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The effect of acute carbohydrate load on the monophasic or biphasic nature of the postprandial lipaemic response to acute fat ingestion in human subjects

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Previous studies in this laboratory have elicited a monophasic response in postprandial plasma triacylglycerol (TAG) level with fat intakes of 0·5 g fat/kg body weight accompanied by about 17 g carbohydrate as lactose. Recent studies involving the same level of fat with a higher level of carbohydrate, 136 g of which 60 g was sucrose, appeared to elicit a biphasic response. The present study compared these two test meals and showed a significant meal × time interaction for plasma total TAG ($P = 0{\cdot}0228$) reflecting a monophasic response with the lower-carbohydrate test meal. The higher-carbohydrate meal induced significantly higher insulin and glucose-dependent insulinotropic polypeptide responses ($P = 0{\cdot}0009$ and $P = 0{\cdot}0041$ respectively). A significant meal × time interaction was seen for plasma non-esterified fatty acids ($P = 0{\cdot}0437$). The biphasic plasma TAG response seen with the high-carbohydrate meal largely reflected the TAG-rich lipoprotein (TRL) or chylomicron fraction, which would tend to suggest a biphasic pattern of absorption. This was borne out by TRL-TAG fatty acid compositions. Both peaks in the biphasic response showed active incorporation of the main dietary fatty acids, 18:1*n*-9, 18:2*n*-6 and 18:3*n*-3 into TRL-TAG. These results indicate that under the specific test-meal conditions used in the present study, a biphasic pattern of fat absorption was seen.

Postprandial metabolism: Carbohydrate: Fat

Many studies have shown that the general pattern of postprandial triacylglycerol (TAG) response to the acute ingestion of fat is that of a gradual rise, a peak between 2 and 5 h after the meal followed by a gradual decline to baseline values by 8 h (Van Amelsvoort *et al.* 1989; Dubois *et al.* 1994; Roche & Gibney, 1995). However, several authors have reported that when the postprandial TAG responses of individuals are examined, many reveal a biphasic rather than the anticipated monophasic response. Olefsky *et al.* (1976) reported a biphasic postprandial TAG response in the majority of their subjects irrespective of their fasting TAG levels. Similar results have been reported by Kayshap *et al.* (1983) and Cohn *et al.* (1988). More recently, Zampelas *et al.* (1994) have shown a mean biphasic pattern of postprandial TAG response to a mixed meal. To date, no satisfactory explanation for this biphasic plasma TAG response is available. Sethi *et al.* (1993) proposed that a multiphasic response could be due to the ingestion of fluids after the test meals which might provoke a secondary influx of fat from the gut. This is an unlikely explanation since a mean monophasic TAG response is consistently seen in studies in this laboratory where subjects have access to water or black unsweetened tea during the period of postprandial observation (Roche & Gibney, 1996). Fielding *et al.* (1996) have provided evidence to suggest that ingested fat may be stored in the gastrointestinal tract or lymphatics and be subsequently released by further fat ingestion. Peel *et al.* (1993) also found that an evening meal produced an early plasma TAG peak which they hypothesized had its origins in fat ingested at an earlier lunch.

More recently, Roche *et al.* (1998*a*) and Zampelas *et al.* (1998) have observed a mean biphasic response using test meals of identical design as part of an international collaborative study. Because these test meals contained higher levels of carbohydrate than normally used in this laboratory (136 *v.* 17 g) where a monophasic response is consistently observed, the present study set out to examine whether this might have been a factor in inducing a biphasic postprandial TAG response. Soyabean oil, containing 18:3*n*-3 (12 g/100 g oil), was used in the present study since the fasting

Abbreviations: AUC, area under the curve; GIP, glucose-dependent insulinotropic polypeptide; HC, high carbohydrate; LC, low carbohydrate; NEFA, non-esterified fatty acids; TAG, triacylglycerol; TRL, TAG-rich lipoproteins.

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TAG concentration of this fatty acid is generally low, thus allowing this fatty acid to be used as a marker of ingested fat. Both insulin and glucose-dependent insulinotropic polypeptide (GIP) stimulate lipoprotein lipase (*EC* 3.1.1.34) in a related fashion (Knapper *et al.* 1996) and because their peak responses differ, insulin earlier and GIP later, the possibility of a differential role for these hormones in shaping a biphasic response was investigated in the present study.

Experimental methods

Subjects

Nine healthy volunteers participated in the study (four males, five females; mean age 23·2 (SD 4·5) years; mean BMI 24·1 (SD 2·5) kg/m^2). Subjects were healthy and were not taking any medication which could interfere with lipid or carbohydrate metabolism. None was taking nutritional supplements.

