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Acute effects of meal fatty acids on postprandial NEFA, glucose and apo E response: implications for insulin sensitivity and lipoprotein regulation?

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Our aim was to determine whether meal fatty acids influence insulin and glucose responses to mixed meals and whether these effects can be explained by variations in postprandial NEFA and Apo, which regulate the metabolism of triacylglycerol-rich lipoproteins (Apo C and E). A single-blind crossover study examined the effects of single meals enriched in saturated fatty acids SFA), *n*-6 PUFA and MUFA on plasma metabolite and insulin responses. The triacylglycerol response following the PUFA meal showed a lower net incremental area under the curve than following the SFA and MUFA meals ($P<0·007$). Compared with the SFA meal, the PUFA meal showed a lower net incremental area under the curve for the NEFA response from initial suppression to the end of the postprandial period (180–480 min; $P<0·02$), and both PUFA and MUFA showed a lower net incremental glucose response ($P<0·02$), although insulin concentrations were similar between meals. The pattern of the Apo E response was also different following the SFA meal ($P<0·02$). There was a significant association between the net incremental NEFA (180–480 min) and glucose response ($r_s=0·409$, $P=0·025$), and in multiple regression analysis the NEFA response accounted for 24 % of the variation in glucose response. Meal SFA have adverse effects on the postprandial glucose response that may be due to greater elevations in NEFA arising from differences in the metabolism of SFA- *v.* PUFA- and MUFA-rich lipoproteins. Elevated Apo E responses to high-SFA meals may have important implications for the hepatic metabolism of triacylglycerol-rich lipoproteins.

Triacylglycerol: Insulin: Apolipoproteins: NEFA

There is direct (Vessby *et al.* 2001) and indirect (Lovejoy *et al.* 2001) evidence to suggest that background diets high in saturated fatty acids (SFA) impair insulin sensitivity. Mechanisms that might link impaired insulin sensitivity and high SFA consumption have not been fully elucidated but include effects on cell signalling via membrane fatty acid compositional changes (Storlien *et al.* 1996) and on transcription factors (Kraegen *et al.* 2002), as well as the over-accumulation of lipids in muscle and liver (Petersen & Shulman, 2002). These mostly reflect chronic effects that would take weeks or months to become evident following dietary change. There are also, however, potential mechanisms that could operate in the short term, for example, following meal ingestion, via differential effects of dietary fatty acids on insulin secretion (Storlien *et al.* 1997) or by effects of the circulating fatty acid concentration on insulin action.

The ease with which diet-induced insulin resistance in rats can be reversed by the withdrawal of high-fat feeding supports the likelihood that at least some of the effects are due to alterations in circulating and tissue concentrations of fatty acids and not secondary to structural membrane changes or changes in gene expression (Oakes *et al.* 1997). During the hydrolysis of postprandial triacylglycerol (TAG)-rich lipoproteins by lipoprotein lipase (LPL; EC 3.1.1.34), a significant proportion of the NEFA are not taken up by adipose tissue but contribute to the rise in plasma NEFA concentration observed in the later postprandial phase, peripheral tissues thereby being exposed to elevated concentrations of dietary fatty acids (Frayn *et al.* 1996). Competition from elevated NEFA for glucose uptake into peripheral tissues via the glucose–fatty acid cycle (Randle *et al.* 1963) as well as direct effects of fatty acids on the downstream signalling response to insulin (Hansen *et al.* 1998) could, theoretically, contribute to variable postprandial insulin and glucose responses to fat-containing meals. This is supported by recent studies that show short-term elevations in NEFA concentrations to be associated with increased glucose-stimulated insulin secretion (Grill & Qvidstad, 2000; Beysen *et al.* 2002). Very little is known about the effects of different meal fatty acids on NEFA concentrations apart from a limited number of studies that show an elevated response with MUFA- or SFA-rich meals (Roche *et al.* 1998; Tholstrup *et al.* 2001; Robertson *et al.* 2002).

It is not known whether meal fatty acid composition alters insulin sensitivity and, if so, whether this could be via differential effects on circulating NEFA concentrations. However, higher postprandial insulin and glucose concentrations have been observed following MUFA- compared with PUFA-rich meals (Joannic *et al.* 1997; Pedersen *et al.* 1999), and higher insulin following SFA- compared with MUFA- and PUFA- enriched meals (Robertson *et al.* 2002). A number of studies have not observed differences in glucose and insulin responses between meals of differing fatty acid composition (Gatti *et al.* 1992; Zampelas *et al.* 1994; Roche *et al.* 1998; Thomson *et al.* 1999; Mekki *et al.* 2002; Macintosh *et al.* 2003).

Abbreviations: AUC, area under the curve; IAUC, incremental area under the curve; LPL, lipoprotein lipase; SFA, saturated fatty acid; TAG, triacylglycerol.

