Acute effects of meal fatty acid composition on insulin sensitivity in healthy post-menopausal women

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Postprandial plasma insulin concentrations after a single high-fat meal may be modified by the presence of specific fatty acids although the effects of sequential meal ingestion are unknown. The aim of the present study was to examine the effects of altering the fatty acid composition in a single mixed fat–carbohydrate meal on glucose metabolism and insulin sensitivity of a second meal eaten 5 h later. Insulin sensitivity was assessed using a minimal model approach. Ten healthy post-menopausal women underwent four two-meal studies in random order. A high-fat breakfast (40 g fat) where the fatty acid composition was predominantly saturated fatty acids (SFA), *n*-6 polyunsaturated fatty acids (PUFA), long-chain *n*-3 PUFA or monounsaturated fatty acids (MUFA) was followed 5 h later by a low-fat, high-carbohydrate lunch (5·7 g fat), which was identical in all four studies. The plasma insulin response was significantly higher following the SFA meal than the other meals after both breakfast and lunch ($P < 0.006$) although there was no effect of breakfast fatty acid composition on plasma glucose concentrations. Postprandial insulin sensitivity ($S_{I(Oral)}$) was assessed for 180 min after each meal. $S_{I(Oral)}$ was significantly lower after lunch than after breakfast for all four test meals ($P = 0.019$) following the same rank order (SFA $<$ *n*-6 PUFA $<$ *n*-3 PUFA $<$ MUFA) for each meal. The present study demonstrates that a single meal rich in SFA reduces postprandial insulin sensitivity with 'carry-over' effects for the next meal.

Fatty acids: Postprandial metabolism: Insulin sensitivity: Post-menopausal women

Insulin insensitivity has been postulated to be the underlying factor linking type 2 diabetes, hypertension and cardiovascular disease (Reaven, 1988). Epidemiological evidence now suggests that a high intake of dietary fat is associated with impaired insulin sensitivity (S_I), which may be modulated by the type of fatty acids in the diet (Vessby, 2000). It has also been shown that postprandial plasma insulin concentrations after a single meal may be modified by the presence of specific fatty acids. However, results have been conflicting. Rasmussen *et al.* (1996) showed that insulin release was stimulated by butter but not by olive oil (rich in monounsaturated fatty acids; MUFA) in patients with type 2 diabetes, whereas in healthy subjects, mixed meals containing MUFA have given higher postprandial plasma insulin and glucose concentrations compared with meals rich in polyunsaturated fatty acids (PUFA) (Joannic *et al.* 1997; Pedersen *et al.* 1999). Other studies, however, have found no effect of meal

fatty acid composition on postprandial insulin or glucose levels when given as part of a mixed meal either in the morning (Thomsen *et al.* 1999) or evening (Zampelas *et al.* 1994). We have emphasized the need to study the effects of sequential meals on postprandial metabolism since this reflects the more usual metabolic state in Westernized societies (Fielding *et al.* 1996; Evans *et al.* 1998). It has already been demonstrated that following sequential meal ingestion, chylomicrons are released rapidly into the circulation following the second meal. Ercan *et al.* (1994) have used a sequential meal protocol to show that plasma glucose and insulin areas under the curve (AUC) were higher in response to a second meal irrespective of the fat content of the first meal. These findings are in contrast with those of Frape *et al.* (1997a,b, 1998), who demonstrated clearly that lunch-time glucose tolerance was impaired by a fatty breakfast. However, neither of these studies addressed the potential influence of breakfast

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fatty acid composition, which may be important as part of the 'second-meal' effect.

The aim of the present study was to examine whether altering the fatty acid composition in a single mixed fat–carbohydrate meal had 'carry-over' effects on glucose metabolism and S_I assessed using the minimal model approach (Caumo *et al.* 2000) for a second meal eaten 5 h later. Other data from the present study on aspects of lipid metabolism have been published previously (Jackson *et al.* 2002; Robertson *et al.* 2002*a*).