Test meals

Two test meals with either high carbohydrate (HC) or low carbohydrate (LC) content were given to the subjects on two occasions. The test meals were standardized with respect to body weight to reduce the possible effect of body size on postprandial metabolism. The HC meal consisted of the following (g/kg body weight): white bread 1·7, strawberry jam 0·6, Nesquik (a flavoured sugar; Nestlé, Vevey, Switzerland) 0·4, skimmed-milk powder 0·5, rapeseed oil 0·5 and water 3·0. This provided 0·53 g fat, 1·93 g carbohydrate and 0·31 g protein per kg body weight. The LC meal consisted of (g/kg of body weight): Nesquik 0·1, skimmed-milk powder 0·5, rapeseed oil 0·5 and water 3·0. This provided 0·50 g fat, 0·28 g carbohydrate and 0·18 g protein per kg body weight. The carbohydrate:fat ratio was 3·5 for the HC meal and 0·6 for the LC meal. The Nesquik, skimmed-milk powder and oil were mixed with the water to form a stable emulsion and were consumed in liquid form. The bread and jam in the HC meal were consumed in solid form together with the liquid formula part of the meal. The mean carbohydrate intake with the HC meal was 136 (SD 19) g of which 40 % was starch, 46 % was sucrose and 14 % was lactose. For the LC meal the mean carbohydrate intake was 23 (SD 3) g of which 73 % was lactose and 27 % was sucrose. The HC meal provided 4·1 (SD 0·6) MJ while the LC meal provided 1·9 (SD 0·2) MJ. These nutrient analyses were obtained from standard food composition tables.

Study design

The study was approved by the Ethics Committee of the Federated Dublin Voluntary Hospitals. The subjects were asked to take a light meal on the evening before the study, to fast from 20.00 hours that evening, to abstain from alcohol and heavy exercise for 24 h before each investigation and to avoid smoking on the study day. Subjects presented themselves between 08.00 and 09.00 hours on each study day and consumed one of the two test meals in random order at intervals of 7–10 d. On arrival, a 21 gauge, 32 mm venous catheter (Abbot Ireland Ltd., Dublin, Republic of Ireland) was placed into the antecubital vein of the forearm. A baseline blood sample was taken immediately before the meal ingestion and the cannula was flushed with normal saline (9 g NaCl/l). Following the consumption of the test meal, the subjects refrained from consuming any food or drink other than water or unsweetened, weak black tea for 8 h. Blood samples were taken at 2, 4, 6 and 8 h following the baseline sample (time 0) with the first 10 ml blood discarded to remove any traces of saline. Blood was collected into 10 ml tubes containing 0·12 ml 0·3 mM-potassium EDTA. The blood samples were immediately centrifuged at 2500 rev./min for 15 min. Plasma for TAG-rich lipoprotein (TRL) separation was stored at 2–5° until the following day. The remainder of each plasma sample was stored in portions at –20°. Plasma TRL were separated using the method of Grundy & Mok (1976) with some modification. In a 4·7 ml Optiseal polyallomer centrifuge tube (Beckman Instruments, Galway, Republic of Ireland), 2·35 ml plasma was overlayed with 2·35 ml saline (density = 1·006 g/ml). Ultracentrifugation at 4° with centrifugal rate at 100 000 rev. /min for 24 min was used for TRL isolation (Beckman Optima TLX ultracentrifuge with TLA 100.4 rotor, Beckman Instruments). The TRL fraction was aspirated and stored at –20° for determination of TAG and fatty acid composition. The TRL fraction is predominantly chylomicrons.

Lipid and hormone concentrations

Enzymic colorimetric assays were used to determine TAG and non-esterified fatty acid (NEFA) concentrations using standard kits. For TAG the method used was a standard coupled enzymic method using lipase (*EC* 3.1.1.3) – glycerol kinase (*EC* 2.7.1.30) (PAP500; Biomerieux, Marcy l'Etoile, France) while the NEFA method used the coupled enzymic method involving acyl-CoA synthetase (*EC* 6.2.1.3) and acyl-CoA oxidase (*EC* 1.3.3.6) (Wako Chemicals GmbH, Neuss, Germany). Plasma insulin was measured by radioimmunoassay double-antibody techniques using guinea-pig anti-human insulin immunoglobulin G and Sepharose sheep anti-guinea-pig immunoglobulin G (Pharmacia AB, Uppsala, Sweden). Plasma GIP was kindly measured by Professor K. Buchanan of The Wellcome Research Laboratories, Royal Victoria Hospital, Belfast using a radioimmunoassay (Alam *et al.* 1992).

Fatty acid composition of plasma and the TRL fraction

Lipids were extracted from the plasma and TRL fractions using the Folch method with 0·1 g butylated hydroxytoluene/kg (Folch *et al.* 1957). NEFA and TAG fractions were isolated from the lipid extracts by TLC on silica-gel-coated plates (Whatman, 9 bridge PL.; Whatman International Ltd., Maidstone, Kent, UK) in a solvent system (Roche & Gibney, 1997) of petroleum ether (b.p. 40–80°) –diethyl ether–formic acid (80:20:2, by vol.) and the TAG and NEFA were identified following a spray of 2,7-dichlorofluorescein (1 g/l in methanol) under u.v. light. Standards were used to identify each fraction. The NEFA and TAG fractions were scraped from the plates and then

washed twice with chloroform–methanol (2:1, v/v). The solvent was then evaporated and the dried samples were stored under N_2 at −20°.