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We recently showed marked differences in the Apo C-II, C-III and E content of TAG-rich lipoproteins in response to meals enriched in SFA, PUFA and MUFA (Jackson *et al.* 2005), which could contribute to variable circulating NEFA and TAG concentrations via their roles in regulating TAG hydrolysis and the hepatic removal of TAG-rich lipoprotein particles. We have now evaluated the impact of meal fatty acids on plasma glucose, insulin, NEFA, TAG and Apo responses in order to further clarify the postprandial relationships between NEFA, glucose and insulin, and to determine the possible role of fatty acid dependent-differences in Apo composition in mediating any differences observed. As well as comparing the response to the three meals, stepwise multiple regression analysis has been undertaken to determine which postprandial variables are the major determinants of plasma glucose and insulin response when meals rich in SFA, *n*-6 PUFA and MUFA are fed.

Subjects and methods

Subjects

Ten healthy, middle-aged men, with a mean age of 48 (SD 9) years and a BMI of 25 (SD 3) kg/m^2 were studied on three occasions. Ethical consent was provided by the University of Reading Ethics Committee, and written informed consent was obtained from the subjects before the study began. Subjects were excluded if they had any metabolic disorders (e.g. diabetes or any other endocrine or liver diseases), were taking dietary supplements (e.g. fish oil), were smokers, regular exercisers (i.e. $>3 \times 30$ min of aerobic exercise per week) or heavy drinkers (i.e. >30 units of alcohol per week), or were taking medication that could affect lipoprotein metabolism. The subjects were recruited following screening for fasting plasma TAG, cholesterol and glucose concentrations, all of which were within normal limits (TAG, 1·3 (SD 0·4) mmol/l; cholesterol, 5·0 (SD 0·7) mmol/l; glucose, 5·7 (SD 0·4) mmol/l). Subjects were asked to maintain their usual exercise patterns and to abstain from alcohol and organised exercise regimens for 24 h prior to each postprandial investigation. A low-fat (<10 g fat) evening meal was consumed on the evening before each study day.

The design of the study was a single-blind, within-subject crossover in which the subjects attended an investigation unit at the University of Reading on three separate occasions separated by at least 1 month. Three test meals of different fatty acid composition were given to the subjects in the form of a warm chocolate drink containing the test oil and toasted white bread with strawberry jam given at approximately 08.00 hours. This meal contained 53 g fat. The fatty acid compositions of the test meals were provided by substituting 50 g of the 53 g fat with different dietary oils. These were:

1. a mixture of palm oil (29 g) and cocoa butter (21 g), rich in SFA (palm oil supplied by Anglia Oils Limited, Hull, East Yorkshire, UK; cocoa butter supplied by ADM Cocoa, Hull, East Yorkshire, UK);
2. safflower oil rich in *n*-6 PUFA (Anglia Oils Limited);
3. olive oil, rich in *n*-9 MUFA (Tesco, Cheshunt, Surrey, UK).

The nutrient and fatty acid composition of the test meals are shown in Tables 1 and 2 respectively.

After a 12 h overnight fast, the subjects attended the investigation unit, where an indwelling cannula was inserted into the antecubital vein of the forearm under local anaesthetic (1 % lignocaine) and two fasting blood samples were taken. The test meal was given and consumed within 20 min. Blood samples were collected at regular intervals for 480 min after the test meal. Samples for the analysis of Apo (B-48, B-100, C-II, C-III and E) were collected at fasting, 60, 180, 300, 360 and 480 min only. No food was allowed during the test period, and decaffeinated sugar-free drinks were consumed at regular intervals. The test meals were well tolerated by the subjects without any unpleasant side effects, and the subjects were unaware of the type of fat given in the meals.

Table 1. Nutritional composition of test meals*

	Carbohydrate (g)	Fat (g)	Protein (g)	Energy (kJ)
Test oil (50 g)	0	50·0	0	1848
Skimmed milk (150 g)	7·5	0·2	5·1	213
Nesquik (15 g)	12·0	0·5	0·5	228
Skimmed milk powder (15 g)	7·8	0·2	5·3	228
White bread (105 g)	73·8	2·3	12·2	1458
Jam (30 g)	27·5	0	0	47
Total	128·6	53·2	23·1	4452

* Determined from manufacturers' data and from food tables (Holland *et al.* 1991).

Plasma separation and analytical methods

Blood samples were transferred to heparin-containing tubes and spun at 1700 ***g*** for 15 min in a bench-top centrifuge at 4°C. Plasma for the analysis of lipids, Apo and insulin were stored at -20°C until analysis. To protect the Apo (B-48, B-100, C-II, C-III and E) from proteolytic cleavage, a preservative cocktail was added to the appropriate tubes before addition of the plasma samples to give a final concentration of 5 % v/v (Edelstein & Scanu, 1986).

TAG, cholesterol (fasting only) and glucose concentrations were measured with an ILAB 600 clinical chemistry analyser (Instrumentation Laboratory, Warrington, Cheshire, UK) using enzyme-based kits supplied by Instrumentation Laboratory. NEFA were analysed as described earlier and Apo C-II, C-III and E by turbidimetric immunoassay using kits supplied by Alpha Laboratories (Eastleigh, Hampshire, UK). All samples for each subject were analysed within a single batch and the interassay CV were less than 6 %.