Subjects and methods

Subjects

Ten healthy post-menopausal women (mean age 56 (range 50–63) years; mean BMI 25·0 (range 20·6–32·0) kg/m^2) were studied on four occasions. All subjects satisfied the following inclusion criteria: they were weight-stable and were not currently taking fatty acid supplements or medication likely to affect either gastrointestinal motility or lipid metabolism (including hormone replacement therapy). They had no previous history of hyperlipidaemia, gastrointestinal or endocrine disease. Subjects were recruited following screening for fasting blood lipid and glucose levels, which were all within normal limits (triacylglycerol, mean 1·2 (range 0·7–1·5) mmol/l; total cholesterol, mean 5·7 (range 4·4–6·5) mmol/l; glucose, mean 5·1 (range 4·5–5·8) mmol/l). Written informed consent was obtained from all subjects and the study was approved by the University of Reading and Central Oxford Research Ethics Committees.

Study design

The experiment was set up as a single-blind, randomized study with four separate postprandial study days occurring at least 1 month apart. Two carbohydrate-rich meals were provided 5 h apart, the first meal high in fat (41 g) and the second meal low in fat (6 g). The fatty acid composition of the high-fat first meal was varied on each visit. Postprandial S_I following each meal was assessed using a minimal model approach (Caumo *et al.* 2000).

Study-day procedures

All studies were performed following a standardized low-fat evening meal (< 10 g fat) and an overnight fast. In the morning, an indwelling intravenous cannula was inserted into an antecubital vein under local anaesthetic (1 % lignocaine). At time zero, subjects were given one of four test meals (Table 1) consisting of Rice Krispies, banana and a drink containing 40 g test oil (Table 2). The test oils were chosen to provide meals enriched with either saturated fatty acids (SFA; palm oil), *n*-6 PUFA (safflower-seed oil; Anglia Oils Ltd, Kingson upon Hull, UK), long-chain *n*-3 PUFA (EPAX 3000TG; Pronova Biocare, Aaslund, Norway. The EPAX 3000TG was supplied under N$_2$ with a new bottle used for each study to minimize oxidation) or MUFA (olive oil; Tesco, Cheshunt, Herts., UK). The EPAX 3000TG fish oil was diluted (1:1) with safflower-seed oil in order to reduce the proportion of SFA in the *n*-3 PUFA test meal and to improve the palatability of the dietary fish oil. Fasting and postprandial blood samples were taken at regular intervals for 300 min. At 300 min, a second meal (low-fat, high-carbohydrate) was consumed by the subjects. This meal was identical on all four visits. Blood samples were taken at 10, 20, 30, 60, 120 and 180 min after the second meal. Between meals subjects did not consume any other food other than water, which was provided *ad libitum*. The test meals were well tolerated by all subjects.

Blood analyses

Whole blood for metabolite and insulin determination was collected into heparinized syringes (Sarstedt, Leicester, Leics., UK and L.I.P., Shipley, W. Yorks, UK). Plasma glucose (Instrumentation Laboratory, Warrington, Ches., UK) and non-esterified fatty acid (NEFA) concentrations (Alpha Laboratories, Eastleigh, Hants, UK) were measured enzymically using an Instrumentation Laboratory Monarch automated analyser. Metabolites were batch-analysed and exhibited an intra-assay variation of less than 2·5 %. Insulin was measured by radioimmunoassay using a commercially available kit (Pharmacia & Upjohn, Milton Keynes,

Table 1. Macronutrient composition of test meals*

	Carbohydrate (g)	Fat (g)	Protein (g)	Energy (kJ)
Breakfast				
Test oil (40 g)	0	40	0	1478
Skimmed milk (250 g)	12·5	0·3	8·3	350
Marvel (10 g)	7·8	0·2	5·3	228
Nesquik (10 g)	8·0	0·3	0·3	152
Rice Krispies (30 g)	26·9	0·3	1·8	472
Banana (100 g)	23·2	0·3	1·2	403
Total	78·4	41·4	16·9	3083
Lunch				
Cheese pizza (200 g)	66·2	5·4	20·0	1670
Lettuce (30 g)	0·5	0·2	0·3	18
Cucumber (20 g)	0·3	0	0·1	8
Tomatoes (45 g)	1·4	0·1	0·3	33
Total	68·4	5·7	20·7	1729

* Determined from food tables and manufacturers' data.