Methyl esters of fatty acids for GLC were synthesized using BF_3 (14 % BF, BDH Chemicals, Poole, Dorset, UK) at 75° for 30 min and the methyl esters were extracted in hexane. The fatty acid compositions of TRL-TAG and NEFA were determined using a Shimadzu GC-14A Series GLC (Mason Technologies, Dublin, Republic of Ireland) fitted with a fused silica capillary column (Phase Sep BP21 polar, 25 m long, 0·22 mm i.d., film thickness 0·25 μm; Phase Separations Ltd., Deeside, Clwyd, UK). The carrier gas was N_2 with injector at 250° and detector at 260°. Analytical conditions were: make-up gas, O_2-free N_2 1 kg/cm^3; column gas, O_2-free N_2 1·25 kg/cm^3; H_2 0·6 kg/cm^3; dry air 0·5 kg/cm^3. The GLC was programmed at: 120°–200° (8°/min), held at 200° for 8 min, raised to 220° (4°/min) and held at 220° for 10 min. The fatty acid methyl esters were identified with a flame ionization detector and recognized by their retention times against known standards (Sigma, Poole, Dorset, UK). Peak areas were calculated by a Shimadzu C-R6A chromatograph integrator (Mason Technologies).

Statistical analysis

The data analyses were completed with Data Desk 4.1 (Data Description Inc., New York, NY, USA). All data are presented as means and standard deviations. Data were transformed to the log (ln) value to give them a normal Gaussian distribution with the exception of plasma NEFA concentrations which were normally distributed. The postprandial data were analysed using ANOVA for repeated measures. The postprandial data were expressed in summary form, i.e. area under the curve (AUC), the incremental AUC, and maximum and minimum postprandial levels. The AUC values were calculated as described by Matthews *et al.* (1990) and the incremental AUC was calculated as AUC minus fasting values. Two-way ANOVA, using subject and meal as the independent variables, was used to investigate significant differences of these summary variables. $P < 0·05$ was accepted as the level of statistical significance. A mean biphasic response was deemed to have occurred if the repeated measures ANOVA revealed a significant meal × time interaction and the subsequent use of the least significant difference test revealed a surge-lull-surge pattern on one treatment.

Results

Results for plasma total and TRL-TAG and for NEFA concentrations are given in Table 1. No significant differences between meals were observed for any of these variables. A significant time effect was seen for total TAG ($P = 0·0024$), TRL-TAG ($P = 0·0015$) and plasma NEFA ($P \leq 0·0001$) concentrations. A significant meal × time interaction was found for total TAG ($P = 0·0228$) and for NEFA ($P = 0·0437$). Postprandial values for glucose, insulin and GIP are given in Table 2. For all these variables a significant time effect was seen ($P < 0·0001$). For both insulin and GIP a significant meal effect was found ($P = 0·0009$ and $P = 0·0041$ respectively). The meal effect for glucose was

Table 1. Postprandial plasma concentrations of total triacylglycerol (TAG), TAG-rich lipoprotein (TRL) and non-esterified fatty acids (NEFA) following a standard fat dose (0·5 g/kg body weight) with a high-carbohydrate (HC) or low-carbohydrate (LC) meal‡
(Mean values and standard deviations for nine subjects)

Time after test meal (h)...		0		2		4		6		8		Statistical significance of effect of:		
	Meal	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	meal ($P =$)	time ($P =$)	meal × time ($P =$)
Plasma TAG (mmol/l)	HC	0·81	0·26	0·98†	0·26	0·89	0·30	1·24*†	0·42	0·88†	0·35	NS	0·0024	0·0228
	LC	0·74	0·23	0·80	0·29	0·97*	0·27	0·94	0·39	0·69	0·30			
TRL (mmol/l)	HC	0·29	0·17	0·42	0·19	0·41*	0·20	0·63*†	0·32	0·37†	0·33	NS	0·0015	NS
	LC	0·20	0·15	0·27	0·17	0·37*	0·22	0·39*	0·30	0·19	0·21			
Plasma NEFA (mmol/l)	HC	0·57	0·24	0·06*†	0·03	0·33*†	0·30	0·76*	0·46	0·70†	0·27	NS	≤0·0001	0·0437
	LC	0·62	0·19	0·32*	0·19	0·60	0·30	0·82*	0·31	0·67	0·23			

Mean values were significantly different from fasting values, * $P < 0·05$.
Mean values were significantly different from those for LC, † $P < 0·05$.
‡ For details of meals and procedures, see pp. 412–413.

