



# Extended effects of evening meal carbohydrate-to-fat ratio on fasting and postprandial substrate metabolism<sup>1-3</sup>

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## ABSTRACT

**Background:** High-fat and high-carbohydrate diets lead to insulin resistance, gastrointestinal adaptation, and high plasma triacylglycerol concentrations. It is unclear, however, how rapidly these changes occur.

**Objective:** We sought to determine the effects of both high-fat and high-carbohydrate evening meals on parameters of insulin resistance, hypertriglyceridemia, and gastrointestinal hormones.

**Design:** Twelve healthy men were studied on 4 separate occasions. On 2 occasions, the subjects received a high-fat evening meal (62% of energy from fat) and on the other 2 occasions the subjects received a low-fat evening meal (16% of energy from fat). The morning after each meal the subjects were administered either an oral-fat-tolerance test or an oral-glucose-tolerance test. Plasma samples were analyzed for glucose, insulin, fatty acids, 3-hydroxybutyrate, triacylglycerol, pancreatic polypeptide, peptide YY, and cholecystokinin. Postchallenge data were analyzed by two-way analysis of variance with interaction and fasting concentrations analyzed by repeated-measures analysis of variance.

**Results:** Fasting plasma concentrations of triacylglycerol were significantly elevated 12 h after each evening meal, but fatty acid and 3-hydroxybutyrate concentrations were reduced. No effects on glucose or insulin concentrations were detected. The high-fat evening meals elevated plasma cholecystokinin concentrations, reduced fasting concentrations of pancreatic polypeptide, and had no significant effect on peptide YY concentrations. The ratio of fat to carbohydrate in the evening meal produced significant effects on plasma triacylglycerol and fatty acids during both the oral-fat-tolerance and oral-glucose-tolerance tests.

**Conclusions:** The present study showed that the effects of high-fat and high-carbohydrate evening meals persist at least overnight and suggests that knowledge of recent dietary history is essential to the effective design of metabolic studies. *Am J Clin Nutr* 2002;75:505-10.

**KEY WORDS** Evening meal, high-carbohydrate meal, high-fat meal, oral glucose tolerance test, oral fat tolerance test, triacylglycerol

## INTRODUCTION

It is the common belief that high-fat diets lead to insulin resistance (1) and that high-carbohydrate diets lead to an eleva-

tion in plasma triacylglycerol concentrations (2), both conditions are recognized as important cardiovascular risk factors. Significant metabolic responses to such diets are evident after 3 d (3), but acute changes within this time frame remain unstudied. Earlier work by Frape et al (4) showed that insulin sensitivity could be reduced at lunch by increasing the proportion of fat consumed at breakfast, the so-called second-meal effect for glucose metabolism. In light of this evidence, it is important to assess the effect of short-term dietary history on both fasting and postprandial measurements.

Furthermore, a high-fat meal alters gastrointestinal function, slowing the passage of a meal out of the stomach. High-fat feeding induces gastrointestinal adaptation, ultimately reversing the slowing effect of a fatty meal and enhancing gastric emptying, intestinal transit, and lipid absorption (5). Such changes are considered to result from changes in peptide hormone receptor density and down-regulation of receptor sensitivity to circulating hormones (6, 7). As gastrointestinal motility is recognized to be an important modulator of postprandial metabolism, nutrient-induced adaptation has been implicated in the etiology of obesity (8). As with the induction of insulin resistance, gastrointestinal adaptation to high-fat diets in both humans and animals has been shown in 3 d of fat feeding (6). The present study was designed to determine how rapidly the effects of both high-fat and high-carbohydrate diets on parameters of insulin resistance, hypertriglyceridemia, and gastrointestinal adaptation become evident. In dietary intervention studies, it is important to know what proportion of the metabolic changes associated with high-fat and high-carbohydrate diets can be attributed simply to the meal eaten before the study period.

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## SUBJECTS AND METHODS

### Subjects

Twelve healthy male subjects aged  $25.6 \pm 1.2$  with a body mass index (in  $\text{kg}/\text{m}^2$ ) of  $23.1 \pm 0.3$  ( $\bar{x} \pm \text{SEM}$ ) were studied on 4 occasions allocated in random order. Subjects had no prior history of gastrointestinal, endocrine, cardiovascular or psychiatric disease and were not taking medication at the time of the study. All medical histories were confirmed with the subjects' general practitioner before inclusion in the study. Diet and exercise diaries were completed for 3 d before the study days for the assessment of typical daily energy expenditure. Subjects were excluded from the study if they typically consumed  $>35\%$  of their total energy as fat. The study protocol was approved by the South Sheffield Ethics Committee and written informed consent was obtained from all subjects.