Apo B-48 was measured by a specific competitive ELISA as previously described (Jackson *et al.* 2002*a*), and plasma samples were diluted 1:6 v/v in PBS containing 10 g human serum albumin/l before addition to the ELISA plate. Apo B-100 was measured using a specific in-house sandwich ELISA (Jackson *et al.* 2005), and plasma samples were diluted 1:32 000 v/v in PBS containing 0·025 % Tween 20 and 0·1 % bovine serum albumin. The interassay

Table 2. Fatty acid composition of test meals (g/50 g)*

	Saturated fatty acid	PUFA	MUFA
Saturated fatty acids	26·1	7·8	7·1
PUFA	3·2	31·8	5·9
MUFA	19·5	8·2	34·9

* Determined from computerised food database (FOODBASE, Institute of Brain Chemistry and Human Nutrition, London, UK).

CV for both the Apo B-48 and Apo B-100 analyses was less than 10 %.

Plasma insulin was measured using a specific ELISA incorporating monoclonal antibodies (Dako Ltd, High Wycombe, Buckinghamshire, UK). The intra-assay CV for insulin was 4·5 %.

Statistical analysis

Data were analysed using SPSS version 11 (SPSS Inc., Chicago, IL, USA). Results presented in postprandial time courses and in tables are mean values with their Standard errors. The area under the curve (AUC) and the net incremental area under the curve IAUC; includes all (IAUC), together with the area below the fasting concentration (Gannon *et al.* 1989; Wolever, 2004) were calculated using the trapezoidal rule (Matthews, 1988). Summary data (AUC, net IAUC, fasting and peak concentrations) for the lipid, Apo and insulin responses were analysed using one-way repeated-measures ANOVA. The postprandial time courses following the three test meals were analysed using two-way repeated-measures ANOVA with interaction. A Bonferroni correction was used for the *post hoc* detection of significant pairwise differences. Simple correlations were performed to determine associations between the lipid, Apo, glucose and insulin responses. Stepwise multiple linear regression was performed, using *P*-in of 0·05 and *P*-out of 0·01 in order to establish the independent associations between the metabolic variables. Data were log-transformed where necessary to render their distribution normal before statistical analysis, except in the case of simple correlations, for which a non-parametric test was used (Spearman's rho). Values of $P \leq 0{\cdot}05$ were taken as significant.

Results

Plasma lipid, glucose and insulin responses

Fasting plasma TAG, NEFA, glucose and insulin concentrations were not significantly different between the study days and are shown in Table 3.

The plasma TAG responses following the SFA, PUFA and MUFA meals are shown in Fig. 1. There was a significant difference between the meals, with the PUFA meal demonstrating lower TAG concentrations than the SFA meal ($P=0{\cdot}016$) and a different pattern of response compared with the SFA and MUFA meals ($P<0{\cdot}02$). The peak TAG concentration reached after the PUFA meal was significantly lower than that following the SFA and MUFA meals ($P<0{\cdot}003$). In addition, the PUFA meal showed a significantly lower AUC compared with the SFA meal ($P=0{\cdot}01$) and a lower net IAUC compared with the SFA and MUFA meals ($P<0{\cdot}007$) (Table 3).

Significant differences were observed in the NEFA responses following the test meals, with the PUFA meal showing a different pattern of response compared with the SFA and MUFA meals ($P<0{\cdot}006$) (Fig. 1). Following the test meals, NEFA concentrations were initially suppressed reaching a nadir at approximately 112 min, and the percentage NEFA suppression from fasting to 120 min was approximately 75 (Table 3). NEFA

Table 3. Summary measures (area under the curve (AUC), net incremental AUC (IAUC), fasting and peak concentrations) for the plasma lipid, glucose and insulin responses after the saturated fatty acid (SFA), PUFA and MUFA meals

(Mean values with their standard errors for the ten healthy, middle-aged men)

	SFA		PUFA		MUFA	
	Mean	SEM	Mean	SEM	Mean	SEM
Triacylglycerol (mmol/l)						
Fasting	1·39	0·19	1·21	0·09	1·25	0·11
Peak	2·88†	0·32	1·87	0·18	2·63†	0·22
AUC	956‡	124	722	68	845	71
Net IAUC	287†	51	140	35	243†	41
NEFA (μmol/l)						
Fasting	390·9	37·4	486·9	89·5	434·2	55·2
% NEFA suppression	−74·6	3·7	−71·9	2·7	−79·3	3·4
Peak	810·6†	70·1	682·6	42·6	656·5	54·4
AUC (180–480 min)	148‡	7	120	9	131	6
Net IAUC (180–480 min)	110†	9	79	6	89	10
Glucose (mmol/l)						
Fasting	5·65	0·14	5·67	0·17	5·79	0·09
Peak	8·66	0·46	8·04	0·35	8·34	0·37
AUC	2879	87	2739	67	2775	55
Net IAUC	169	58	17§	67	−6‖	47
Insulin (pmol/l)						
Fasting	34·0	4·6	37·8	5·1	38·9	7·9
Peak	415·0	53·5	392·0	39·7	432·2	61·9
AUC	51 500	5369	47 685	6109	50 309	7081
Net IAUC	35 165	4499	29 524	4921	31 626	4444

AUC and net IAUC for the triacylglycerol and glucose responses are expressed as mmol/l × 480 min, and for insulin as pmol/l × 480 min. For NEFA, AUC and IAUC (180–480 min) are expressed as mmol/l × 300 min, and % NEFA suppression is calculated from fasting to 120 min.

