Insulin response and changes in composition of non-esterified fatty acids in blood plasma of middle-aged men following isoenergetic fatty and carbohydrate breakfasts

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It was previously shown that a high plasma concentration of non-esterified fatty acids (NEFA) persisted after a fatty breakfast, but not after an isoenergetic carbohydrate breakfast, adversely affecting glucose tolerance. The higher concentration after the fatty breakfast may in part have been a result of different mobilization rates of fatty acids. This factor can be investigated as NEFA mobilized from tissues are monounsaturated to a greater extent than those deposited from a typical meal. Twenty-four middle-aged healthy Caucasian men were given oral glucose tolerance tests (OGTT), and for 28 d isoenergetic breakfasts of similar fat composition but of low (L) or moderate (M) fat content. The composition of NEFA in fasting and postprandial plasma was determined on days 1 and 29. No significant treatment differences in fasting NEFA composition occurred on day 29. During the OGTT and 0–1 h following breakfast there was an increase in plasma long-chain saturated NEFA but a decrease in monounsaturated NEFA (μg/100 μg total NEFA; $P < 0.001$). Between 1 and 3 h following breakfast treatment differences occurred for total saturated and total monounsaturated fatty acids (μg/100 μg total NEFA; $P < 0.05$), expressed as an increase in 18:1 and decreases in 16:0 and 17:0 in treatment M relative to treatment L ($P < 0.05$). Serum insulin attained 35 and 65 mU/l in treatments M and L respectively during this period. Negative correlations were found between 16:0 in fasting plasma and both waist:hip circumference ($P = 0.0009$) and insulin response curve area during OGTT (within treatment M, $P = 0.0001$). It is concluded that a normal postprandial insulin response is associated with a rapid change in plasma saturated:monounsaturated NEFA. It is proposed that this change is the result of a variable suppression of fat mobilization, which may partly account for a large difference in postprandial total plasma NEFA between fatty and carbohydrate meals.

Non-esterified fatty acids: Insulin: Dietary fat: Dietary carbohydrate

High circulating concentrations of non-esterified fatty acids (NEFA) have emerged as important risk factors for a variety of complex metabolic disorders. These disorders include non-insulin-dependent diabetes mellitus, dyslipidaemia, insulin resistance, hyperinsulinaemia and atherosclerotic cardiovascular disease (Frayn *et al.* 1997; Byrne *et al.* 1998). In a concentration-dependent manner NEFA decrease insulin binding and receptor-mediated insulin

degradation in liver cells (Svedberg *et al.* 1990). The importance of elevated portal NEFA is supported by the observation that obese subjects, with high circulating NEFA concentrations, have fewer binding sites for insulin in the hepatocytes (Arner *et al.* 1986).

Chylomicrons are hydrolysed at the capillary endothelium by the action of lipoprotein lipase. This process causes a continuous influx of fatty acids into adipocytes. However,

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leak-back occurs, which augments the efflux entering the venous plasma as a result of the activity of intracellular hormone-sensitive lipase HSL. Thus, adipose tissue triacylglycerols (TAG) exist in a continuous dynamic state (Frayn, 1993). Following a fatty meal the influx greatly exceeds the efflux, as the efflux is suppressed by the action of insulin on HSL. Circulating NEFA are rapidly metabolized by other tissues, as they have a turnover of only a few minutes (Friderickson & Gordon, 1958*a*,*b*). Consequently, they influence the availability of glucose by a fatty acid-mediated inhibition of plasma glucose clearance and cellular uptake in the insulin-stimulated state (Wolfe, 1998). We previously found that a relatively small increase in fat consumption from isoenergetic breakfasts was associated with a relatively large increase in plasma NEFA concentration that persisted for at least 5 h. The NEFA were then associated with reduced glucose tolerance that was caused either by the breakfast on day 29 or by the effect of all breakfasts for days 1–29 (Frape *et al.* 1998).

Following consumption of a fatty meal containing 80 g fat and at least 80 g carbohydrate (*v.* 5·5 and 25·7 g fat and 113 and 56 g carbohydrate in the two meals in the present study) by healthy subjects, Griffiths *et al.* (1994) and Frayn *et al.* (1997) concluded that more than 80 % of the venous NEFA draining adipose tissue were meal-derived through the activity of lipoprotein lipase. We questioned whether there may be an endogenous origin for a significant proportion of the raised postprandial NEFA following a moderately fatty breakfast. The origin of this NEFA may be elucidated by determining its fatty acid composition, and specifically the relative proportions of long-chain saturated and monounsaturated fatty acids. This procedure was previously adopted by Connor *et al.* (1996).

It was surmised that the difference between treatments in the carbohydrate content of the isoenergetic breakfasts, and the resulting insulin responses, would have been closely associated with a difference in the composition of postprandial NEFA in middle-aged men. This composition was now determined in an endeavour to understand the large treatment difference in plasma total NEFA concentration. Evidence from experiments on rabbits indicates that mobilized endogenous fatty acids are considerably less saturated than the fatty acids of adipose tissue sources from which they originate (Connor *et al.* 1996). Insulin response associated with insulin resistance, or the consumption of a low-carbohydrate meal, may fail to adequately suppress postprandial intracellular fat mobilization. Consequently, monounsaturated fatty acids, as a proportion of total plasma NEFA, should not decrease to the extent that they may do following a carbohydrate meal.