Table 2. Fatty acid composition (g/100 g fatty acids) of breakfast*

Mixed-meal enriched with...	n-6 PUFA	n-3 PUFA	MUFA	SFA
SFA	11	19	17	50‖
MUFA	15	20	72§	40
n-6 PUFA	74†	39	11	10
n-3 PUFA	0	22‡	0	0

PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids.
* Determined by GC.
† Comprises 100 % linoleic acid (18 : 2n-6).
‡ Comprises 50 % eicosapentaenoic acid (20 : 5n-3) and 32 % docosahexaenoic acid (22 : 6n-3).
§ Comprises 99 % oleic acid (18 : 1n-9).
‖ Comprises 86 % palmitic acid (16 : 0).

Bucks., UK), exhibiting both inter- and intra-assay CV of less than 10 %.

Insulin sensitivity

S_I was assessed in the postprandial state using a recently described minimal model index (Caumo *et al.* 2000). The model provides an estimate of S_I following carbohydrate ingestion in each individual and following each meal. The S_I calculated by this method utilizes cumulative integrated AUC measures of both insulin and glucose concentration assuming that the total glucose disposal from the system after 240 min (or when basal values have been reached) equals the glucose entering the peripheral circulation allowing for first-pass extraction by the liver. Insulin-independent mechanisms also contribute to total glucose disposal and a constant rate of glucose effectiveness (GE) has been assumed for the whole time interval.

$$S_{I(oral)} = f \times D_{oral} \frac{\frac{AUC(\Delta g(t)/g(t))}{AUC(\Delta g(t))} - GE \times AUC(\Delta g(t)/g(t))}{AUC(\Delta i(t))}$$

(1)

where D_{Oral} is the dose of ingested carbohydrate/unit of body weight (mg/kg) and f is the fraction of ingested carbohydrate reaching the peripheral circulation as glucose. AUC was calculated from time zero until the end of the test, and GE was fixed at 0·0024 litres/kg × min (Best *et al.* 1996). The test meals provided in our study contained a significant proportion of starch as the source of carbohydrate, and as the appearance of certain starch-derived glucose fractions into the circulation has been shown to be slower than that of glucose, a nominal value for f of 0·6 was chosen for all subjects (Robertson *et al.* 2002b).

Statistical analysis

Time-course data were analysed by repeated measures ANOVA when normally distributed with *post-hoc* least significant difference method where appropriate using SPSS (SPSS Inc., Chicago, IL, USA). A level of $P < 0.05$ was taken as significant.

Results

There was no difference in the apparent palatability of the test meals due to the use of a deodorized fish oil. The protocol adopted for the present study proved acceptable to the subjects and all elements of the protocol were completed.

Plasma insulin

Fig. 1 illustrates the postprandial insulin response following the four high-fat breakfast meals of differing fatty acid composition (at time zero) followed by four identical lunches (at 300 min). All subjects exhibited the typical biphasic pattern with concentrations returning to baseline between the two meals. The postprandial response to the first high-fat meal was both of greater magnitude ($P = 0.019$) and earlier ($P = 0.004$) than the second low-fat meal. The response following the breakfast rich in SFA was significantly different from the other three high-fat meals ($P = 0.006$, repeated measures ANOVA) demonstrated
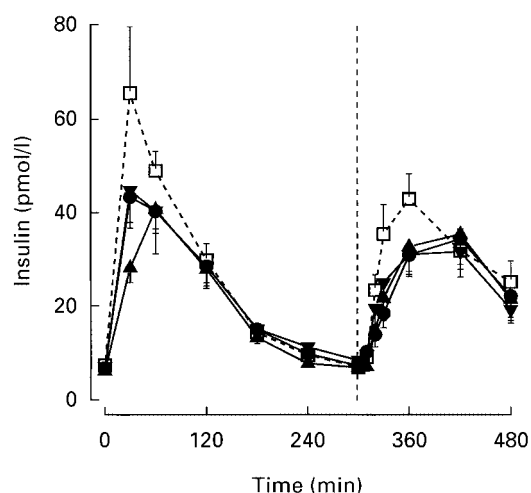