Mean values were significantly different from PUFA meal (one-way repeated-measures ANOVA followed by Student's *t* test with Bonferroni correction): †$P<0{\cdot}008$, ‡$P<0{\cdot}02$.

Mean values were significantly different from SFA meal (one-way repeated measures ANOVA followed by Student's *t* test with Bonferroni correction): §$P=0{\cdot}002$, ‖$P=0{\cdot}013$.

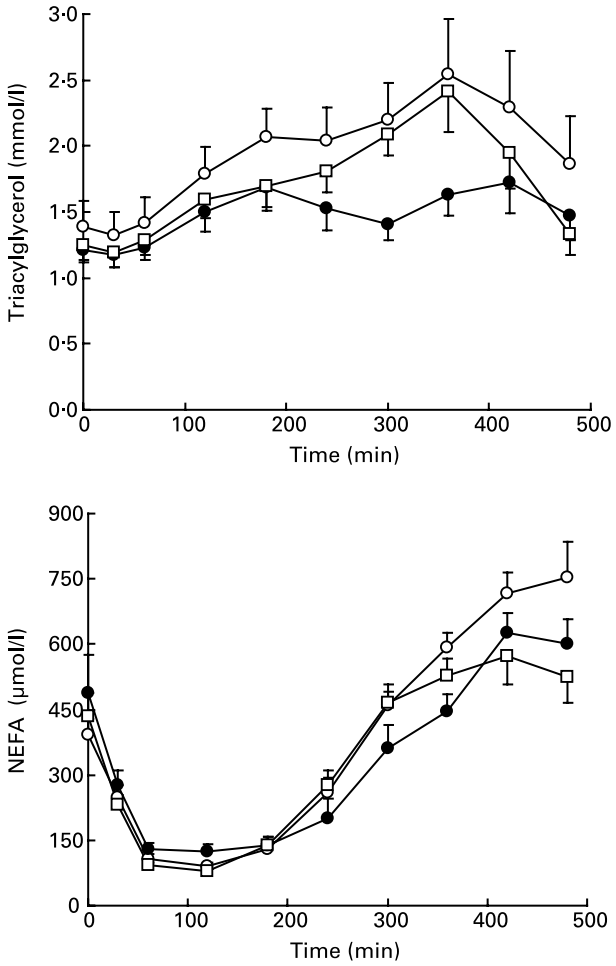

Fig. 1. Plasma triacylglycerol and NEFA responses following test meals enriched in saturated fatty acids (SFA; ○), PUFA (●) and MUFA (□). Values are means for the ten healthy, middle-aged men, with their standard errors represented by vertical bars. Postprandial samples were collected over a 480 min period during which subjects consumed sugar-free, decaffeinated drinks every 2 h and maintained normal sedentary activity. For the plasma triacylglycerol and NEFA responses, there was a significant time effect ($P<0·001$) and a meal × time interaction ($P<0·008$) by repeated-measures ANOVA.

concentrations increased following the nadir, reaching peak concentrations at approximately 420 min. From 240 min onwards, the NEFA concentrations remained lower following the PUFA meal than the SFA and MUFA meals, although from 300 min onwards for the MUFA meal, the NEFA concentration did not continue to rise and was similar to the PUFA meal for the late postprandial period. The SFA meal showed a significantly greater peak concentration compared with the PUFA meal ($P=0·014$) (Table 3). At the end of the postprandial period, the NEFA concentration 480 min following the SFA meal was significantly higher compared with the PUFA and MUFA meals ($P<0·01$) (Fig. 1). The total NEFA AUC and net IAUC (0–480 min) were not significantly different following the meals (data not shown). However, the AUC and net IAUC calculated between 180 and 480 min (the time during which NEFA concentrations begin to rise from the nadir) were significantly higher following the SFA than the PUFA meal ($P<0·02$) (Table 3).

Glucose and insulin concentrations rose sharply following the test meals, peaking at approximately 60 min, with the PUFA meal showing a tendency for a second peak at 120 min before returning to baseline concentrations between 240 and 300 min (Fig. 2). Although there were no significant differences in the overall responses (for glucose, two-way repeated-measures ANOVA showed a trend for a difference between the meal fatty acids; meal effect $P=0·07$) or in the AUC (0–480 min) between the meals, the net IAUC for the glucose response following the SFA meal was significantly greater compared with the PUFA and MUFA meals ($P<0·02$; Table 3). There were no significant differences in the peak insulin and glucose concentrations after the SFA, PUFA and MUFA meals (Table 3).

Plasma Apo responses

There were no significant differences in the fasting concentrations for Apo B-48, B-100, C-II, C-III and E between study days (data not shown).