### Test meal composition and protocol

In the 24 h preceding all 4 study meals, subjects consumed both a standard breakfast and lunch before reporting to the Royal Hallamshire Hospital, Sheffield, United Kingdom, in the evening. Subjects were instructed to avoid the consumption of alcohol and excessive exercise. On 2 occasions subjects were provided with a high-fat evening meal (62% of energy from fat and 31% of energy from carbohydrate) and on another 2 occasions they were provided with a low-fat evening meal (16% of energy from fat and 76% of energy from carbohydrate). Both the high- and low-fat evening meals were very low in dietary fiber. The meals comprised a main course of pasta (Heinz Weight Watchers, Uxbridge, United Kingdom) followed by a dairy dessert (Angel Delight; Birds, United Kingdom) that was made with either skim milk or double cream (**Table 1**). For the meals to be isoenergetic, a high-carbohydrate (glucose) drink was provided with the low-fat meal. Subjects were instructed to consume only water before reporting back to the unit the next morning. On the study day, an indwelling retrograde cannula was inserted into the back of the hand and heated to provide arterialized venous blood samples. Fasting blood samples were drawn 12 h after the evening meal and immediately before the study breakfast. Breakfast consisted of either an oral fat tolerance test (OFTT), consisting of 40 g fat in the form of dairy cream, or an oral glucose tolerance test (OGTT), consisting of 100 g glucose given in liquid form, that were randomized with the study meals. Blood samples were drawn at regular intervals for 6 h after the oral tolerance tests.

### Blood collection and analytic methods

Blood samples were collected into lithium heparin-coated tubes (Sarstedt, Leicester, United Kingdom) for the analysis of plasma glucose, insulin, fatty acids, 3-hydroxybutyrate, and triacylglycerol. Plasma for the measurement of pancreatic polypeptide, peptide YY, and cholecystokinin was collected into potassium EDTA-coated tubes containing 200 kallikrein inactivator units aprotinin/mL whole blood (Bayer Plc, Newbury, United Kingdom). Blood samples for the analysis of gut hormones and 3-hydroxybutyrate were collected during the fasting period only.

Plasma glucose, fatty acids (Roche, Lewes, United Kingdom), and triacylglycerol (Biostat, Stockport, United Kingdom) concentrations were measured by enzymatic colorimetric methods with the use of an automated analyzer (Cobas Mira; Roche) by using commercially available kits. Plasma for 3-hydroxybutyrate determination was deproteinized with 7% (wt:vol) perchloric

acid and concentrations were measured by enzymatic methods on an IL-Monarch analyzer (Instrumentation Laboratory, Warrington, United Kingdom). The intraassay CV's for all metabolite assays were  $<2\%$ .

Plasma insulin (Pharmacia and UpJohn, Milton Keynes, United Kingdom), pancreatic polypeptide, cholecystokinin (IDS, Boldon, United Kingdom), and peptide YY (Peninsula Labs, Merseyside, United Kingdom) were analyzed by using commercially available radioimmunoassays. Insulin and pancreatic polypeptide analysis were undertaken directly on unextracted plasma. Samples for cholecystokinin measurement underwent ethanol extraction (9) before analysis, and samples for peptide YY were purified by solid-phase extraction by using Sep-Pak Vac columns (Millipore Corp, Bedford, MA). The intra- and interassay CVs for all radioimmunoassays were  $<10\%$ .

### Statistical analysis

Time course data were analyzed by two-way repeated measures analysis of variance with interaction when normally distributed by using the SPSS statistical package (version 10; SPSS Inc, Chicago). The postchallenge data are also presented in tables and text in summary form, ie, fasting and peak values. Summary data were analyzed by paired Student's *t* tests. *P* values of  $<0.05$  were considered to be significant.

## RESULTS

### Fasting metabolite and hormone concentrations

Fasting metabolite and hormone concentrations measured from blood drawn 12 h after the evening meals are shown in **Table 2**. Fasting plasma triacylglycerol concentrations were significantly higher and fatty acid and 3-hydroxybutyrate concentrations significantly lower after consumption of the high-carbohydrate, low-fat evening meal than after the low-carbohydrate, high-fat evening meal. The fat-to-carbohydrate ratio of the evening meal, however, did not appear to significantly affect either the fasting plasma glucose or insulin concentration.

