Timing of vagal stimulation affects postprandial lipid metabolism in humans

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ABSTRACT

Background: Vagal stimulation combined with an oral fat load enhances postprandial lipemia in animals and humans. Objective: We assessed whether the observed postprandial increase in plasma lipids could be explained by changes in exogenous (chylomicron) or endogenous (VLDL) lipid metabolism and whether the timing of vagal stimulation in relation to fat intake was important. Design: Vagal stimulation was achieved by using the modified sham feeding (MSF) technique, in which food is tasted and chewed but not swallowed. Seven healthy men consumed an oral fat load (50 g) on one occasion (control protocol). On 2 other occasions, they consumed an oral fat load combined with MSF of an appetizing meal. MSF was performed for either 1 h before or 1 h after the oral fat load. Blood was collected for 7 h and was analyzed for hormones and metabolites. Results: The postprandial triacylglycerol response differed significantly ($P < 0.001$) between the 3 protocols. Both MSF studies resulted in significantly higher plasma pancreatic polypeptide concentrations compared with the control. Compared with MSF for 1 hour after fat intake, MSF for 1 h before fat intake resulted in significantly higher plasma insulin concentrations ($P = 0.013$), a more rapid rise in chylomicron triacylglycerol concentrations ($P = 0.04$), and higher VLDL triacylglycerol and apolipoprotein B-100 concentrations. Conclusions: Vagal stimulation enhanced postprandial lipemia via effects on both chylomicron and VLDL metabolism. MSF before fat intake had more dramatic effects on postprandial lipemia than did MSF after fat intake, possibly because of increased parasympathetic activity at the time of ingestion. Am J Clin Nutr 2002;76:71–7.

KEY WORDS Triacylglycerol, pancreatic polypeptide, insulin, apolipoprotein B-100, very-low-density lipoprotein, VLDL, chylomicron, postprandial lipemia, cardiovascular disease, sham feeding, vagal stimulation, men

INTRODUCTION

The magnitude and duration of postprandial lipemia are increasingly being recognized as important indicators of the risk of cardiovascular disease (1). It has been proposed that successive, daily exposure to atherogenic lipoprotein remnants in the postprandial period may lead to gradual formation of atherosclerotic lesions (2). Understanding the nutritional factors that influence postprandial lipemia is therefore of considerable interest. Although some research has been done on the effects of different dietary components on postprandial lipemia, less attention has been paid to modulation of gastrointestinal involvement in this response (3).

Gastrointestinal responses to food are generally divided into the cephalic, gastric, and intestinal phases. Cephalic-phase responses are physiologic changes initiated by the parasympathetic nervous system when there are food cues such as the thought, sight, smell, and taste of palatable food (4). The primary metabolic changes occurring in this early ingestive period are gallbladder contraction (5), gastrin and gastric acid production (6), and stimulation of both the exocrine and endocrine pancreas (7). The cephalic phase of insulin secretion is now regarded as important in determining postprandial glucose tolerance (8, 9).

Evidence also suggests that orosensory stimuli may modulate postprandial lipemia (10). Plasma triacylglycerol concentrations were shown to increase in both animal (11) and human (10) studies when cephalic-phase stimuli followed a gastric fat load, as compared with giving the fat load alone. Insulin plays a major role in the coordination of postprandial lipid metabolism, and insulin resistance is associated with impairment of lipid tolerance (12). This suggests that orosensory stimulation that occurs before ingestion of a fat load and raises the plasma insulin concentration might have even more marked effects than orosensory stimulation that occurs after fat ingestion, although this has not been tested.

The postprandial increase in plasma triacylglycerol concentration can be of both exogenous (dietary) origin and endogenous (hepatic) origin, although the effects of orosensory stimuli on
these individual components of lipemia remain unknown. Whether orosensory factors increase plasma concentrations of lipoproteins that raise atherogenic risk in humans warrants investigation.

Therefore, the aim of the present study was to further examine the origin of the increase in plasma triacylglycerol concentration observed after an oral fat load with vagal stimulation and also to investigate whether the timing of the cephalic phase in relation to fat intake is an important factor in the augmentation of postprandial lipemia. Vagal stimulation was achieved by using the modified sham feeding (MSF) technique, as described previously (13, 14). However, in the present study, the timing of MSF was varied. For each subject, on 3 occasions in random order, MSF preceded an oral fat load for 1 h, followed the oral fat load for 1 h, or was absent altogether. Results from this study were published previously in abstract form (15).

SUBJECTS AND METHODS

Subjects

Seven men (± age: 22.7 y, range: 19–29 y) participated in this study. The baseline characteristics of the subject group are shown in Table 1. All the subjects were healthy and none were taking any medications. All subjects gave their written, informed consent, and the study was approved by the Central Oxfordshire Research Ethics Committee.