Plasma Apo B-48, B-100, C-II and C-III responses following the SFA, PUFA and MUFA meals were not significantly different.

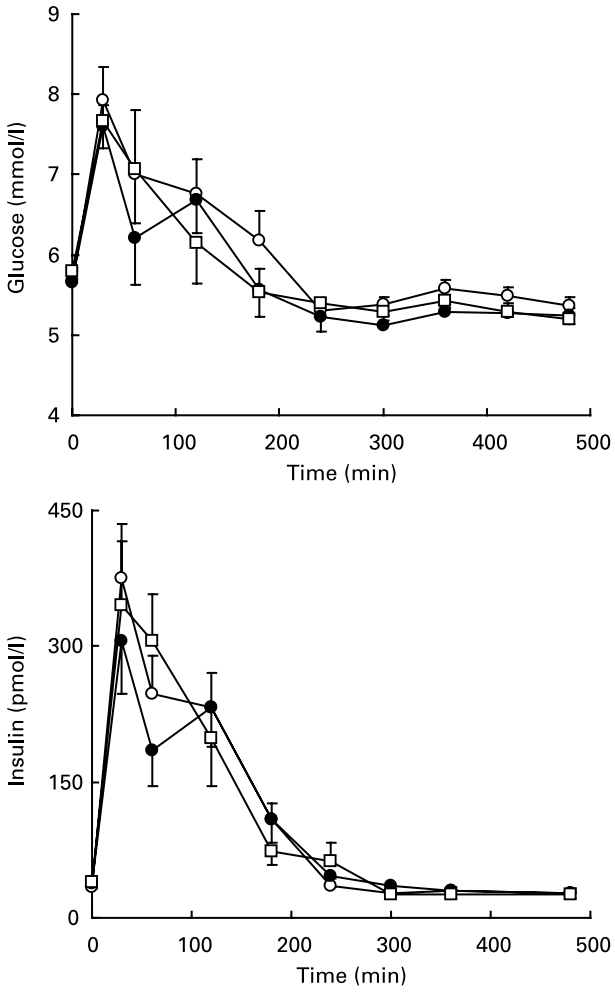

Fig. 2. Plasma glucose and insulin responses following test meals enriched in saturated fatty acids (SFA; ○), PUFA (●) and MUFA (□). Values are means for the ten healthy, middle-aged men, with their standard errors represented by vertical bars. For the glucose response, there was a trend for a significant meal effect ($P=0·07$), and for the insulin and glucose responses, there was a significant time effect ($P<0·001$) by repeated-measures ANOVA.

There was a significant difference in the Apo E responses following the three meals, with the SFA meal showing a different pattern of response compared with the PUFA and MUFA meals (Fig. 3). AUC, net IAUC and peak responses were not significantly different after the SFA, PUFA and MUFA meals for any of the plasma Apo (data not shown).

Simple correlations and regression analysis

In bivariate Spearman's correlation analysis, the only incremental postprandial variable to correlate with the net incremental glucose response was NEFA (180–480 min) (r_s=0·409, P=0·025) (Table 4). None of the net incremental postprandial variables correlated with the insulin response, although a moderate negative association was observed between the net incremental insulin and Apo B-48 responses (r_s = 0·350, P=0·058). A number of other significant associations between postprandial variables were observed for TAG, NEFA and Apo (C-II, C-III, E, B-48 and B-100) responses (Table 4). These were entered into stepwise multiple regression analysis to determine, for each variable, the percentage variation in response that could be explained by other net incremental postprandial variables (Table 5). NEFA (180–480 min) and NEFA (0–480 min) explained 24 % of the variation in postprandial glucose response. Apo B-48 explained 10 % of the variation in the insulin response, whereas Apo E and NEFA (180–480 min) explained 42 % of the variation in the TAG response. Of the variation in apo C-II, 50 % could be explained by Apo C-III, whereas for Apo C-III, the Apo C-II and Apo E responses explained 66 % of the variation in response. Of the apo E ressponse, 65 % could be explained by the responses of Apo C-III, NEFA (0–480 min) and insulin, and Apo B-100 explained 11 % of the variation in Apo B-48 response. For NEFA (0–480 min), Apo E explained 28 % of the response, and for NEFA (180–480), glucose explained 12 % of the response (Table 5).