Experimental methods

Subjects and diets

Full details of design, procedures, statistical methods and composition of the test meals have been reported previously (Frape *et al.* 1998). Twenty-four middle-aged non-smoking healthy Caucasian men aged 35–65 years (Table 1) were given low-fat (L; composed of 86 g corn

Table 1. Characteristics of the twenty-four male subjects on day 1
(Values are means and their standard deviations for twelve subjects)

Treatment group…	L		M	
	Mean	SD	Mean	SD
Age (years)	48·1	8·2	50·6	8·0
Weight (kg)	79·7	6·5	80·3	7·9
BMI (kg/m²)	26·8	2·7	25·2	2·5
Waist:hip circumference	0·900	0·038	0·901	0·053
Fasting blood values				
Insulin (mU/l)	6·55	6·43*	8·65	3·78*
Glucose (mmol/l)	5·10	0·58	4·98	0·42
TAG (mmol/l)	1·30	1·17	1·32	0·73
NEFA (mmol/l)	0·47	0·31	0·41	0·11
Total cholesterol (mmol/l)	5·69	1·41	5·85	1·08
LDL-cholesterol (mmol/l)	3·83	1·17	3·97	0·80
Areas under the curve during OGTT				
Glucose (mmol/l.min)	824·8	195	739·3	99
TAG (mmol/l.min)	158·2	132	160·1	87
NEFA (mmol/l.min)	24·6	17	23·0	13

L, low-fat breakfast; M, moderate-fat breakfast; TAG, triacylglycerols; NEFA, non-esterified fatty acids; OGTT, oral glucose tolerance test.
* Median and interquartile range.

flakes + 300 ml semi-skimmed milk + 250 ml orange juice, providing 5·5 g fat and 113 g carbohydrate), or moderately fatty (M; a pastie weighing 86 g, composed of a lean meat–vegetable mixture in a pastry covering, in which the fat was predominately butter, individually prepared by Country & Colonial, 18300 Ménétréol-sous-Sancerre, France and providing 26 g fat and 56 g carbohydrate) isoenergetic breakfasts for 28 d with control over the other two daily meals (Frape *et al.* 1998). The fatty acid composition of the two breakfast menus (Table 2) was determined by a GC method of Medina *et al.* (1992).

There were twelve pairs of men matched according to the characteristic: waist:hip circumference × fasting LDL-cholesterol. The two dietary treatments were allocated at random within pairs. The treatment breakfasts were each taken with a cup of tea or coffee on days 2–28 at home before 09.00 hours. Tea and coffee were not given on day 29. All subjects were provided with a low-fat cereal evening meal that was eaten exclusively at 19.00 hours on days 0 and 28. Subjects were then fasted until 09.00 hours on days 1 and 29. On days 2–28, some freedom was given in the composition of the two other daily meals taken at midday and in the evening. The content of these meals was recorded daily by each subject in a diary. High-fat foods were avoided, fish was excluded and all meals were taken at home. No subject received prescription drug treatment or took any drug containing salicylic acid or sodium salicylate during the course of the study.

On day 1 fasting blood characteristics were measured and all subjects were given an oral glucose tolerance test (OGTT) at 09.00 hours. The glucose load was 66 g glucose monohydrate (60 g glucose) in approximately 400 ml water. On day 29 each subject received their previously allocated L or M breakfast at 09.00 hours, followed at 13.30 hours by an OGTT. Water was available for drinking on both days 1 and 29. All test meals and metabolic measurements were carried out in Papworth Hospital and were synchronized within pairs on days 1 and 29.

Body weight, height and waist and hip circumferences

Table 2. Fatty acid compositions (μg/100 μg total fatty acids) of the breakfast meals and the fasting plasma triacylglycerols (TAG) and non-esterified fatty acids (NEFA) for healthy middle-aged men on day 29 of the study*†

(Plasma values are means with their standard errors for twelve subjects)

| | Meal | | TAG | | | | NEFA | | | |
| | | | L | | M | | L | | M | |
	L	M	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
14:0	8.7	9.1	2.0	0.23	2.3	0.21	3.0	0.29	2.7	0.24
16:0	24.4	25.1	30.6	0.82	28.6	1.01	32.7	1.09	30.9	0.88
16:1	2.3	3.1	3.2	0.27	3.8	0.38	2.4	0.20	3.4	0.48
17:0	0	1.3	0.4	0.10	0.4	0.08	0.8	0.09	0.8	0.06
18:0	9.6	12.2	5.1	0.58	6.1	1.12	16.5	1.09	16.0	1.01
18:1	29.6	29.3	42.8	1.56	42.2	1.93	31.9	1.50	33.4	1.52
18:2	12.2	5.3	14.7	1.26	15.2	1.39	12.1	1.12	12.2	1.05
20:4	0	0.3	0.7	0.11	0.9	0.18	0.5	0.16	0.4	0.09
22:6	0	0.1	0.4	0.09	0.4	0.04	0.1	0.04	0.1	0.05

L, low-fat breakfast; M, moderate-fat breakfast.
* For details of subjects and procedures, see Table 1 and p. 738.
† There were no significant differences between treatments for any fatty acid: $P > 0.05$.