Table 2. Postprandial concentrations of plasma glucose, insulin and glucose-dependent insulinotropic polypeptide (GIP) following a standard fat dose (0·5 g/kg body weight) with a high-carbohydrate (HC) or low-carbohydrate (LC) meal‡
(Mean values and standard deviations for nine subjects)

Time after test meal (h)...		0		0·5		1		2		4		6		8		Statistical significance of effect of:		
	Meal	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	meal ($P =$)	time ($P =$)	meal × time ($P =$)
Glucose (mmol/l)	HC	5·09	0·35	5·63*†	1·07	5·17†	0·79	5·20†	0·45	4·96	0·38	4·85	0·39	4·83	0·24	NS	0·0001	0·0472
	LC	4·86	0·50	6·16*	1·43	4·48	0·30	4·67	0·46	4·77	0·35	4·57	0·36	4·55	0·36			
Insulin (μU/ml)	HC	7·9	1·9	48·9*†	18·5	44·3*†	25·6	32·4*†	16·1	9·1	2·9	6·6	2·1	5·9	1·4	0·0009	0·0001	0·0001
	LC	8·9	4·3	33·4*	22·1	12·7	4·5	8·6	2·8	6·2	1·9	6·0	1·9	5·0	1·3			
GIP (ng/l)	HC	7·8	2·6	83·9*†	73·9	75·6*†	71·2	64·0*†	46·0	67·2*†	82·7	26·1	20·4	11·7	7·5	0·0041	0·0001	NS
	LC	5·6	1·7	31·7*	38·6	35·6*	42·9	24·4	12·9	26·7*	26·2	16·7	9·4	6·7	2·5			

Mean values were significantly different from fasting values, $^*P < 0{\cdot}05$.
Mean values were significantly different from those for LC, †$P < 0{\cdot}05$.
‡For details of meals and procedures, see pp. 412–413.

not statistically significant, but approached it ($P = 0{\cdot}0583$). A significant meal × time interaction was, however, observed for glucose ($P = 0{\cdot}0472$) and the meal × time interaction for plasma insulin was highly significant ($P < 0{\cdot}0001$). Summary postprandial variables are given in Table 3. The AUC for glucose, insulin and GIP were significantly higher with the HC meal ($P < 0{\cdot}05$, $P < 0{\cdot}001$ and $P < 0{\cdot}05$ respectively) than the LC meal. The minimum NEFA values were significantly lower with the HC meal ($P < 0{\cdot}05$).

The fatty acid compositions of TRL-TAG and NEFA (g/100 g total fatty acids) are given in Table 4. These fatty acids represent > 90 % of the test-meal fatty acids (18:1*n*-9, 56 %; 18:2*n*-6, 24 %; 18:3*n*-3, 12 %). For both TRL-TAG and NEFA, the concentration of each fatty acid was influenced by time ($P = 0{\cdot}0065$ to $P < 0{\cdot}0001$). A significant meal effect was seen for TRL-TAG 18:3*n*-3 ($P = 0{\cdot}0353$), and for NEFA a significant meal effect was observed for both 18:2*n*-6 ($P = 0{\cdot}0036$) and 18:3*n*-3 ($P = 0{\cdot}0127$). Only in the case of 18:1*n*-9 in TRL-TAG, was a significant meal × time interaction found ($P = 0{\cdot}02$). The following fatty acids showed a significant decline in TRL over the postprandial period: 14:0, 16:0, 16:1, 18:0. Together, these fatty acids comprised only 7 g/100 g of the test meal but comprised 42 g/100 g of fasting TRL fatty acids. Table 5 gives results for the plasma concentrations (units/l) of these fatty acids in the TRL and NEFA pools. Again, time was a significant factor influencing variance for all variables studied. A significant meal effect was seen for TRL-TAG 18:2*n*-6 ($P = 0{\cdot}0208$) and 18:3*n*-3 ($P = 0{\cdot}009$) and for NEFA 18:2*n*-6 ($P = 0{\cdot}0202$) and 18:3*n*-3 ($P = 0{\cdot}0453$). For all of the fatty acids, a significant meal × time interaction was found in the NEFA fraction ($P = 0{\cdot}0225$–$0{\cdot}0001$). In the present study both males and females were included but no significant differences in fasting plasma TAG (0·78 *v.* 0·77 mmol/l) were noted. No attempt was made to examine the possible different responses of the two sexes to the test meals because the numbers did not justify this.

Discussion

Although the majority of studies of postprandial lipaemia show a monophasic response, several authors have reported a mean biphasic response or a mean monophasic response with some individuals showing a biphasic response. In some instances, the biphasic response was observed when the test meal was given 5–6 h after an earlier meal, either breakfast (Fielding *et al.* 1996) or lunch (Peel *et al.* 1993; Zampelas *et al.* 1994). In others, the biphasic response was noted after an overnight fast (Olefsky *et al.* 1976; Kayshap *et al.* 1983; Cohn *et al.* 1988). However, the great majority of studies which have examined the postprandial lipaemic response after an overnight fast have not noted a biphasic-type response. The present study represents the third experiment in which the specific ingredients used in the HC test meal have elicited a mean biphasic lipaemic response (Roche *et al.* 1998*a*; Zampelas *et al.* 1998). In each case, the test meal was administered after an overnight fast and it is possible, if not probable, that the basis of this biphasic response will differ from that observed in studies with meals administered 5–6 h apart. This biphasic response is illustrated in Fig. 1,