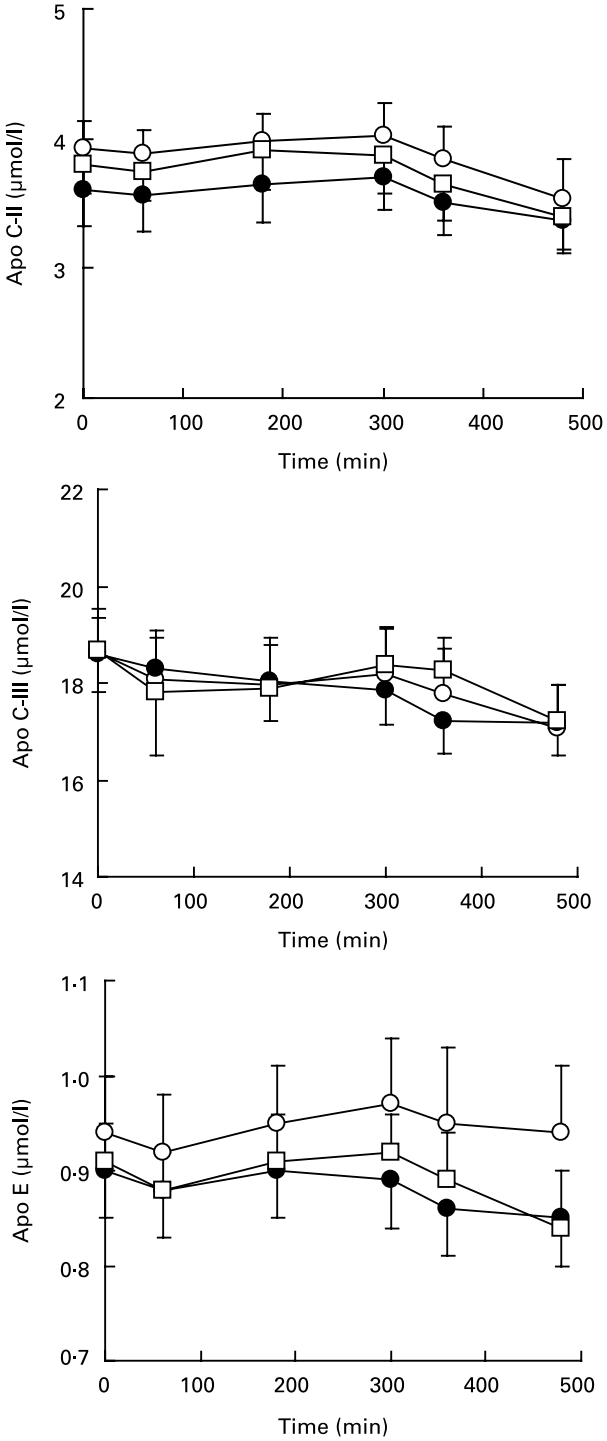

Fig. 3. Plasma apo C-II, C-III and E responses following test meals enriched in saturated fatty acids (SFA; ○), PUFA (●) and MUFA (□). Values are means for the ten healthy, middle-aged men, with their standard errors represented by vertical bars. For the apo E response, there was a significant meal × time interaction (P=0·021), and for the apo C-II, C-III and E responses, there were significant time effects (P<0·03) by repeated-measures ANOVA.

Discussion

The findings reported here confirm and extend previous reports of meal fatty-acid-dependent differences in postprandial TAG and NEFA response (Williams, 1997). Although some other studies have reported similar plasma TAG concentrations following *n*-6 PUFA compared with SFA- or MUFA-rich oils (Sanders *et al.* 2000; Tholstrup *et al.* 2001; Jackson *et al.* 2002*b*; Mekki *et al.* 2002), other authors have shown attenuated TAG responses to PUFA-rich meals with and without PUFA-rich background diets (Weintraub *et al.* 1988; Demacker *et al.* 1991; Zampelas *et al.* 1994).

The effect of meal fatty acids on circulating NEFA levels has been investigated in only a small number of studies to date (Roche *et al.* 1998; Tholstrup *et al.* 2001; Robertson *et al.* 2002). Roche *et al.* (1998) found that the consumption of a test meal rich in MUFA showed less suppression of the NEFA response compared with a meal rich in SFA, which was not found here. In the study of Robertson *et al.* (2002), the rise in plasma NEFA following initial suppression was significantly higher following an SFA-rich breakfast compared with *n*-6 PUFA-, *n*-3 PUFA- and MUFA-rich breakfast meals. Recent findings by Tholstrup and co-workers (2001) have shown that the type of SFA can influence the 4 h postprandial NEFA concentration, with a mixture of palmitic and myristic acid showing higher, and stearic acid lower, NEFA concentrations compared with meals rich in palmitic acid, PUFA and MUFA. In the present study, ingestion of the SFA-rich meal showed a different pattern of NEFA response compared with the PUFA meal, the SFA meal demonstrating a greater rise and significantly higher peak concentration following initial suppression.

Table 4. Bivariate correlation coefficients (Spearman's rho (r_s)) for the associations between the net incremental lipid, apo, glucose and insulin responses

	TAG	Apo C-II	Apo C-III	Apo E	Apo B-48	Apo B-100	NEFA (0–480)	NEFA (180–480)	Glucose	Insulin
TAG	1·000	0·260	0·451[b]	0·465[b]	0·148	0·067	0·553[a]	0·240	0·190	0·215
Apo C-II	0·260	1·000	0·661[a]	0·353	0·044	0·062	0·167	0·208	0·211	0·304
Apo C-III	0·451[b]	0·661[a]	1·000	0·552[a]	0·062	0·044	0·289	0·111	0·177	0·196
Apo E	0·465[b]	0·353	0·552[a]	1·000	0·135	−0·104	0·664[a]	−0·067	0·295	−0·176
Apo B-48	0·148	0·050	0·062	0·135	1·000	−0·388[c]	−0·098	0·000	−0·118	−0·350
Apo B-100	0·067	0·069	0·044	−0·104	−0·388[c]	1·000	−0·001	0·067	−0·089	0·190
NEFA (0–480)	0·553[a]	0·167	0·289	0·664[a]	−0·098	−0·001	1·000	0·042	0·337	0·104
NEFA (180–480)	0·240	0·208	0·111	−0·067	0·000	0·067	0·042	1·000	0·409[c]	0·224
Glucose	0·190	−0·023	−0·191	−0·271	−0·028	−0·111	−0·123	0·409[c]	1·000	0·257
Insulin	0·215	0·304	0·196	−0·176	−0·350	0·190	0·104	0·224	0·261	1·000