(measured at the levels of the umbilicus and the greater trochanter of the femur respectively) were recorded in fasting subjects by the same nurse at the same time of day during the medical examination on days 1 and 29. Waist:hip circumference varied from 0.80 to 1.05. Comparisons between treatments were made over the entire response period, but particularly 12.00 hours on day 29, at which time it was previously found (Frape *et al.* 1998) that total plasma NEFA values differed between treatments to the greatest extent. The null hypothesis was that the fatty acid profile of the NEFA would be the same for the two treatments, as the dietary fats had a similar fatty acid composition (Table 2). Blood was sampled repeatedly following both the OGTT and meals. The procedures for the withdrawal and preparation of blood samples, and the measurement of total NEFA content of the samples have been described previously by Frape *et al.* (1997).

Analytical procedure

The plasma samples for the fatty acid analyses were held at −70°C before analysis. The fatty acid composition (μg/100 μg) of the NEFA and fasting TAG samples was determined by GC after preparative TLC (one subject per plate) of the plasma lipids extracted by a modification of the procedure of Bligh & Dyer (1959). All reagents were analytical grade; in addition, chloroform and diethyl ether were further purified by redistillation. Water was glass distilled. Analytical processing was carried out in Decon 90 (BDH, Poole, Dorset, UK)-cleaned chloroform-rinsed glass tubes. TLC plates were cleaned by pre-elution with solvent mixture of the composition to be used for chromatography, then air-dried at room temperature before application of samples. Plasma (200 μl) was added to 4 ml saline (0.9 % NaCl, w/v), which was then acidified (using a few drops of 1 M-HCl) to pH 4.0–4.5. Methanol (10 ml) and chloroform (5 ml) were added, and the mixture was mixed thoroughly. A further 5 ml saline and 5 ml chloroform were then added, vortexed and centrifuged (1500 rpm, 4°C, 10 min).

The upper aqueous layer was removed by aspiration and the lower chloroform layer containing the lipids was collected and evaporated to dryness under a gentle stream of O_2-free N_2. The dry sample was redissolved in a small volume of chloroform (approximately 100 μl) for TLC using Whatman TLC plates (20 cm × 20 cm × 1000 μm, Type code PK6F silica gel 60A°; Whatman International Ltd., Maidstone, Kent, UK). The plates were developed in hexane–chloroform–diethyl ether–glacial acetic acid (120:15:15:1.5, by vol.) The NEFA and TAG bands (R_f values of 0.28 and 0.45 respectively) were identified in u.v. light and scraped into glass tubes. The samples were extracted with chloroform, evaporated under Ar and stored at −70°C in screw-capped vials. The TAG were hydrolysed overnight under N_2 in the dark at room temperature, using 3 ml of a solution containing 1 g KOH, 10 ml diethyl ether, 10 ml methanol, 2 ml water. Water (1 ml) was added and the samples were acidified with 3 M-HCl and extracted twice with 3 ml diethyl ether. The diethyl ether extracts were washed with water, and evaporated under a stream of N_2. The samples were redissolved in 2 ml dichloromethane and transferred to screw-capped vials, in which they were stored under Ar at −70°C. Before derivatization the samples were evaporated to dryness under a gentle stream of N_2. A few drops of methanol were then added to both the NEFA and the TAG hydrolysate samples, followed by 1 ml freshly prepared diazomethane (solution in diethyl ether) to convert the fatty acids to their methyl esters. After 10 min the excess diazomethane was evaporated under a gentle stream of N_2 and the fatty acid methyl esters redissolved in hexane and stored at −20°C under Ar before GC analysis. GC was carried using a Vega 6130 gas chromatograph (Carlo Erba Instruments, Milan, Italy), with a 30 m DB-1 fused silica capillary column (i.d. 0.32 mm and film thickness 0.1 μm; J&W Scientific, Folsom, CA, USA). The temperature was programmed to increase from 50 to 120°C at 10°C/min, from 120 to 200°C at 4°C/min, from 200 to 280°C at 3°C/min, and then at 280°C for 10 min. H_2 carrier gas was used at an inlet pressure of 80 kPa. A 2 μl portion of each sample was injected using an AS800 autosampler (Carlo Erba Instruments) via a split–splitless injector operated in the splitless mode. Detection was by a flame-ionization detector, and the output recorded using a DP800 integrator (Carlo Erba Instruments). The injector and detector temperatures were 270 and 280°C respectively.

Identification of peaks was achieved by GC retention times with reference to authentic standards, and by GC–MS. The retention times, areas and peak heights of the predominant fatty acid peaks were recorded. Each peak was expressed both as a percentage (μg/100 μg) of the total area and height of all the predominant and identified peaks, and in μg/l plasma, for two blood sampling times on day 1 and five sampling times on day 29.