Table 3. Summary postprandial variables for plasma triacylglycerol (TAG), TAG-rich lipoproteins (TRL), and plasma glucose, insulin, glucose-dependent insulinotropic polypeptide (GIP) and non-esterified fatty acids (NEFA) following a standard fat dose (0·5 g/kg body weight) with a high-carbohydrate (HC) or low-carbohydrate (LC) meal†

(Mean values and standard deviations for nine subjects)

		Total AUC (units/1·8 h)		AUC (units/1·8 h)		Maximum value (units)	
		Mean	SD	Mean	SD	Mean	SD
Plasma TAG (mmol/l)	HC	7·9	2·1	1·4	1·9	1·3	0·4
	LC	6·9	2·1	0·9	1·1	1·1	0·4
TRL-TAG (mmol/l)	HC	3·6	1·4	1·2	1·4	0·7	0·3
	LC	2·4	1·5	0·9	0·7	0·5	0·3
Glucose (mmol/l)	HC	40·2*	1·6	9·7	2·5	5·9	0·8
	LC	37·9	2·7	16·0	11·3	6·3	1·2
Insulin (μU/ml)	HC	146***	53	98**	52	55*	25
	LC	71	24	18	19	34	22
GIP (ng/l)	HC	395*	332	348*	327	102*	77
	LC	174	120	141	120	47	40
						Minimum value	
NEFA (mmol/l)	HC	3·6	1·7	–0·96	1·25	0·06*	0·03
	LC	4·8	1·7	–0·15	1·75	0·31	0·15

Mean values were significantly different from those for LC: *$P < 0·05$, **$P < 0·005$, ***$P < 0·001$.
† For details of meals and procedures, see pp. 412–413.

which represents the change in plasma TAG over each consecutive 2 h period. The LC meal led to a gradual rise and fall, whereas the HC meal showed a surge between 0 and 2 h, a lull between 2 and 4 h and a substantial surge between 4 and 6 h.

The study of Fielding *et al.* (1996) clearly indicates that the ingestion of a lunch, 5 h after a breakfast, caused a rise in plasma TAG which could be clearly identified, on the basis of the fatty acid composition, as being derived from the breakfast fat. The authors consider the possibility that fat ingested at breakfast can, in part, be stored in the enterocytes or lymphatics and its release into the circulation could be stimulated by the ingestion of the later meal at lunchtime. In a recent review, Bergeron & Havel (1997) drew attention to earlier work by Mendeloff (1954) which showed that intestinal lacteals of animals remain engorged with fat long after fat digestion is completed. Recent work by Mattes (1996) showed that sham feeding with cream cheese and crackers, which were chewed but not swallowed, significantly augmented the postprandial lipaemic response to 50 g safflower oil. Clearly oronasal stimuli alone can contribute to the release of lacteal lipid originating from an earlier meal. Whilst it is possible that this phenomenon could have played a role in the present study, it cannot explain why it would have operated in one treatment but not the other.

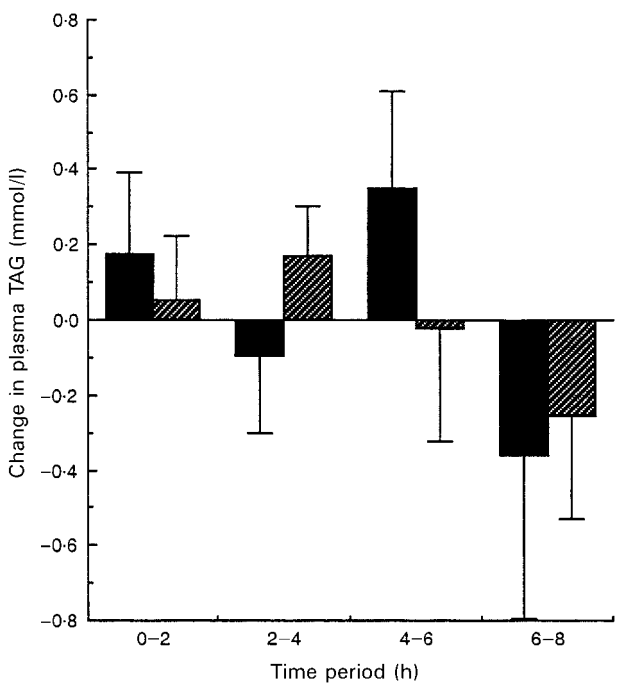

Fig. 1. Change in postprandial plasma triacylglycerol (TAG) concentrations following a standard fat dose (0·5 g/kg body weight) with a high-carbohydrate (■) or a low-carbohydrate (▨) meal. Values are means for nine subjects, with standard deviations indicated by vertical bars.

