Rapid chylomicron appearance following sequential meals: effects of second meal composition

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Previous studies have noted the presence of an early postprandial peak in plasma triacylglycerol concentrations following successive fat-rich meals. An earlier study has shown that the triacylglycerol in this early peak originates from a previous meal. The present study was performed to investigate the effects of different second meals on the plasma triacylglycerol response. Six healthy subjects were studied on four occasions each. At 5 h following a fat-rich breakfast they ingested one of the following in a balanced design: a fat-rich meal, a low-fat meal, water or nothing by mouth. Blood samples were taken for 2·5 h following the second meal. An early peak in chylomicron and plasma triacylglycerol concentrations was seen following both low-fat and fat-rich second meals but not following water. During studies investigating postprandial lipaemia, further meals must be avoided, even if they contain no fat, although water may be allowed.

Postprandial metabolism: Plasma: Meal eating

There is accumulating evidence that postprandial triacylglycerol concentrations may be more closely related to atherogenic risk than those observed in the post-absorptive state when measurements of triacylglycerol concentrations are usually made (Patsch *et al.* 1992). Following a fat-rich meal the plasma triacylglycerol concentration rises to a broad peak at 3–4 h and is then gradually cleared from the circulation. While most studies have shown a single peak, some studies have shown two or more peaks in triacylglycerol concentration (Cohn *et al.* 1988; Williams *et al.* 1992; Peel *et al.* 1993). These peaks have corresponded to peaks in chylomicron triacylglycerol concentration (Cohn *et al.* 1989; Peel *et al.* 1993). Some peaks include apoliproprotein B-100-containing lipoproteins in addition (Cohn *et al.* 1989). In a previous study we have shown an early peak in triacylglycerol concentrations when a mixed meal was given 5 h after a fat-rich breakfast (Fielding *et al.* 1996). The chylomicron triacylglycerol in this early peak was predominantly from the first meal. It is not known if the composition of the second meal is important in determining this response. We have therefore compared the effects of a mixed meal, low-fat meal or water given as second meals following a fat-rich first meal.

Subjects and methods

Protocol

Six healthy volunteers (four male) aged 21–44 years, with BMI 20·8–27·2 kg/m^2 were each studied on four occasions following an overnight fast. All subjects were normolipidaemic and normoglycaemic. Subjects refrained from strenuous exercise, smoking or alcohol for 24 h before each study and were given instructions to consume a low-fat meal on the evenings before the studies. At 07.00 hours they consumed a high-fat breakfast at home. This meal consisted of scrambled eggs on toast, together with jam on toast and contained 38 g protein, 63 g fat and 80 g carbohydrate (Table 1). Subjects were supplied with the food for their breakfast together with instructions on its preparation. The nutrient composition of meals was estimated from the manufacturer's data and from food tables (Holland *et al.* 1991). During the morning the subject came to the laboratory and a cannula was inserted into an antecubital vein and kept patent by a continuous slow infusion of saline (9 g NaCl/l). Blood samples were taken at 11.30 and 12.00 hours (just before the second meal).

At 12.00 hours subjects consumed a high-fat lunch

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Table 1. Nutrient composition of test meals*

	Carbohydrate (g)	Fat (g)	Protein (g)	Energy (kJ)
Breakfast				
Safflower oil, 55 g	0	55·0	0	2033
Egg yolk, 6·9 g	0	4·3	2·3	197
Egg white, 27·5 g	0	0	21·2	360
Bread, 150 g	66·6	3·6	14·3	1509
Jam, 20 g	13·4	0	0	229
Total	80	62·9	37·8	4328
High-fat lunch				
Full-cream milk, 300 g	14·1	12·0	9·6	849
Cornflakes, 45 g	36·9	0·3	3·6	698
Full-fat ice-cream, 60 g	13·1	6·4	2·3	500
Double cream, 80 g	2·1	38·0	1·4	1465
Total	66·2	56·7	16·9	3511
Low-fat lunch				
Skimmed milk, 300 g	14·1	0·3	9·6	438
Cornflakes, 45 g	36·9	0·3	3·6	698
Fat-free ice-cream†, 85 g	13·2	0·3	2·2	275
Total	64·2	0·9	15·4	1411

* Determined from manufacturer's data and from food tables (Holland *et al.* 1991).
†Too Good to be True (Birds Eye Wall's Ltd, Walton-on-Thames, UK).

containing 17 g protein, 57 g fat and 66 g carbohydrate (Table 1), a low-fat lunch containing 15 g protein, 1 g fat and 64 g carbohydrate (Table 1), 330 ml water, or were given nothing. In order to avoid dehydration, subjects receiving nothing by mouth were given 1 litre saline intravenously over the course of the experiment. Both the high-fat and low-fat lunches were given as a milk shake and cornflakes with milk. The four studies were performed in random order. Following the second meal blood samples were taken for a further 150 min (every 15 min for 90 min, then at 120 and 150 min).