Statistical analysis

Responses for all repeated measurement variables were summarized at individual subject level. Variables not normally distributed were log$_e$-transformed towards normality. The responses from fasting of total saturated and

monounsaturated acids, and 16 : 0/18 : 1, were analysed separately by ANOVA. Time means for individual fatty acids were compared by *t* test, where ANOVA indicated a significant treatment effect. Areas under the curve (AUC) and incremental areas from the baseline were calculated by applying the trapezium rule to time points (Altman, 1991). Statistical significance was accepted at $P < 0.05$. Standard errors of the mean indicate the variation within treatment. The Pearson correlation coefficient was employed to assess associations. All comparisons were carried out with SPSS for Windows version 9.0 (SPSS Inc., Chicago, IL, USA) and, where appropriate, by Microsoft Excel 97 for Windows software (Microsoft Corporation, Seattle, WA, USA).

Ethical considerations

The protocol for the experiment was approved by the Huntingdon Area Medical Ethics Committee, and all subjects gave informed written consent to participate. The investigations were carried out in accordance with the principles of the Declaration of Helsinki.

Results

Fatty acid comparisons

We compared long-chain saturated (C_{14}, C_{16}, C_{17} and C_{18}) and monounsaturated (C_{16} and C_{18}) fatty acids, constituting 89·9 % of the total measured fatty acids of plasma NEFA during the OGTT and during the postprandial period following breakfast. All plasma values given in tables and figures represent the means of twelve subjects. The fatty acid compositions of the two breakfast meals given at 09.00 hours on days 1–29 were very similar, and are shown in Table 2. Neither the fatty acid composition of the fasting plasma NEFA nor that of the fasting plasma TAG measured either as µg/100 µg total fatty acids or as µg/l plasma, differed significantly between treatments on day 29 (Table 2; µg/l plasma values are not shown).

We have demonstrated previously (Frape *et al.* 1998) that the difference between treatments L and M on day 29 with regard to total NEFA content of plasma reached a maximum at 12.00 hours, 3 h following the meal. Furthermore, over the period 09.00–13.20 hours, the NEFA AUC with treatment L was only 59 % of that with treatment M. In the present study, long-chain saturated fatty acids were examined separately from monounsaturated fatty acids. ANOVA indicated significant treatment differences over the postprandial period following breakfast on day 29 for total saturated ($P = 0.027$) and total monounsaturated ($P = 0.0026$) fatty acids expressed as µg/100 µg NEFA. The responses at 10.00 hours did not differ significantly from those at 12.00 hours. The values for individual fatty acids at 12.00 hours are given in Fig. 1.

The insulin responses during the OGTT on days 1 and 29 were considerable with both treatments (see p. 741) and so differences were not expected in the associated responses of any fatty acid to the OGTT over 120 min. This was the case and so the values for the two treatments and for days 1 and 29 were combined (Table 3). The proportion of the saturated fatty acids increased and that of monounsaturated fatty acids decreased following the oral glucose dose ($P < 0.002$). This response was in accordance with that observed 1 h after consumption of the breakfast meal for both treatments (Table 4; Fig. 2 for monounsaturated fatty acids). Saturated fatty acids gave a fractional response which was approximately 1−x (data not shown) of the monounsaturated fatty acid response (x). At 1 h after the meal the fatty acid composition of the NEFA for the two treatments, L and M, diverged. At 12.00 hours, 3 h after the treatment L meal, the proportions of 16 : 0 and 17 : 0 were greater ($P = 0.026$ and 0·0055 respectively) and that of 18 : 1 was lower ($P = 0.0027$) than those for treatment M (Fig. 1).
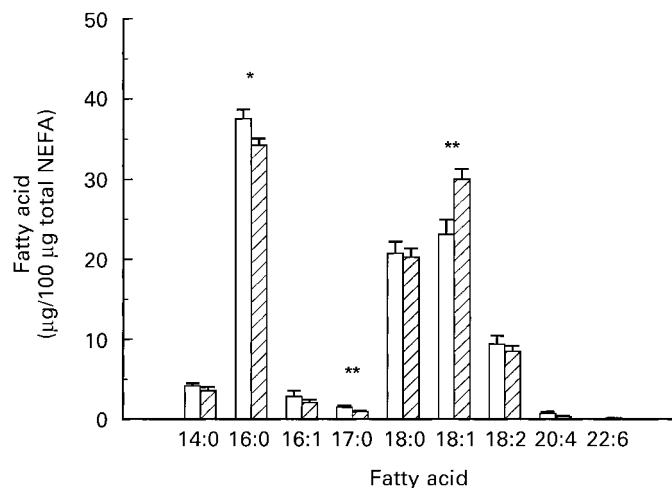

Fig. 1. Non-esterified fatty acid (NEFA) composition of plasma (µg/ 100 µg total NEFA) in healthy middle-aged men at 12.00 hours, 3 h after consumption of either a low-fat (L; □) or moderate-fat (M; ▨) breakfast meal on day 29. For details of the breakfast meals and procedures, see Table 2 and p. 738. Values are means with their standard errors represented by vertical bars for twelve subjects. Mean values were significantly different between treatments: *$P < 0.05$, **$P < 0.01$.