In the present study, rapeseed oil was used to provide a marker of the test-meal fat because whereas fasting TRL-TAG contains < 2 g/100 g as 18:3*n*-3, this oil contains 12 g 18:3*n*-3/100 g. Thus, this fatty acid could track the movement of exogenous (ingested) lipid within the endogenous plasma pool. If the first peak in the biphasic response was due to the release of fat from lacteals stored there overnight, then the level of 18:3*n*-3 in plasma TRL-TAG would have been diluted. Equally if the first peak represented NEFA recycled into VLDL-TRL-TAG, dilution of 18:3*n*-3 would also be expected. In fact, with the HC test meal, the level of 18:3*n*-3 in TRL-TAG more than doubled in the first 2 h. Again, with the HC meal, the levels of TRL-TAG and the concentration of 18:3*n*-3 in that fraction remained static in the period of hour 2 to hour 4. Then, just as TRL-TAG showed a second surge in hours 4–6 to create the second peak, the level of 18:3*n*-3 in the TRL-TAG also showed a second surge. Within the plasma NEFA fraction, 18:3*n*-3 also exhibited this surge-lull-surge pattern with the HC test meal which contrasts with the gradual rise and fall pattern observed with the LC test meal. Taken together these data support the view that, for this particular HC test meal, the biphasic pattern involves the test-meal lipid rather than

Table 4. Postprandial fatty acid (18:1*n*-9, 18:2*n*-6 and 18:3*n*-3) composition (g/100 g total fatty acids) of plasma triacylglycerol (TAG)-rich lipoprotein (TRL) TAG fraction and non-esterified fatty acids (NEFA) fraction following a standard fat dose (0·5 g/kg body weight) with a high-carbohydrate (HC) or low-carbohydrate (LC) meal‡

(Mean values and standard deviations for nine subjects)

Time after test meal (h)...		0		2		4		6		8		Statistical significance of effect of:		
	Meal	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	meal ($P =$)	time ($P =$)	meal×time ($P =$)
TRL-TAG														
18:1*n*-9	HC	36·4	3·7	37·7	3·0	39·0*†	3·9	43·8*	2·0	43·3*	4·0	NS	0·0001	0·020
	LC	35·9	5·1	39·6*	3·9	41·5*	5·8	41·7*	3·2	41·1*	3·3			
18:2*n*-6	HC	17·6	6·5	20·4*	5·0	19·2*	4·3	21·0*	3·0	20·9*	3·1	NS	0·0065	NS
	LC	16·7	4·8	18·1	2·9	19·4*	2·1	19·9*	1·9	20·2*	2·5			
18:3*n*-3	HC	14·2	0·89	3·13*	0·62	3·37*	1·42	4·98*	1·01	4·63*	1·32	0·0353	0·0001	NS
	LC	1·36	0·36	2·52*	0·84	3·44*	1·26	4·01*	0·61	3·52*	0·75			
NEFA														
18:1*n*-9	HC	37·0	2·9	35·2	3·2	38·5	3·8	42·0*	5·7	43·4*	3·1	NS	0·0001	NS
	LC	37·5	2·4	36·8	5·1	39·3	3·1	42·9*	2·9	40·7*	3·2			
18:2*n*-6	HC	12·37	3·4	15·6	3·6	14·3	2·0	16·3*	3·2	18·8*	9·9*	0·0036	0·0071	NS
	LC	11·6	3·0	12·7	1·9	13·4	1·2	14·9	1·8	13·4	1·8			
18:3*n*-3	HC	1·02	0·43	2·23*	0·80	2·13*	0·79	3·13*	0·98	3·03*	1·26	0·0127	0·0001	NS
	LC	1·02	0·40	1·58*	0·69	2·14*	0·97	2·56*	1·29	2·00*	0·52			

Mean values were significantly different from fasting values, *$P < 0·05$.
Mean values were significantly different from those for LC,†$P < 0·05$.
‡ For details of meals and procedures, see pp. 412–413.

Table 5. Postprandial fatty acid (18:1*n*-9, 18:2*n*-6 and 18:3*n*-3) concentrations (units/l) in the triacylglycerol (TAG)-rich lipoprotein (TRL) TAG fraction and in the non-esterified fatty acids (NEFA) fraction of plasma following a standard fat dose (0·5 g/kg body weight) with a high-carbohydrate (HC) or low-carbohydrate (LC) meal‡

(Mean values and standard deviations for nine subjects)

Time after test meal (h)...		0		2		4		6		8		Statistical significance of effect of:		
	Meal	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	meal ($P =$)	time ($P =$)	meal×time ($P =$)
TRL-TAG (μmol/l)														
18:1*n*-9	HC	109	66	161	81	165	90	275*†	93	164†	158	NS	0·0016	NS
	LC	69	53	107	70	155*	99	157*	118	78	91			
18:2*n*-6	HC	58	59	86	42	82	52	131*†	64	72	58	0·0208	0·0019	NS
	LC	33	30	50	32	69*	40	75*	55	38	47			
18:3*n*-3	HC	5·2	7·2	13·2	6·7	15·5*	13·5	32·5*†	18	19·4*†	21·9	0·009	0·0002	NS
	LC	2·8	2·6	6·7	4·7	12·6	8·1	15·0*	11·7	6·5	8·1			
NEFA(μmol/l)														
18:1*n*-9	HC	214	99	22*†	10	128*†	115	316*	174	304*	115	NS	0·0001	0·0225
	LC	232	80	116*	59	239	123	344*	111	271	100			
18:2*n*-6	HC	70	37	9*	5	50	50	131*	107	132*†	79	0·0202	0·0001	0·0001
	LC	71	33	42	30	81	40	118	39	91	38			
18:3*n*-3	HC	5·8	3·9	1·3	0·6	7·6	8·2	24·1*	15·8	21·0*†	11·6	0·0453	0·0001	0·0019
	LC	6·4	3·8	5·1	3·6	12·7	7·1	19·1*	8·3	13·4*	6·0			