The high-fat evening meal resulted in higher fasting cholecystokinin concentrations the following morning, whereas the reverse was observed for pancreatic polypeptide (**Table 2**). There was no detectable difference in the peptide YY concentration after the 2 evening meals.

### Glucose and insulin concentrations

The OGTT produced a significant plasma glucose response, irrespective of the previous evening meal (**Figure 1**). Higher circulating glucose concentrations were attained when the OGTT was preceded by the high-fat, low-carbohydrate evening meal then when preceded by the low-fat, high-carbohydrate evening meal (8.8 compared with 7.8 mmol/L,  $P = 0.006$ ). The circulating insulin concentrations followed plasma glucose concentrations closely, peaking 60 min after the start of the OGTT (**Figure 2**). There was no significant effect of the evening meal composition on the plasma insulin excursion. The OFTT had little effect on plasma glucose or insulin concentrations.

### Plasma triacylglycerol and nonesterified fatty acid concentrations

The fat-to-carbohydrate ratio of the evening meal had a significant effect on plasma triacylglycerol concentrations during the



**TABLE 1**  
Macronutrient composition of the evening meal<sup>1</sup>

Evening meal	Protein	Fat	Glucose	Starch	Fiber	Energy
	g	g	g	g	g	kJ
High carbohydrate, low fat						
Vegetable lasagna	10.9	9.0	6.0	27.8	1.3	1113
Skim milk dessert	6.3	6.2	23.3	7.7	0	521
Orange drink	0.1	0	91.0	0	0	1533
Total	17.3	15.2	120.3	35.5	1.3	3524
High fat, low carbohydrate						
Vegetable lasagna	10.9	9.0	6.0	27.8	1.3	1113
Cream dessert	4.7	47.8	20.5	7.7	0	2360
Orange drink	0.1	0	1.2	0	0	42
Total	15.7	56.8	27.7	35.5	1.3	3515

<sup>1</sup>Values calculated from manufacturer's data.

OGTT (**Figure 3**). The lower fasting triacylglycerol concentration after consumption of the high-fat evening meal was maintained for another 6 h when only dietary carbohydrate was consumed. Forty-five minutes after the OGTT was administered there was a distinct peak in plasma triacylglycerol concentrations after consumption of the high-fat evening meal, but this peak was not apparent after consumption of the high-carbohydrate evening meal. There was a significant suppression of plasma fatty acid concentrations during the OGTT, although there was no detectable difference between the 2 evening meals (**Figure 4**).

There was a significant rise in plasma triacylglycerol concentrations after the OFTT (**Figure 3B**) after both evening meals. There was a trend, however, for the difference in triacylglycerol concentrations observed in the fasting state to be maintained, with higher triacylglycerol concentrations after the high-carbohydrate evening meal. Plasma fatty acids were also suppressed during the OFTT, although substantially less than during the OGTT. Before the OFTT, the high-carbohydrate evening meal was associated with lower fatty acid concentrations than was the high-fat evening meal. This lower fatty acid concentration was reestablished 6 h into the OFTT (**Figure 4**).

## DISCUSSION

Alterations in the fat-to-carbohydrate ratio in the evening meal had significant metabolic effects on both the morning fasting and the postchallenge concentrations after both the OFTT and OGTT.

### Fasting metabolite and hormone concentrations

Morning triacylglycerol concentrations were significantly higher and fatty acid and 3-hydroxybutyrate concentrations were significantly lower after the high-carbohydrate meal than after that the high-fat evening meal. Our primary hypothesis was that the fat-to-carbohydrate ratio of the evening meal and the type of carbohydrate consumed significantly affect hepatic fatty acid partitioning. We predicted that high nocturnal glucose and insulin concentrations would lead to a reduction in fatty acid oxidation (and hence ketogenesis). Fatty acids in the liver may then have been driven toward esterification and thus new triacylglycerols would form, which would be exported to the plasma as VLDL triacylglycerol. Fasting fatty acid concentrations were also suppressed after the high-carbohydrate meal, a further probable effect of high nocturnal insulin concentrations. Insulin significantly

affects lipase activity, serving to lower circulating fatty acids. Hormone sensitive lipase would be suppressed, reducing the quantity of fatty acid liberated from adipose tissue (10). Furthermore, adipose tissue lipoprotein lipase activity would be increased by insulin, which would favor the uptake of fatty acids into adipose tissue for triacylglycerol storage. Studies of the effects of carbohydrates consumed in the evening are not new (11). Improvements in morning glucose tolerance were evident after healthy subjects (12) and type 2 diabetics (13) consumed low-glycemic-index foods in the evening, although there is less evidence of the effects of high-glycemic-index carbohydrates consumed in the evening. In the case of consuming a simple carbohydrate, however, glucose would likely have a greater effect on the evening insulin excursion than would similar complex carbohydrate loads.