Table 3. Plasma non-esterified fatty acids (μg/100 μg total non-esterified fatty acids) in healthy middle-aged men before and 120 min after a 60 g oral glucose dose*

(Values are means with their standard errors for twenty-four subjects)

Fatty acid	Pre-dose†		Post-dose†		Statistical significance of difference between means: P
	Mean	SEM	Mean	SEM	
14:0	3·27	0.17	4·46	0·34	<0·0001
16:0	32·16	0·53	35·40	0·85	<0·0001
16:1	2·63	0·23	2·16	0·16	0·002
17:0	0·76	0·07	1·20	0·13	0·0002
18:0	14·82	0·79	20·03	0·90	<0·0001
18:1	33·49	1·03	25·04	1·04	<0·0001
18:2	12·27	0·83	10·82	0·98	0·054
20:4	0·35	0·05	0·67	0·18	0·04

* For details of procedures, see p. 738.
† Values are pooled from both treatment groups and from days 1 and 29 as individual values did not differ significantly.

Insulin response

Before treatment allocation there was no significant difference in the plasma insulin response to an oral glucose dose on day 1. However, there were significant differences between treatments in the plasma insulin response to the breakfast (incremental $\log_e$ AUC, $P = 0.051$) and to the oral glucose dose ($\log_e$ AUC, $P = 0.038$) on day 29. On day 29, comparisons between treatments at 10.00 ($P = 0.014$), 14.30 ($P = 0.062$) and 15.00 ($P = 0.024$) hours indicated differences in insulin response ($\log_e$ mU/l) (Fig. 3).

16:0/18:1 response

From fasting to 13.30 hours on day 29, the 16:0/18:1 plasma values indicated a difference in response between treatments ($P < 0.01$). From fasting to 3 h after the breakfast, this ratio increased from 1·06 (SE 0·084) to 1·78 (SE 0·214) for treatment L (a 67 % increase), but it increased by only 23 % from 0·95 (SE 0·064) to 1·17 (SE 0·068) for treatment M (Fig. 4). Adipose tissue fat has been reported to have a mean 16:0/18:1 of 0·49 (range 0·46–0·56; Heffernan, 1963; Brook, 1971; Kokatnur et al. 1979; Pittet et al. 1979; Malcom et al. 1989), compared with the corresponding values for the fat in meals of 0·83 for L and 0·86 for M. The plasma ratio for treatment M was closer to

that for adipose tissue fat and less than that for treatment L at 12.00 hours ($P = 0.012$). At 13.30 hours serum insulin had returned to fasting concentrations, at which time the saturated and monounsaturated fatty acid profiles were very similar between treatments. The subsequent increases in 16:0/18:0 during the OGTT were from 1·00 (SE 0·037) to 1·62 (SE 0·175) for treatment L and from 1·03 (SE 0·046) to 1·66 (SE 0·151) for treatment M. These increases were similar to those for treatment L after the breakfast (Fig. 4).

Associations between 16:0 and insulin and between 16:0 and waist:hip circumference

16:0 represented a major fraction of the NEFA, especially during the insulin response to digestible carbohydrate. It was expected that its concentration (μg/100 μg total NEFA) would be negatively correlated, especially within treatment M, to insulin AUC response after the breakfast meal. 16:0 was correlated with the contemporary plasma $\log_e$ insulin AUC (r −0·47, $P = 0.02$) at 11.00 hours on day 1, before treatment allocation, during the OGTT. At 12.00 hours on day 29, following the meal, the correlation of plasma 16:0 with the contemporary $\log_e$ insulin AUC was stronger within treatment M (r −0·91, $P = 0.0001$) than within treatment L (r −0·52, $P = 0.08$). For times 10.00 and 12.00 hours combined, the corresponding values

Table 4. Plasma non-esterified fatty acids (μg/100 μg total non-esterified fatty acids) in healthy middle-aged men immediately before (09.00 hours) and 1 h after (10.00 hours) consumption of a low-fat (L) or moderate-fat (M) breakfast meal on day 29*

(Values are means with their standard errors for twelve subjects)

Treatment group…	L				Statistical significance of difference between means: P=	M				Statistical significance of difference between means: P=
	09.00 hours		10.00 hours			09.00 hours		10.00 hours		
	Mean	SEM	Mean	SEM		Mean	SEM	Mean	SEM	
14:0	2·97	0·29	4·14	0·34	0·008	2·69	0·24	3·60	0·27	0·003
16:0	32·69	1·09	36·23	1·61	0·013	30·93	0·88	33·59	0·94	0·018
16:1	2·43	0·20	1·94	0·18	0·040	3·40	0·48	2·37	0·24	0·007
17:0	0·84	0·09	1·11	0·20	0·130	0·81	0·06	1·05	0·09	0·010
18:0	16·52	1·09	21·21	1·36	0·0003	16·01	1·01	19·28	1·31	0·058
18:1	31·89	1·50	24·76	1·82	0·0003	33·4	1·52	29·17	1·50	0·022
18:2	12·10	1·12	9·84	1·47	0·027	12·21	1·05	10·02	0·80	0·017
20:4	0·48	0·16	0·61	0·14	0·320	0·43	0·09	0·66	0·13	0·120

* For details of breakfast meals and procedures, see Table 2 and p. 738.