Fig. 1. Plasma insulin concentrations following a high-fat breakfast (at time zero) and a low-fat lunch (at 300 min, ---) where the breakfast meal was rich in saturated fatty acids (□), n-6 polyunsaturated fatty acids (▼), n-3 polyunsaturated fatty acids (▲) and monounsaturated fatty acids (●). The lunch was identical for all four visits. Mean values for ten women are shown and SEM are represented by vertical bars. Repeated measures ANOVA showed a significant time effect ($P < 0.001$) and a significant meal effect ($P = 0.006$). *Post-hoc* analysis (least significant difference method) showed that the insulin response following the saturated fatty acid meal was significantly different from the other three meals ($P < 0.05$).
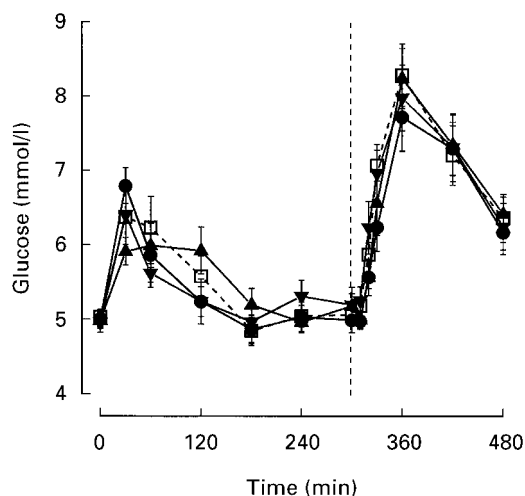
Fig. 2. Plasma glucose concentrations following a high-fat breakfast (at time zero) and a low-fat lunch (at 300 min, ---) where the breakfast meal was rich in saturated fatty acids acids (□), *n*-6 polyunsaturated fatty acids (▼), *n*-3 polyunsaturated fatty acids (▲) and monounsaturated fatty acids (●). The lunch was identical in all four studies. Mean values for ten women are shown and SEM are represented by vertical bars. Repeated measures ANOVA showed a significant time effect only ($P < 0.001$).
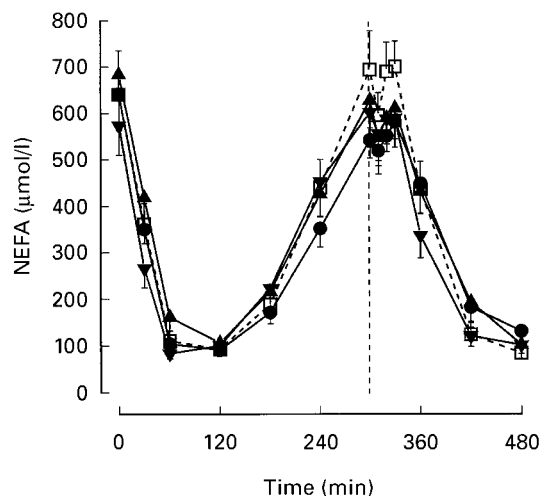

Fig. 3. Plasma non-esterified fatty acid (NEFA) concentrations following a high-fat breakfast (at time zero) and a sequential low-fat lunch (at 300 min, --) where the breakfast meal was rich in saturated fatty acids acids (□), *n*-6 polyunsaturated fatty acids (▼), *n*-3 polyunsaturated fatty acids (▲) and monounsaturated fatty acids (●). The lunch was identical in all four studies. Mean values for ten women are shown and SEM are represented by vertical bars. Repeated measures ANOVA showed a significant effect of time ($P < 0.001$) and a significant time *v.* meal interaction ($P = 0.003$).

by higher insulin concentrations after both breakfast and lunch.