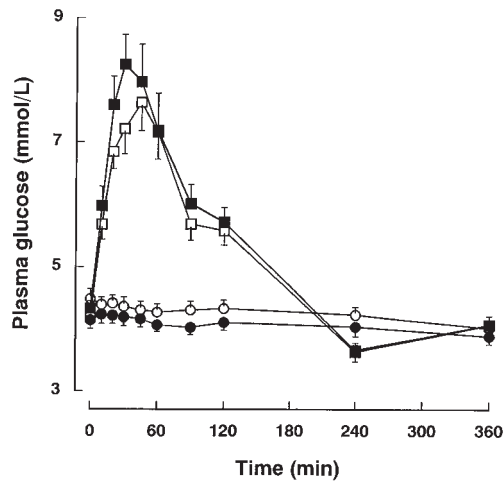
After the high-fat evening meal, it is likely that plasma fatty acid concentrations would have risen because of the spillover from triacylglycerol lipolysis and the liberation of fatty acids from adipose tissue. In the absence of significant insulin concentrations, circulating fatty acids are likely to have undergone  $\beta$ -oxidation. This theory is supported by the higher 3-hydroxybutyrate concentrations observed in the fasting state after the high-fat evening meal.

There is known to be a marked day-to-day variability in fasting metabolite concentrations (14), which, in light of these data, may be largely attributable to the nutrient composition of the previous evening meal. An energy challenge in the form of the

**TABLE 2**Morning metabolite and hormone concentrations after consumption of evening meals with differing fat-to-carbohydrate ratios<sup>1</sup>

Fasting sample	Evening meal	
	High fat-to-low carbohydrate	High carbohydrate-to-low fat
Triacylglycerol (mmol/L)	0.9 ± 0.1	1.1 ± 0.1 <sup>2</sup>
Fatty acids (μmol/L)	520 ± 33	420 ± 23 <sup>2</sup>
Glucose (mmol/L)	4.2 ± 0.1	4.3 ± 0.1
3-Hydroxybutyrate (μmol/L)	144 ± 38	65 ± 20 <sup>3</sup>
Insulin (pmol/L)	50.2 ± 5.2	53.2 ± 4.5
Cholecystokinin (pmol/L)	1.5 ± 0.4	1.1 ± 0.4 <sup>4</sup>
Pancreatic polypeptide (pmol/L)	29.5 ± 3.6	37.3 ± 4.4 <sup>4</sup>
Peptide YY (pmol/L)	19.2 ± 2.3	20.8 ± 2.3

<sup>1</sup> $\bar{x} \pm \text{SEM}$ ;  $n = 12$ .<sup>2-4</sup>Significantly different from the high-fat, low-carbohydrate meal (repeated-measures ANOVA of mean values from the 2 visits at which the fat-to-carbohydrate ratios were identical: <sup>2</sup> $P < 0.001$ , <sup>3</sup> $P < 0.01$ , <sup>4</sup> $P < 0.05$ ).

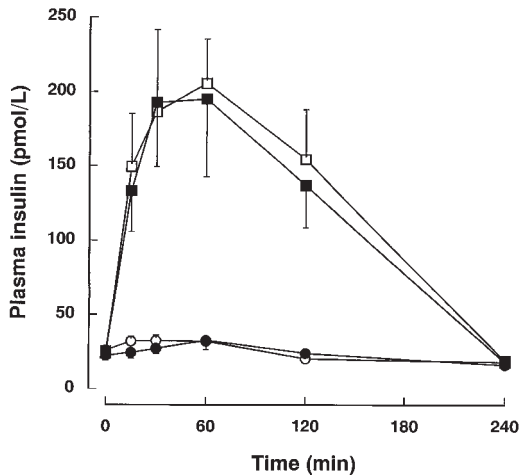


**FIGURE 1.** Mean ( $\pm$ SEM) plasma glucose concentrations during an oral-glucose-tolerance test (OGTT) when preceded by either a high-fat (■) or a high-carbohydrate (□) evening meal and during an oral-fat-tolerance test (OFTT) when also preceded by either a high-fat (●) or a high-carbohydrate (○) evening meal,  $n = 12$ . After administration of the OGTT, there was a significant time effect ( $P < 0.001$ ) and a significant evening meal  $\times$  time interaction ( $P = 0.05$ ). There were no significant effects after administration of the OFTT.