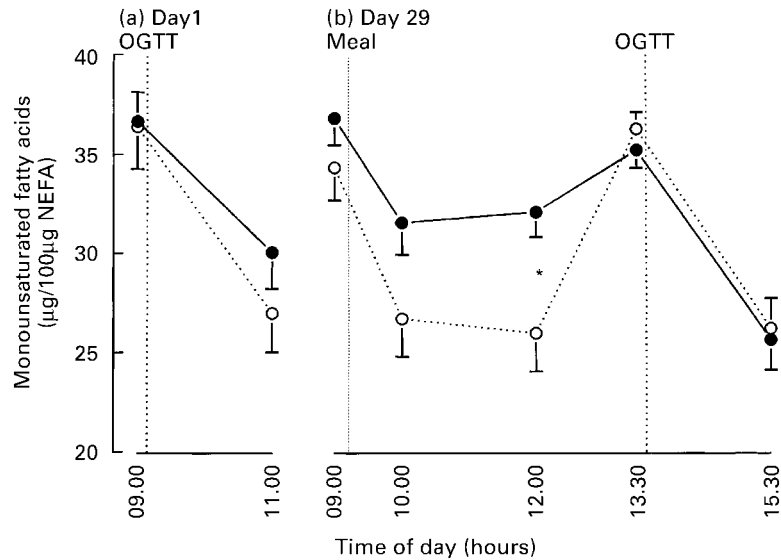**Fig. 2.** Monounsaturated $(16:1+18:1)$ non-esterified fatty acid (NEFA) composition of plasma (μg/100 μg total NEFA) in healthy middle-aged men before (day 1; a) and after (day 29; b) consuming a low-fat (L; $\bigcirc$) or moderate-fat (M; $\bullet$) breakfast meal for 28 d. Fasting fatty acid compositions are shown at 09.00 hours on both days. An oral glucose dose was given at 09·00 hours on day 1 and at 13.30 hours on day 29. The breakfast meal was consumed at 09.00 hours on day 29. For details of the breakfast meals and procedures, see Table 2 and p. 738. Values are means with their standard errors represented by vertical bars for twelve subjects. The change in plasma monounsaturated fatty acid compositions from fasting to 13.30 hours on day 29 was significantly different between treatments ($P = 0.0026$). Mean values were significantly different between treatments within times: *$P < 0.05$. Saturated fatty acids ($C_{14} + C_{16} + C_{17} + C_{18}$) gave a fractional response which was approximately $1-x$ (data not shown) of the monounsaturated fatty acids response (x). OGTT, oral glucose tolerance test.

were $r\ -0.79$, $P = 0.002$ and $r\ -0.57$, $P = 0.051$ for treatments M and L respectively.

Intra-abdominal fat is subject to a high flux (Arner, 1995). A high waist:hip circumference should therefore be associated with a relatively higher proportion of $18:1$ and a lower proportion of $16:0$ (μg/100 μg plasma total NEFA), especially when plasma insulin is at post-absorptive concentrations (Arner, 1995). Indeed, we found that fasting $16:0$ on day 1 was correlated with waist:hip circumference on day 1, $r\ -0.63$, $P = 0.0009$ (principally an effect of differences in waist measurement; $r\ -0.57$, $P = 0.003$), whereas the corresponding values for $18:1$ were $r\ 0.43$, $P = 0.04$.

Discussion

The saturated and monounsaturated fatty acid composition of NEFA is the result of: (1) different individual mobilization rates of fatty acids by HSL (Connor *et al.* 1996); (2) the composition and extent of leak-back of fatty acids from lipoprotein lipase activity (Fielding & Frayn, 1998); (3) differential clearance rates, (not differentiated in our data). Nevertheless, our two diets had very similar fatty acid compositions, and plasma TAG concentrations, of low chylomicron content, did not differ significantly between treatments throughout day 29 (Frape *et al.*1998). Leak-back composition should not have been very different between treatments, and the composition of fasting plasma TAG and

NEFA, and the fasting insulin were all very similar between treatments (Table 2 and Fig. 3). Turn-over rates of isotopically-labelled $16:0$ and $18:1$ were found to be similar (Friderickson & Gordon 1958*a,b*), as were the rates of $^{13}CO_2$ recovery in breath of absorbed labelled $16:0$ and $18:1$ (Jones *et al.* 1999). Thus, disposal rates of these two predominant circulating fatty acids are similar. Although an influence in our data from leak-back and clearance cannot be excluded, a large difference in the tendency of individual fatty acids to be mobilized has been reported (see p. 742).