TAG, triacylglycerol.
Values with superscript letters were significantly correlated [a]$P<0·003$. [b]$P<0·03$ [c]$P<0·03$.

These findings are in agreement with those of Robertson *et al.* (2002) and suggest that the type of fatty acids fed in meals influences the rising part of the NEFA response, which reflects the relative contribution of NEFA from adipose tissue lipolysis, the release of NEFA from circulating TAG-rich lipoproteins and the uptake of NEFA into the peripheral tissues and liver. Our data suggest that, in the late postprandial phase, there is either a greater overspill of NEFA due to a continuing active hydrolysis of SFA-rich, TAG-rich lipoproteins and/or a lesser uptake of NEFA rich in SFA by adipose tissues, other peripheral tissues or the liver. It is notable that, following the SFA and MUFA meals, superimposable NEFA responses were observed up to 300 min, the MUFA meal thereafter showing a plateau in NEFA concentration between 300 and 480 min, whereas the SFA meal showed a continuing rise. The postprandial TAG concentrations for the SFA and MUFA meals were also similar up to 360 min, when a greater decrease in TAG concentration was observed for the MUFA (360–480 min) compared with the SFA meal. TAG responses returned to fasting levels for PUFA and MUFA by 480 min but were still elevated in the case of the SFA meal. The slower clearance of TAG-rich lipoprotein particles in the late postprandial phase following the SFA meal may provide more substrate for LPL-mediated hydrolysis of SFA during the latter part of the study period and may thereby contribute to the continuing increase in plasma NEFA concentration seen at this time.

Studies in healthy human subjects examining the effects of meal fatty acids on postprandial glucose and insulin have generated conflicting findings, which may be due to variations in meal conditions other than fatty acid type; for example, the amount of fat and the amount and type of carbohydrate in the meals (Gatti *et al.* 1992). The addition of SFA (butter) to mashed potato was shown to increase insulin and C-peptide responses (Gannon *et al.* 1993), whereas the addition of PUFA (maize oil) was shown to reduce the insulin response in a separate study (Welch *et al.* 1987). In the study of Robertson *et al.* (2002), higher insulin concentrations were observed following a meal rich in SFA (palmitic acid) compared with PUFA and MUFA meals, but there was no effect on glucose responses. In the present study, a significantly greater net incremental glucose response was observed following the SFA meal compared with the PUFA and MUFA meals, although differences were not observed in the insulin responses. These findings are similar to those of Gatti *et al.* (1992), who found a reduction in glucose responses following the addition of PUFA and MUFA to white bread but not with the addition of SFA; there were no differences in insulin response. These data suggest a greater sensitivity to the glucose-lowering actions of insulin in the presence of unsaturated fatty acids rather than SFA.

The possibility that the meal-induced differences in glucose response in the present study were mediated by differences in circulating NEFA concentrations is supported by the findings obtained from stepwise multiple regression analysis. The total NEFA response (0–480 min) and the late NEFA response (180–480 min) were independently associated with the postprandial glucose response and together explained 24 % of the variation in glucose response. Although the most obvious explanation

Table 5. Relationship between the incremental postprandial variables measured using stepwise multiple regression analysis

Dependent variable	Independent variable	Standardised coefficient	Adjusted r^2	*P*-value
TAG	Apo E	0·603	0·341	0·000
	and NEFA (180–480 min)	0·307	0·419	0·039
Apo C-II	Apo C-III	0·729	0·515	0·000
Apo C-III	Apo C-II	0·729	0·515	0·000
	and apo E	0·448	0·666	0·001
Apo E	Apo C-III	0·690	0·458	0·000
	and NEFA (0–480 min)	0·373	0·573	0·007
	and insulin	−0·301	0·653	0·012
Apo B-48	Apo B-100	−0·383	0·116	0·037
NEFA (0–480 min)	Apo E	0·552	0·280	0·002
NEFA (180–480 min)	Glucose	0·397	0·128	0·030
Glucose	NEFA (180–480 min)	0·397	0·128	0·030
	and NEFA (0–480 min)	0·363	0·237	0·034
Insulin	Apo B-48	−0·366	0·103	0·047

TAG, triacylglycerol.

for this relationship is that high NEFA concentrations inhibit the uptake and oxidation of glucose via the glucose–fatty acid cycle, *in vivo* studies in human subjects are revealing other mechanisms to explain the adverse effects of high circulating NEFA levels on glucose utilisation by the peripheral tissues. Using ^{13}C-NMR, recent studies have demonstrated an adverse impact of acute elevations in NEFA concentrations on skeletal muscle insulin sensitivity, reflected in a reduced glucose-6-phosphate concentration and glycogen accumulation (Roden *et al.* 1996). Insulin insensitivity appeared to be due to the impaired activation of phosphatidyl inositol-3 kinase, a crucial enzyme required to mediate the insulin-induced translocation of GLUT-4 to the plasma membrane (Dresner *et al.* 1999). These and our own data support the view that the effects of dietary fatty acid on insulin sensitivity may be mediated via acute fat ingestion, as well as by longer-term effects dependent upon dietary impacts on membrane fatty acid composition or transcriptionally induced alterations in gene expression.