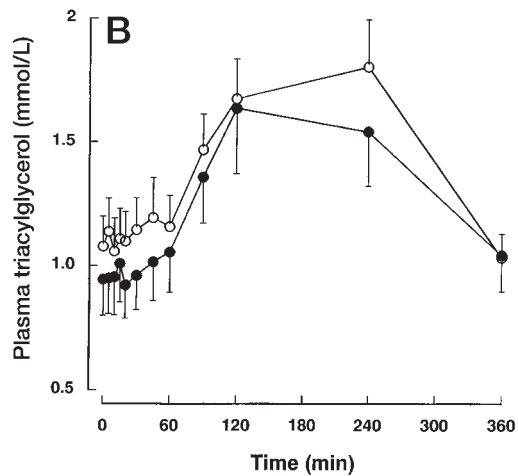
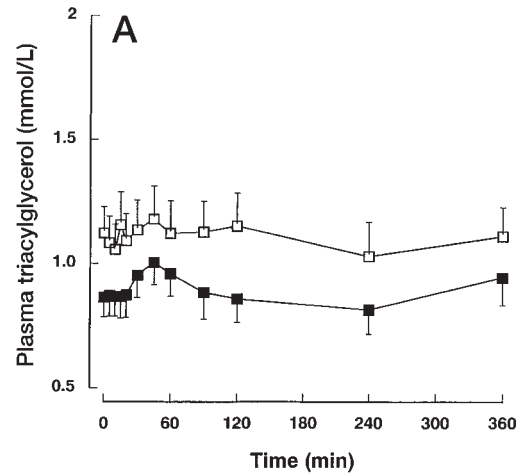
OFTT and OGTT led to metabolism that could be compared with the second-meal effect (4, 15).

#### Oral-glucose-tolerance test

Glucose tolerance after the OGTT was improved after the high-carbohydrate evening meal, although it may in fact be the case that glucose tolerance was impaired by the high-fat evening meal. One possible explanation is that high nocturnal insulin concentrations primed the system, so with insulin already present within the cellular compartment, the subjects were more



**FIGURE 2.** Mean ( $\pm$ SEM) plasma insulin concentrations during an oral-glucose-tolerance test (OGTT) when preceded by either a high-fat (■) or a high-carbohydrate (□) evening meal and during an oral-fat-tolerance test (OFTT) when also preceded by either a high-fat (●) or a high-carbohydrate (○) evening meal,  $n = 12$ . There was a significant time effect ( $P < 0.001$ ) after administration of the OGTT but no significant effects after the OFTT.

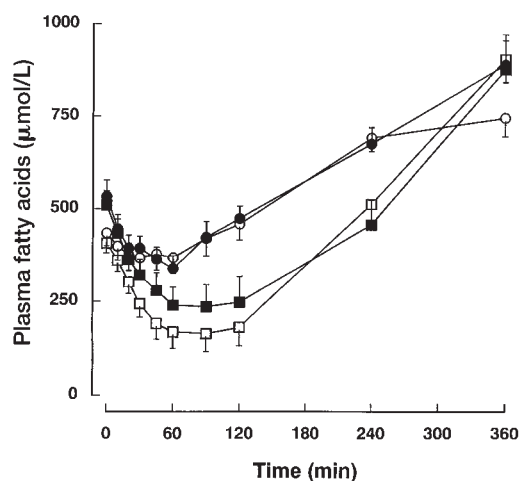


**FIGURE 3.** Mean ( $\pm$ SEM) plasma triacylglycerol concentrations during an oral-glucose-tolerance test (OGTT) when preceded by either a high-fat (■) or a high-carbohydrate (□) evening meal (A) and during an oral-fat-tolerance test (OFTT) when also preceded by either a high-fat (●) or a high-carbohydrate (○) evening meal (B),  $n = 12$ . After administration of the OGTT, there was a significant time effect ( $P = 0.002$ ), effect of evening meal ( $P < 0.001$ ), and a time  $\times$  meal interaction ( $P = 0.05$ ). After administration of the OFTT, there was a significant time effect ( $P < 0.001$ ) and a nearly significant evening meal effect ( $P = 0.078$ ).

insulin sensitive, enhancing fatty acid suppression and insulin-independent glucose disposal and inhibiting hepatic glucose production. Note, however, that fatty acid concentrations, suppressed after the high-carbohydrate evening meal, which were suppressed again 6 h after the OGTT (Figure 4). This observation is yet to be explained. Another concept is that the high-fat evening meal impaired morning glucose tolerance. Frape et al (4, 16, 17) showed in a series of experiments that began in the late 1980s that the ingestion of a fatty breakfast impairs glucose tolerance at lunchtime, a result attributable primarily to increased fatty acid concentrations. It is yet to be determined whether elevations in fasting fatty acids alone could be responsible for the apparent changes in insulin sensitivity in this study.