Mobilization of tissue fatty acids

HSL activity is inhibited in healthy young subjects when plasma insulin concentration exceeds 13–16 mU/l (Frayn *et al.* 1997). Nevertheless, there is a dose–response relationship between serum insulin concentration and the postprandial appearance of plasma fatty acids, a relationship that is more pronounced in middle-aged and obese subjects. A small increment in plasma insulin concentration caused a marked suppression of palmitate ($16:0$) flux in lean women, but the flux remained substantial in obese women despite greater plasma insulin concentrations (Jensen *et al.* 1989). Moreover, the rate of lipolysis of stored fat and the rate of appearance of plasma fatty acids depend on the plasma glucose and insulin concentrations, but the relationship between insulin sensitivity and plasma
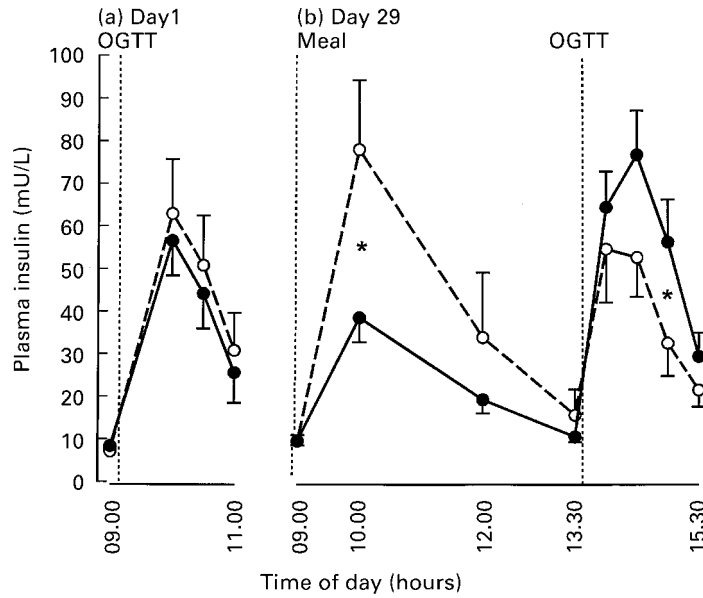
Fig. 3. Plasma insulin concentration (mU/l) in healthy middle-aged men before (day 1; a) and after (day 29; b) consuming a low-fat (L; ○) or moderate-fat (M; ●) breakfast meal for 28 d. Fasting insulin levels are shown at 09.00 hours on both days. An oral glucose dose was given at 09.00 hours on day 1 and at 13.30 hours on day 29. The breakfast meal was consumed at 09.00 hours on day 29. For details of the breakfast meals and procedures, see Table 2 and p. 738. Values are means with their standard errors represented by vertical bars for twelve subjects. ANOVA of $\log_e$ transformed values showed significant differences between treatments on day 29 from 09.00 to 13.30 hours and from 13.30 to 15.30 hours ($P = 0.05$). Mean values were significantly different between treatments within times: $*P < 0.05$. OGTT, oral glucose tolerance test.
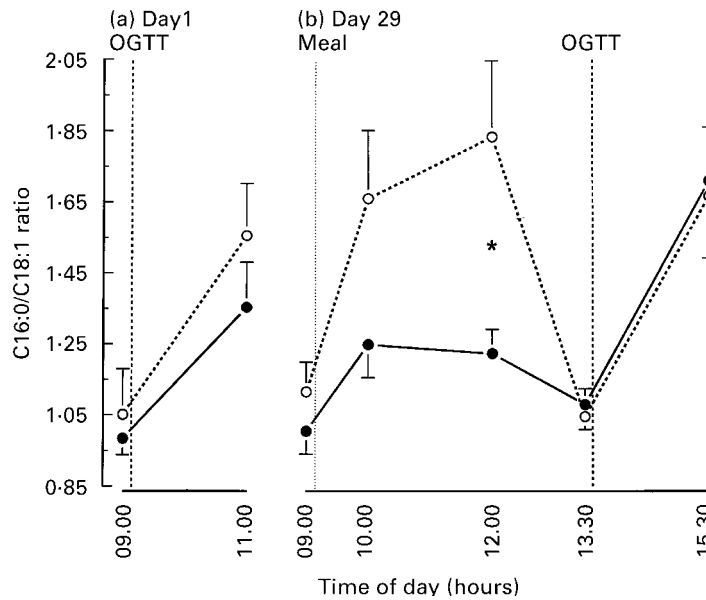

Fig. 4. Plasma non-esterified fatty acid ratios, 16 : 0/18 : 0 ($\mu g/\mu g$), in healthy middle-aged men before (day 1; a) and after (day 29; b) consuming a low-fat (L; ○) or moderate-fat (M; ●) breakfast meal for 28 d. Fasting ratios are shown at 09.00 hours on both days. An oral glucose dose was given at 09.00 hours on day 1 and at 13.30 hours on day 29. The breakfast meal was consumed at 09.00 hours on day 29. For details of the breakfast meals and procedures, see Table 2 and p. 738. Values are means with their standard errors represented by vertical bars for twelve subjects. ANOVA showed significant differences between treatments on day 29 from 09.00 to 13.30 hours ($P < 0.01$). Mean values were significantly different between treatments within times: $*P < 0.05$. OGTT, oral glucose tolerance test.

NEFA concentration is more pronounced during the postprandial period than during fasting (Ferrannini *et al.* 1997). At plasma glucose and insulin concentrations of 7·2 mmol/l and 20–25 mU/l respectively, both lipolysis and the rate of appearance of plasma fatty acids were suppressed. However, when plasma glucose and insulin were held at 10·5 mmol/l and 40 mU/l respectively, the rate of appearance of plasma fatty acids was further suppressed (Wolfe & Peters, 1987). Thus, postprandial intracellular fat mobilization rate depends on plasma insulin concentration and is suppressed to a lesser extent in obese and older subjects than in lean and younger subjects.