The study was approved by the Central Oxford Research Ethics Committee and all subjects gave informed consent.

Analytical methods

Blood samples were collected into heparinized syringes and plasma was separated rapidly by centrifugation at 4°. Chylomicron-rich fractions were prepared from 0·75 ml plasma by using a solution with a density of 1·006 kg/l and centrifugation at 12 600 ***g*** for 120 min as previously described (Griffiths *et al.* 1994). Triacylglycerol concentrations were measured in plasma and chylomicron-rich fractions using a lipase (*EC* 3.1.1.3)–glycerol kinase (*EC* 2.7.1.30) method (Instrumentation Laboratory, Warrington, UK) and glucose concentrations measured in plasma using a hexokinase (*EC* 2.7.1.1) method (Instrumentation Laboratory) using an IL Monarch centrifugal analyser (Instrumentation Laboratory). Plasma non-esterified fatty acid concentrations were measured enzymically (WAKO NEFA C kit; Alpha Laboratories Ltd, Eastleigh, UK; adapted for use in the centrifugal analyser). Plasma insulin was measured by radioimmunoassay (Kabi Pharmacia Ltd, Milton Keynes, UK).

Statistical methods

Repeated-measures ANOVA with SPSS (SPSS UK Ltd, Chertsey, UK) was used to test for the significance of changes in plasma concentrations with time and meals. Fisher's protected least significant difference test was used to compare differences in incremental area under the curve between meals.

Results

Mean chylomicron triacylglycerol concentrations rose following both the low-fat and high-fat lunches to a peak at 45–60 min; there were no increases in chylomicron triacylglycerol concentrations following water or no lunch (Fig. 1, Table 2). Similar, although less pronounced, changes occurred in plasma triacylglycerol concentrations. Differences between meals were not significant.

Mean plasma non-esterified fatty acid concentrations rose following water or no lunch but fell following low-fat or high-fat lunches, although there was a large degree of inter-individual variation (Fig. 2; repeated-measures ANOVA, $P = 0{\cdot}007$ for meal × time effect).

As expected plasma glucose concentrations rose following both low-fat and high-fat meals, and fell following water or no lunch (Fig. 3(A), Table 2). Insulin concentrations followed a similar pattern (Fig. 3(B), Table 2).

Discussion

In agreement with our previous findings, chylomicron triacylglycerol concentrations clearly demonstrated an early peak at 45–60 min following a high-fat lunch, which had been preceded by a high-fat breakfast (Fielding *et al.* 1996). There was a similar peak at 45–60 min following the low-fat lunch. The later triacylglycerol peak seen at

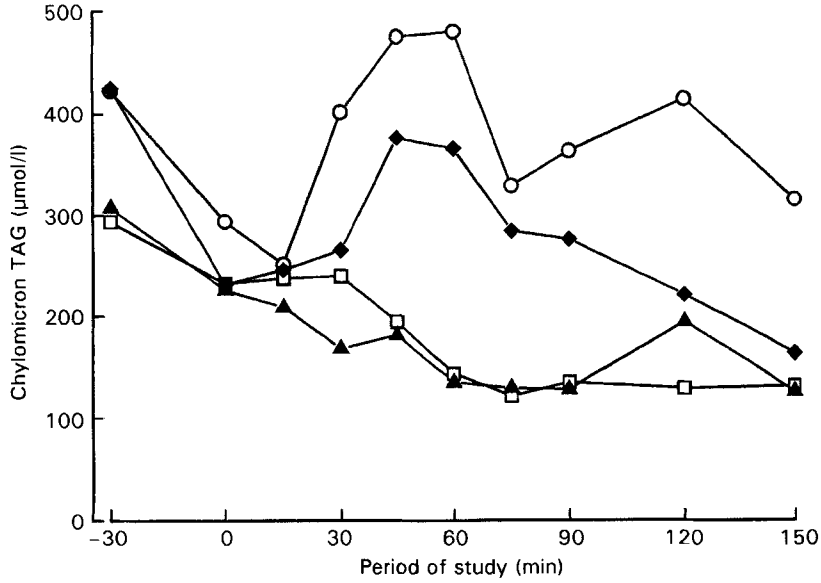

Fig. 1. Chylomicron triacylglycerol (TAG) concentrations in six healthy volunteers after a high-fat breakfast, followed 5 h later (time 0) by various second meals: saline (9 g NaCl/l; ▲), water (□), low-fat (◆), high-fat (○). For details of subjects and procedures, see pp. 425–426 and for details of composition of test meals, see Table 1.