Mean values were significantly different from fasting values, *$P < 0·05$.
Mean values were significantly different from those for LC, †$P < 0·05$.
‡ For details of meals and procedures, see pp. 412–413.

residual lipid stored in the lacteals from the previous meal or hepatic synthesis of TAG.

Quite why this particular design of HC test meal elicits such a response is difficult to explain. Murphy *et al.* (1995) found a clear monophasic response with levels of carbohydrate higher than those used in the present study (168 *v.* 136 g). Van Amelsvoort *et al.* (1989) demonstrated a consistent monophasic response following four meals which varied in fat and carbohydrate content and found that the magnitude of the TAG response was inversely related to the carbohydrate content of the test meal. However, several studies (Mann *et al.* 1971; Cohen & Schall, 1988; Grant *et al.* 1994) have shown a mean monophasic lipaemic response with sucrose intakes in the range of 50–114 g which embraces the level of 60 g or so used in the present investigation. Studies which used this latter amount of carbohydrate entirely as fructose have also not noted a postprandial biphasic response (Cohen & Schall, 1988; Jeppesen *et al.* 1995). Clearly, neither the amount nor the nature of the carbohydrate used in the present study provides an acceptable explanation for the biphasic response with the HC test meal. Neither do the more pronounced and prolonged responses in plasma glucose, insulin and GIP provide an acceptable explanation for this observation. Equally, to discuss the possibility of differential rates of gastric emptying of fat in this HC test meal, which had both a solid and liquid component, would be speculative.

One area which might merit further exploration is that in the two previous experiments which used this particular HC test-meal formulation, a biphasic response was noted only in subjects habituated to a north-European diet relatively high in saturated fatty acids (Zampelas *et al.* 1998). Southern European subjects with a high intake of olive oil or northern European subjects adapted to such a diet over an 8-week period showed a monophasic response to this particular test meal (Roche *et al.* 1998*b*). Thus, the particular features of the present HC test meal which cause a biphasic response are influenced by chronic dietary fatty acid intake. It is interesting to note that van Greevenbroek *et al.* (1996), working with the human enterocyte cell line, CaCo-2, found that cells pre-incubated with olive-oil fatty acids showed a higher rate of secretion of TAG in response to a tracer fatty acid, and the secreted chylomicrons were richer in TAG per unit apolipoprotein B compared with cells pre-incubated with maize-oil fatty acids. It may be that in the present study the HC test meal led to a rapid rate of gastric emptying and fat digestion which could only be absorbed in two waves because of some limiting factor conditional on chronic fatty acid intake.

In conclusion this study shows that the specific formulation used for the HC test meal leads to a biphasic pattern of fat absorption which might be exploited to further elucidate the complexity of fat digestion, absorption and disposal.

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References

Alam MJ, Kerr JI, Cormican K & Buchanan KD (1992) Gastric inhibitory polypeptide (GIP) response in diabetes using a highly specific antiserum. *Diabetic Medicine* **9**, 542–545.

Bergeron H & Havel RJ (1997) Assessment of postprandial lipaemia: nutritional influences. *Current Opinion in Lipidology* **8**, 43–52.

Cohen JC & Schall R (1988) Reassessing the effects of simple carbohydrates on the serum triglyceride responses to fat meals. *American Journal of Clinical Nutrition* **48**, 1031–1034.

Cohn JS, McNamara JR, Cohn SD, Ordovas JM & Schaefer EJ (1988) Plasma apolipoprotein changes in the triglyceride-rich lipoprotein fraction of human subjects fed a fat-rich meal. *Journal of Lipid Research* **29**, 925–936.

Dubois C, Armand M, Azais–Braesco V, Portugal H, Pauli A-M, Bernard P-M, Latge C, Lafont H, Borel P & Lairon D (1994) Effects of moderate amounts of emulsified dietary fat on postprandial lipemia and lipoproteins in normolipidemic adults. *American Journal of Clinical Nutrition* **60**, 374–382.

Fielding BA, Callow J, Owen RM, Samra JM, Matthews DR & Frayn KN (1996) Postprandial lipaemia: the origin of an early peak studied by specific dietary fatty acid intake during sequential meals. *American Journal of Clinical Nutrition* **63**, 36–41.

Folch J, Lees M & Stanley GHS (1957) A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry* **226**, 497–509.