During the first 90 min of the OGTT the serum insulin exceeded 35 mU/l with both treatment groups on days 1 and 29. This response was associated, proportionately and between treatments, with similar increases in saturated fatty acids and decreases in monounsaturated fatty acids in the NEFA ($P < 0.002$). However, following consumption of the breakfast meals, the insulin response was elevated in both treatment groups, but was significantly higher for treatment L ($P = 0.05$). Over this meal period the fatty acids 16:0 and 18:1 contributed over 60 μg/100 μg total measured plasma NEFA. 18:1 was significantly higher ($P < 0.01$), whereas 16:0 was significantly lower ($P < 0.05$) for treatment M than treatment L (Fig. 1).

The mobilization of fatty acids by HSL in adipose tissue of laboratory animals is positively correlated with unsaturation (Raclot & Groscolas, 1993; Connor *et al.* 1996). This observation is consistent with our evidence that a meal of carbohydrate should be closely associated with a decrease in the relative proportions of monounsaturated fatty acids and an increase in the relative proportions of saturated fatty acids in total NEFA.

As the proportions of saturated fatty acids relative to monounsaturated fatty acids in the NEFA differed between treatments following breakfast at 12.00 hours but not in the fasting plasma, an explanation was sought. Circulating 17:0 fatty acid was measured, as odd-numbered -C fatty acids are not synthesized endogenously and must be of dietary origin. Indeed, it has been proposed that 17:0 in subcutaneous adipose tissue (Wolk *et al.* 1998) and 15:0 in serum (Smedman *et al.* 1999) might be valid biological markers of long-term milk-fat intake. 17:0 was not detectable either in the evening meal for both treatment groups on day 28 or in the breakfast for treatment L on day 29. By contrast, the proportion of 17:0 in the NEFA was greater for treatment L than for treatment M at 12.00 hours on day 29 ($P = 0.0055$). This proportional response was consistent with that of other saturates, indicating suppression of intracellular fat mobilization. Consumption of 17:0 during days 2–28 was assessed blind from the individual food diaries. Dietary 17:0 was found to be positively correlated, within treatment, to plasma 17:0 (μg/100 μg NEFA) when plasma insulin was at post-absorptive concentrations on day 29 (r 0·46, $P < 0.05$), indicating tissue storage as its source. These data support a conclusion that the raised total NEFA content of treatment M plasma 3 h after the meal was derived to a measurable extent from fatty acids mobilized in storage tissues.

Associations amongst response characteristics

Arner (1995) observed that the rate of lipolysis by HSL is low in the subcutaneous femoral and gluteal regions, intermediate in the subcutaneous abdominal region and high in the visceral (omental) region, and Coon *et al.* (1992) found a correlation between waist:hip circumference and the rate of decline of plasma NEFA concentration during euglycaemic hyperinsulinaemic clamping. Byrne *et al.* (1998) reported that fasting insulin levels and waist:hip circumference predicted percentage NEFA suppression in men. The negative correlations in the present study between fasting 16:0 and waist:hip circumference ($P = 0.0009$), and between 16:0 and contemporary $\log_e$ insulin AUC ($P = 0.0001$) are consistent with this evidence, but not proof of cause and effect. A raised proportion of NEFA as 16:0 may reflect a suppression of endogenous fat mobilization. The mobilization of omental fat is resistant to suppression by raised postprandial plasma insulin, especially in subjects with a high waist:hip circumference.

Conclusions

The net outward flux of NEFA was greater, and its profile different, during the 1–3 h postprandial period for treatment M than for treatment L. This difference occurred at a time when plasma insulin concentration was lower for treatment M, but which greatly exceeded 13–16 mU/l in both groups. Thus, an adverse effect of a fatty meal on glucose tolerance and insulin function may be expressed by its failure to adequately inhibit intracellular fat mobilization.

It is concluded that a substantial rise in postprandial plasma insulin concentration is associated with a rise in the proportion of saturated fatty acids and a decrease in the proportion of monounsaturated fatty acids in plasma NEFA. This difference does not persist following a fatty meal amongst middle-aged men. It is proposed that the subjects who received a breakfast providing 26, rather than 5 g fat, especially those with an excessive waist:hip circumference, may have been less able to suppress postprandial intracellular fat mobilization. They appear to have expressed insulin resistance, notably by a strong negative correlation between the postprandial plasma concentrations of the NEFA 16:0 and insulin. Insulin resistance and an excessive waist:hip circumference appear to be associated with elevated postprandial plasma NEFA that is relatively rich in monounsaturated fatty acids. These observations are in accordance with recent conclusions from a cross-sectional survey by Han *et al.* (1999) that type 2 diabetes is strongly associated with a high waist:hip circumference. Further investigation is required to clearly differentiate between rates of mobilization and clearance of postprandial NEFA in subjects receiving isoenergetic fatty and carbohydrate meals.

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