The metabolism of TAG-rich lipoproteins is influenced by their Apo C (C-II and C-III) and E composition, since these proteins have a number of regulatory functions. Apo C-II activates LPL, whereas Apo C-III inhibits the binding and hydrolysis of lipoproteins by LPL and hepatic lipase (EC 3.1.1.3), and inhibits the uptake of lipoproteins by the liver (Jong *et al.* 1999). Apo E plays a crucial role in mediating the hepatic recognition and uptake of remnant particles by receptor-mediated processes (Blum, 1982). There is, however, very little information in the literature regarding the effects of meal fatty acids on exchangeable Apo concentrations in plasma. Of the limited studies conducted, there were no apparent effects of PUFA- and MUFA-enriched emulsions on Apo C-II concentrations (Brouwer *et al.* 1993), although in another study there was a trend for Apo E concentrations to be higher for SFA than PUFA meals (Mero *et al.* 1998).

In the present study, differences were observed in the Apo E response following the meals, the SFA meal showing higher concentrations than the PUFA and MUFA meals. Although there were no significant differences in the Apo C-II and C-III responses, the SFA meal showed a tendency towards higher Apo C-II concentrations over the postprandial period, whereas for Apo C-III, the PUFA meal showed a tendency towards higher concentrations in the earlier part of the postprandial period, the MUFA meal being higher during the latter part of the study period (data included in Fig. 3). The concentrations of Apo E and C-III in total plasma have previously been shown to correlate with the level of plasma TAG (Batal *et al.* 2000). In the present study, the plasma TAG response was shown to be positively correlated with both the Apo C-III ($r_s = 0{\cdot}451$, $P < 0{\cdot}05$) and Apo E ($r_s = 0{\cdot}465$, $P < 0{\cdot}05$) responses, and the Apo E response accounted for 34 % of the variation in TAG response in multiple regression analysis. There was, however, a significant negative correlation between Apo E and total NEFA response ($r_s = -0{\cdot}664$, $P < 0{\cdot}001$), and in multiple regression analysis the Apo E response accounted for 28 % of the variation in total NEFA response.

In studies with HepG2 cells, we showed that TAG-rich lipoproteins prepared from human plasma following SFA, PUFA and MUFA meals inhibited the uptake of ^{125}I-labelled LDL in the order SFA > MUFA = PUFA. These differences in uptake were shown to be dependent upon the Apo E content of the TAG-rich lipoproteins (unpublished results). We propose that this reflects greater affinity by the LDL receptor for SFA-containing TAG-rich lipoproteins due to their higher Apo E content, and that other non-Apo E-dependent pathways may be responsible for the rapid uptake of MUFA and PUFA TAG-rich lipoprotein particles. Competition between LDL and SFA-rich, TAG-rich lipoproteins for removal by the LDL receptor may therefore result in the impaired removal of SFA-rich, TAG-rich lipoproteins in the late postprandial phase, resulting in greater rates of NEFA generation. These interpretations are consistent with our overall findings and with the associations between Apo E, postprandial TAG and NEFA that we have observed here.

In conclusion, this study has demonstrated meal fatty acid-induced differences in plasma TAG, NEFA and glucose response that are consistent with adverse effects of meal SFA content on postprandial insulin sensitivity for glucose disposal. The differential responses do not appear to be due to dietary fatty acid-induced differences in the particle content of the stimulatory and inhibitory LPL co-factors Apo C-II and Apo C-III. We speculate that unsaturated fatty acid-rich TAG-rich lipoproteins, particularly PUFA-rich particles, may be more rapidly metabolised via LPL and their remnants more readily taken up by non-Apo E-dependent receptor pathways. In contrast, we propose that the uptake of SFA-rich, TAG-rich lipoproteins by the Apo E-dependent LDL receptor and competition between LDL and SFA-rich, TAG-rich lipoprotein particles impairs TAG-rich lipoprotein removal, allowing a continued release of NEFA into the circulation that can compete with glucose for uptake into peripheral tissues, for example skeletal muscle. The data presented here suggest that the chronic consumption of meals rich in SFA may contribute to the impairment of insulin sensitivity that arises from the consumption of diets that are also high in total fat. However, the small number of studies and the inconsistency of findings from studies, due to differences in experimental design, fat content and specific fatty acids used, currently make it difficult to extrapolate these findings to dietary policy.

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