Grant KI, Marais MP & Dhansay MA (1994) Sucrose in a lipid-rich meal amplifies the postprandial excursion of serum and lipoprotein triglyceride and cholesterol concentration by decreasing triglyceride clearance. *American Journal of Clinical Nutrition* **59**, 853–860·

Grundy SM & Mok HYI (1976) Chylomicron clearance in normal and hyperlipidemic men. *Metabolism* **25**, 1225–1239.

Jeppesen J, Chen Y-DI, Zhou M-Y, Schaaf P, Coulston A & Reaven GM (1995) Postprandial triglyceride and retinyl ester responses to oral fat: effects of fructose. *American Journal of Clinical Nutrition* **61**, 787–791.

Kashyap ML, Barnhurt RL, Srivastava LS, Perisutti G, Allen C, Hogg E, Glueck CJ & Jackson RL (1983) Alimentary lipemia: plasma high-density lipoproteins and apolipoproteins CII and CIII in healthy subjects. *American Journal of Clinical Nutrition* **37**, 233–243.

Knapper JME, Morgan LM & Fletcher JM (1996) Nutrient-induced secretion and metabolic effects of glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1. *Proceedings of the Nutrition Society* **55**, 291–305.

Mann JI, Truswell AS & Pimstone BL (1971) Different effects of oral sucrose and glucose on alimentary lipaemia. *Clinical Science* **41**, 123–129.

Mattes RD (1996) Oral fat exposure alters postprandial lipid metabolism in humans. *American Journal of Clinical Nutrition* **63**, 911–917.

Matthews JNS, Altman DG, Campbell MJ & Royston P (1990) Analysis of serial measurements in medical research. *British Medical Journal* **300**, 230–235.

Mendeloff AL (1954) The effects of eating and sham feeding upon the absorbance of vitamin A palmitate in man. *Journal of Clinical Investigation* **33**, 1015–1021.

Murphy MC, Isherwood S, Sethi S, Gould BJ, Wright JW,

Knapper JA & Williams CM (1995) Postprandial lipid and hormone responses to meals of varying fat contents: modulatory role of lipoprotein lipase? *European Journal of Clinical Nutrition* **49**, 579–588.

Olefsky JM, Crapo P & Reaven GM (1976) Postprandial plasma triglyceride and cholesterol responses to a low fat meal. *American Journal of Clinical Nutrition* **29**, 535–539.

Peel AS, Zampelas A, Williams CM & Gould BJ (1993) A novel antiserum specific to apolipoprotein B-48: application in the investigation of postprandial lipidaemia in humans. *Clinical Science* **85**, 521–524.

Roche HM & Gibney MJ (1995) Postprandial triacylglycerolaemia – nutritional implications. *Progress in Lipid Research* **34**, 249–266.

Roche HM & Gibney MJ (1996) Postprandial triacylglycerolaemia: the effect of low-fat dietary treatment with and without fish oil supplementation. *European Journal of Clinical Nutrition* **50**, 617–624.

Roche HM & Gibney MJ (1997) Postprandial coagulation factor VII activity: the effect of monounsaturated fatty acids. *British Journal of Nutrition* **77**, 537–549.

Roche HM, Zampelas A, Jackson KG, Williams CM & Gibney MJ (1998*a*) The effect of test meal MUFA:SFA ratio on postprandial lipid metabolism. *British Journal of Nutrition* **79**, 419–424.

Roche HM, Zampelas A, Knapper JME, Webb D, Brooks C, Jackson KG, Wright JW, Gould BJ, Kafatos A, Gibney MJ & Williams CM (1998*b*) Effect of long-term olive oil dietary intervention on postprandial triacylglyerol and factor VII metabolism. *American Journal of Clinical Nutrition* (In the Press).

Sethi S, Gibney MJ & Williams CM (1993) Postprandial lipoprotein meabolism. *Nutrition Research Reviews* **6**, 161–183.

Van Amelsvoort JMM, Van Stratum P, Kraal JH, Lussenburg RN & Houtsmuller UMT (1989) Effects of varying the carbohydrate : fat ratio in a hot lunch on postprandial variables in male volunteers. *British Journal of Nutrition* **61**, 267–283.

Van Greevenbroek MMJ, van Meer G, Erkelens DW & de Bruin TWA (1996) Effects of saturated, mono- and polyunsaturated fatty acids on the secretion of apo B containing lipoprotein by Caco-2 cells. *Atherosclerosis* **121**, 139–150.

Zampelas A, Peel AS, Gould BJ & Williams CM (1994) Polyunsaturated fatty acids of the *n*-6 and *n*-3 series: effects on postprandial lipid and lipoprotein levels in healthy men. *European Journal of Clinical Nutrition* **48**, 842–848.

Zampelas A, Roche HM, Kapsolefalou M, Knapper JME, Jackson KG, Pentaris E, Tornaritis M, Hatzis C, Gibney MJ, Kafatos A, Gould BJ, Wright J & Williams CM (1998) Differences in postprandial lipaemic responses, but not traditional plasma lipid coronary risk indicators between Northern and Southern Europeans. *Atherosclerosis* (In the Press).