When the high-carbohydrate evening meal was consumed, plasma triacylglycerol concentrations remained elevated for 6 h after administration of the OGTT (Figure 3A). High insulin





**FIGURE 4.** Mean ( $\pm$ SEM) plasma fatty acid concentrations during an oral-glucose-tolerance test (OGTT) when preceded by either a high-fat (■) or a high-carbohydrate (□) evening meal and during an oral-fat-tolerance test (OFTT) when also preceded by either a high-fat (●) or a high-carbohydrate (○) evening meal,  $n = 12$ . After administration of the OGTT, there was a significant time effect ( $P < 0.001$ ). After administration of the OFTT there was a significant time effect ( $P < 0.001$ ) and a significant evening meal  $\times$  time interaction ( $P = 0.001$ ).

concentrations may have led to fatty acid esterification rather than to continued oxidation during the OGTT.

A second-meal effect of plasma triacylglycerol concentrations rising rapidly after the consumption of a meal when a high-fat meal has been eaten previously is also well documented (15, 18). However, in earlier studies this early plasma triacylglycerol peak occurred at lunch, 5–6 h after a fatty breakfast. It is important to note that the effect is still present 12 h after a meal rich in fat, can be triggered by the ingestion of glucose alone, and does not need to be in the presence of additional dietary fat. The origin of this triacylglycerol peak is still under debate. The first possibility is that eating a large, high-fat evening meal may have resulted in partial gastric retention until the morning because the delay in gastric emptying is exaggerated when the meal is consumed late in the evening (19). Another possibility is that preformed chylomicron-triacylglycerol are released from another enteric store (either from the lymph fluid or from within the enterocyte itself) and stimulated by either glucose or insulin. Insulin and glucose were shown to raise plasma triacylglycerol in other studies (20), although the metabolic implications of this remain unknown.


#### Oral-fat-tolerance test

The decrease in fatty acid concentrations after the OFTT was greater after the high-fat evening meal had been consumed. This effect may have been due primarily to differences in the original fasting concentration and was noted previously (21). In both instances, the OFTT resulted in circulating fatty acid concentrations in excess of fasting concentrations. This was likely due to spillover from chylomicron-triacylglycerol lipolysis after a large fat load rather than to any increase in fatty acid mobilization from adipose tissue.

The pattern of triacylglycerol concentrations found in the present study followed the overall pattern reported after both short-term and longer-term (2) adaptation to high-carbohydrate diets. In the present study, the higher triacylglycerol and lower fatty

acid concentrations after consumption of the high-carbohydrate evening meal than after the high-fat meal were likely due to acute nocturnal priming of the insulin system in a similar way to that described after the administration of the OGTT.

The small but significant elevation in fasting cholecystokinin observed in this study could indicate that peptide secretion and motility adaptation begin within hours of consuming a high-fat meal, or it may simply be due to the residual amount of fat left-over from the evening meal. There is limited information on the effects of diet on pancreatic polypeptide concentrations in humans, although a high protein content is believed to be the main stimulus for pancreatic polypeptide release (22). Pancreatic polypeptide has been strongly implicated as a satiety hormone, with lower concentrations being noted during obesity (23) and now, in the present study, after a single high-fat evening meal. The experimental evidence that the nutrient composition of an evening meal may influence both appetite and food intake the next day confirms previous qualitative observations and warrants further in depth investigation.

The composition of the evening meal (fat or carbohydrate) affected the opposite substrate the next day, ie, a high-fat meal negatively influenced glucose metabolism the next day and a high-carbohydrate meal had the strongest effect on subsequent triacylglycerol metabolism. The so-called second meal effect for plasma triacylglycerol release was shown to occur 12 h after consumption of the first high-fat meal, an important observation in this research area. As such, postprandial studies failing to recognize the importance of recent dietary history may add yet another variable to an already complex situation. There is limited evidence from the present study to show that short-term gut adaptation, known to occur with a high-fat diet, commences after the first meal, although more work is needed to determine the sequence of events that lead to changes in motility, transit, and absorption. 

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