Plasma metabolites

The plasma glucose response following the four high-fat breakfast meals of differing fatty acid composition and the four identical low-fat lunch meals are shown in Fig. 2. All subjects again exhibited a biphasic pattern with glucose concentrations returning to baseline between the two meals. The peak postprandial response following the second low-fat meal (68 g carbohydrate) was significantly greater than that following the first high-fat meal (78 g carbohydrate) ($P < 0.001$). The fatty acid composition of the test breakfast had no effect on the postprandial plasma glucose response at breakfast or lunch.

Fig. 3 shows the plasma NEFA response following the four high-fat breakfast meals. NEFA levels were initially suppressed reaching a nadir at 120 min before increasing to reach pre-meal levels again by 300 min. After the second low-fat, high-carbohydrate meal there was a peak in NEFA concentration before levels were again suppressed. Following the breakfast rich in SFA the rise in plasma NEFA following initial suppression was significantly higher ($P = 0.003$) with a higher peak after the lunch meal 30 min after ingestion.

Insulin sensitivity

The S_I following the SFA-rich breakfast was significantly lower than with the other three high-fat meals (SFA, 1·38; *n*-6 PUFA, 1·87; *n*-3 PUFA, 1·96 and MUFA, 2·13 × 10^{-4} litres/kg/min per mU/l, $P = 0.038$). The S_I following lunch was lower than that following breakfast ($P = 0.009$) irrespective of breakfast fatty acid composition. However,

the S_I for the four breakfast test meals exhibited the same rank order after both breakfast and lunch (Fig. 4).

Discussion

In the present study we have demonstrated that the acute ingestion of a mixed meal rich in SFA results in significantly elevated postprandial insulin levels compared with meals enriched with other dietary fats (MUFA, *n*-6 PUFA or *n*-3 PUFA). Utilizing a minimal model approach (Caumo *et al.* 2000), the palm-oil meal resulted in a lower
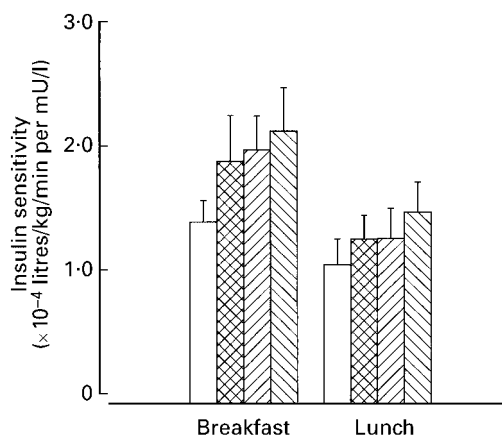

Fig. 4. Oral insulin sensitivity following a high-fat breakfast and a sequential low-fat lunch where the breakfast meal was rich in saturated fatty acids acids (□), *n*-6 polyunsaturated fatty acids (▨), *n*-3 polyunsaturated fatty acids (▨) and monounsaturated fatty acids (▨). Mean values for ten women are shown and SEM are represented by vertical bars. Repeated measures ANOVA showed a significant effect between breakfast and lunch ($P = 0.009$) and a significant difference between the breakfast fatty acid composition ($P = 0.038$).

postprandial S_I after breakfast. It is accepted that a fatty breakfast has deleterious effects on glucose tolerance and S_I to a lunch-time meal, presumably through effects on NEFA concentrations (Frape et al. 1998). In the present study we were able to clarify the effects of breakfast fatty acid composition on these parameters. Although insulin levels and S_I were reduced at lunch-time compared with breakfast irrespective of breakfast fatty acid composition, the S_I following lunch showed the same rank order as after breakfast, despite the lunch-time meal being identical in all cases (Fig. 4).