Table 2. Post-hoc significance tests* for chylomicron triacylglycerol and plasma glucose and insulin concentrations in six healthy volunteers after a high-fat breakfast followed 5 h later by a second meal of saline (9 g NaCl/l), water, low-fat or fat-rich†

	Repeated-measures ANOVA	Saline *v.* water	Saline *v.* low-fat	Saline *v.* fat-rich	Water *v.* low-fat	Water *v.* fat-rich	Low-fat *v.* fat-rich
Chylomicron triacylglycerol	< 0·001	NS	< 0·05	< 0·05	< 0·05	< 0·05	NS
Glucose	0·001	NS	< 0·001	< 0·001	< 0·001	< 0·001	NS
Insulin	< 0·001	NS	< 0·001	< 0·001	< 0·001	< 0·001	NS

*Repeated-measures ANOVA; significance levels (*P*) for meal effects. Significance levels determined using Fisher's protected least significant difference test for analytes showing a significant difference between meals, using repeated measures ANOVA on incremental areas under the curve.
†For details of subjects and procedures, see pp. 425–426 and for details of composition of test meals, see Table 1.

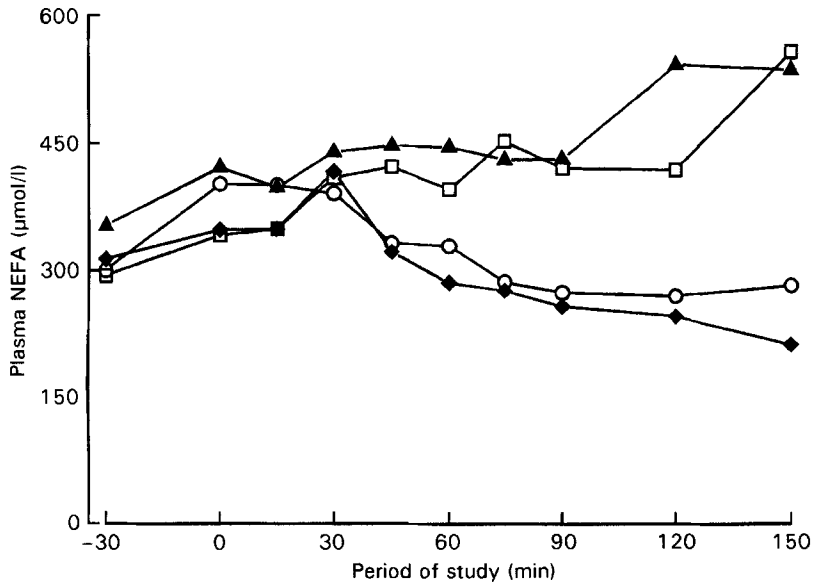

Fig. 2. Plasma non-esterified fatty acid (NEFA) concentrations in six healthy volunteers after a high-fat breakfast followed 5 h later (time 0) by various second meals: saline (9 g NaCl/l; ▲), water (□), low-fat(◆), high-fat (○). For details of subjects and procedures, see pp. 425–426 and for details of composition of test meals, see Table 1.