The effects of meal fatty acid composition on acute glucose-stimulated insulin levels following a single meal have been demonstrated previously. Gannon et al. (1993) found that adding butter (high in SFA) to a potato meal gave a higher insulin and C-peptide response than when the potato was given alone. However, when Welch et al. (1987) added maize oil (high in PUFA) to a potato meal they found the insulin response to be reduced. Comparison between such mixed-meal studies is difficult due to differences in test meal composition, in particular variations in the amount of fat, fatty acid enrichment and type of carbohydrate (Gatti et al. 1992). It might be supposed that variations in the secretion of gut hormones (glucagon-like peptide 1, cholecystokinin and glucose insulinotropic polypeptide) could potentiate differential insulin secretion according to fatty acid composition (Zampelas et al. 1994). Whilst it is true that stimulation of these hormones by fat-containing meals could explain the enhanced insulin response compared with when fat is absent from a meal, gut hormone concentrations measured during the present study show that palm oil is not a more potent secretagogue than the other dietary oils (Robertson et al. 2002a). Other possibilities include variable effects of meal fatty acid composition on circulating plasma NEFA, which have been shown to be stimulatory towards the pancreatic β-cell (Greenough et al. 1967). Short-term (3 h) elevation of NEFA in rats (Grill & Qvigstad, 2000) and human subjects (Beysen et al. 2002) increases glucose-stimulated insulin secretion although different fatty acids may not be equal in their insulinotropic potency. In this respect the results from the literature are contradictory. In vitro studies have clearly shown that SFA are the most potent stimulators of insulin secretion in isolated rat islets (Stein et al. 1997), whereas in human plasma there is some evidence that MUFA dominate in terms of insulinotropic potential (Beysen et al. 2002).

In the plasma, dietary chylomicron-triacylglycerol is acted upon by lipoprotein lipase to release NEFA and up to 50 % of these liberated NEFA escape 'capture' by tissues and enter the plasma NEFA pool directly (Coppack et al. 1992). Although specific fatty acid measurements were not made in the present study, similar studies using high-fat, high-carbohydrate test meals have shown postprandial changes in the plasma NEFA profile to resemble the fatty acid composition of the test meal (Fielding et al. 1996). Therefore in the postprandial period following each of our high-fat breakfasts, we suggest that due to the action of insulin in suppressing hormone-sensitive lipase, the majority of the NEFA pool would be of dietary origin. A high saturated fatty acids:unsaturated fatty acids ratio in the NEFA pool following the palm-oil breakfast may therefore have promoted hypersecretion of insulin (Stein et al. 1997). This is contrary to other work by our group (Beysen et al. 2002), in which MUFA were found to promote greater insulin secretion than SFA. However, in that study NEFA levels were artificially elevated with an infusion of heparin. In the present study the absolute concentration of plasma NEFA fell in the postprandial period and so whether the composition of the NEFA pool is as important as the combined concentration of all circulating NEFA remains to be determined.

The ability to calculate S_I from glucose and insulin measurements following mixed meals adds a new dimension to postprandial experiments (Caumo et al. 2000). In this setting the minimal model approach has distinct advantages over the classic hyperglycaemic–hyperinsulinaemic clamp methods as it allows gastrointestinal factors (gastric emptying and gastrointestinal hormone release) to be accounted for in the physiological non-steady state. However, the value obtained from the minimal model cannot be readily compared with those obtained from clamp experiments. The true relationship between the plasma glucose and insulin levels may be masked by the secondary effects of hormones such as glucagon-like peptide 1 on concentrations of insulin, glucagon, glucose and NEFA levels postprandially.

In the acute situation there is reported to be a 3–4 h delay between the rise in plasma NEFA and the inhibition of insulin-stimulated glucose uptake (Boden et al. 2001), making a direct effect of NEFA on insulin action in the present study unlikely. Another possible explanation could be a reduction in muscle S_I due to the accumulation of intramyocellular fat within muscle tissue, although whether this is relevant in the acute postprandial period remains to be established.

In conclusion, ingestion of a single meal rich in SFA led to an elevated insulin concentration with a reduction in S_I when compared with other dietary fats with follow-on effects for a subsequent meal.

Acknowledgements

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