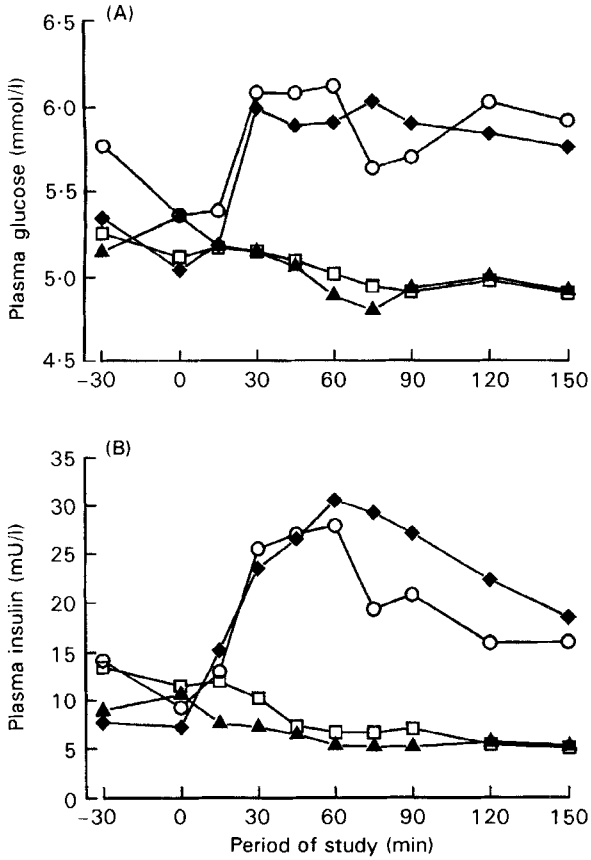

Fig. 3. Plasma glucose (A) and insulin (B) concentrations in six healthy volunteers after a high-fat breakfast, followed 5 h later (time 0) by various second meals: saline (9 g NaCl/l; ▲), water (□), low-fat (◆), high-fat (○). For details of subjects and procedures, see pp. 425–426 and for details of composition of test meals, see Table 1.

120 min following the high-fat lunch is not seen following the low-fat lunch, which is consistent with the later triacylglycerol peak originating from fat ingested in the second meal. An early plasma triacylglycerol response after an overnight fast has occasionally been seen following a low-fat meal (Griffiths *et al.* 1994) or an oral glucose tolerance test (Coppack *et al.* 1989), probably due to fat ingested the previous evening.

We have previously shown that the early peak in chylomicron triacylglycerol concentration after lunch is composed of fat ingested at the previous meal (Fielding *et al.* 1996). The mechanism whereby fat ingested at one meal appears in chylomicrons following a subsequent meal is not clear. An early peak in chylomicron concentration suggests the release into the circulation of pre-formed chylomicrons, rather than new chylomicron formation. One hypothesis is that fat in the second meal displaces fat remaining in the enterocytes from the first meal. The present results make this unlikely, as there was a clear early rise in chylomicron triacylglycerol following a low-fat second meal. However, an increase in intestinal lymph flow may accelerate the rate of entry of pre-formed chylomicrons in the lacteals or lymph ducts into the plasma. In support of this, intestinal lymph flow has been shown to increase following the duodenal infusion of fatty acids in rats (Kalogeris *et al.* 1996). This may be mediated by an increase in small intestinal blood flow or intestinal muscle contractions (Schmid-Schonbein, 1990). An alternative explanation is that intestinal retention of hydrolysed fat is responsible for the early appearance of chylomicrons in the plasma following the second meal. Sham feeding of an attractive meal has also been shown to increase plasma concentrations of retinyl ester ingested previously (Mendeloff, 1954). More recently, it was found that a significant change in the postprandial lipaemia resulting from the ingestion of safflower oil capsules could be provoked by the repeated oral exposure to cream cheese with different fat contents (Mattes, 1996). It seems more plausible, therefore, that the second meal causes early chylomicron secretion by an increase in small intestinal blood flow and an increase in lymphatic drainage, and that this process may also be stimulated by the smell or taste of food.

Plasma non-esterified fatty acid concentrations usually fall following a mixed meal, due to insulin suppression of lipolysis (Coppack *et al.* 1990; Griffiths *et al.* 1994), and this was seen in the present study following both low-fat and high-fat meals. This was in contrast to our previous findings (Fielding *et al.* 1996) in which we reported a rise in some subjects and no significant change in mean values after two high-fat meals. This may be due to the higher carbohydrate content of the second meal in the present study (66 g) compared with the previous study (35 g).

Our results have a clear practical implication. With increasing interest in determination of postprandial lipaemic responses, it is essential to understand factors that may affect the response. Had we shown that ingestion even of a glass of water was capable of eliciting a further rise in plasma triacylglycerol concentration, subjects for such experiments, which often last 8 h or even 12 h following the meal, would have had to refrain from drinking over that period. It is clear that simple water ingestion has no such effect. However, when the aim of a study is to investigate the lipaemic response to fat ingested on a single occasion, then no other meals, whatever their nutrient composition, should be consumed during the study.

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