Study protocol

To standardize the subjects’ nutritional state before the study, all subjects consumed a low-fat (<10 g fat) evening meal and were then instructed to fast overnight (for 12–14 h) and to avoid both alcohol and exercise. The next morning, an antecubital cannula was inserted under local anesthetic (1% lidocaine). The subjects were supervised until the first fasting blood sample was obtained at t = 0. Plasma glucose and triacylglycerol concentrations were measured with kits from Instrumentation Laboratory (Warrington, United Kingdom). Plasma glucose and triacylglycerol concentrations were measured with kits from Alpha Laboratories (Eastleigh, United Kingdom). All the metabolites were measured enzymatically with an IL Monarch automated analyzer (Instrumentation Laboratory). Insulin was measured with a radioimmunoassay by using a commercially available kit (Pharmacia & Upjohn, Milton Keynes, United Kingdom). Metabolites were batch-analyzed and exhibited an intraassay CV of <2.5%. Whole blood for pancreatic polypeptide (PP) determination was collected into plain tubes for serum radioimmunoassay (Eurodiagnostica, Boldon, United Kingdom). All samples for hormone analysis were frozen according to the kit manufacturers’ instructions and batch-analyzed; these analyses had both inter- and intraassay CVs of <10%.

Triacylglycerol-rich lipoprotein fractions were prepared from plasma as follows. Chylomicron-rich (S > 400) fractions were prepared by layering 0.75-mL portions of plasma underneath a solution with a density of 1.006 kg/L. The samples were then centrifuged at 55,000 × g for 20 min at 4°C (Optima TLX centrifuge; Beckman Instruments UK, High Wycombe, United Kingdom). The chylomicron-rich fraction was then separated by using a CentriTube tube slicer (Beckman Instruments UK). The VLDL-rich fraction (S: 60–400) was prepared from the infranate prepared as described previously. Samples were centrifuged in a TLA 100.4 rotor (GPR centrifuge; Beckman Instruments UK) at 41,700 × g for 2.5 h at 4°C. The lipid-rich fraction was again separated by tube-slicing.

The concentration of apolipoprotein B-100 was measured in the triacylglycerol-rich lipoprotein fractions by using analytical SDS-PAGE as described previously (16). Lipoprotein samples were first delipidated by using a methanol-diethyl ether solvent system. The resulting protein material was rediluted in a 0.15 mol/L sodium phosphate buffer containing 5% β-mercaptoethanol. After subsequent denaturation, samples were frozen at −20°C. Electrophoresis was performed with precast 3–27% acrylamide mini-gels (ICN, Basingstoke, United Kingdom) by using the mini-Protein II vertical electrophoresis equipment (BioRad Laboratories, Hemel Hempstead, United Kingdom) connected to an PowerPac 300 (BioRad Laboratories) power supply.

Modified sham feeding analysis

The MSF meal was weighed before and after mastication. The expectorated meal was then homogenized in a Waring blender (Dynamics Corporation of America, New Hartford, CT) and a sample was analyzed for dietary triacylglycerol content. The lipid in the sample was extracted in a solution of 2:1 chloroform methanol followed by transesterification with methanolic sulfuric acid. Total fatty acid analysis was performed with the Chrompack 9000 gas chromatograph with heptadecanoic acid as the internal standard (Chrompack, London) (17).
RESULTS

Expectorated meals

The weights of the MSF meal before and after chewing were compared; this yielded a mean (±SEM) recovery rate of 103.4 ± 3.2% compared with 97.6 ± 2.9% when the recovery was calculated from gas chromatography analysis.

Plasma triacylglycerol and lipoproteins

Oral fat ingestion resulted in an elevation of plasma triacylglycerol concentrations compared with fasting concentrations during all 3 protocols (Figure 1), although the pattern of the lipemic response differed significantly between the test protocols (P < 0.001). Postprandial concentrations remained above fasting concentrations for the duration of the study.

Triacylglycerol concentrations in the chylomicron-rich fraction (S_f > 400) rose after all 3 protocols (Figure 2). The results indicate a similar pattern to that observed for plasma triacylglycerol. When the oral fat load was preceded by MSF (protocol B), there was a more rapid rise in the triacylglycerol concentration, which peaked sooner than when oral fat was given without MSF (control, protocol A) (P = 0.04). There were no significant differences between the 3 protocols, however, in the peak values attained or the integrated responses (ie, IAUCs).

The pattern of VLDL-triacylglycerol (S_f: 60–400) concentrations was also similar to that observed for plasma triacylglycerol concentrations. Both of the MSF protocols (B and C) resulted in an increase in VLDL triacylglycerol (Figure 3A); however, because of a high degree of interindividual variation, only protocol B resulted in a significantly higher integrated response compared with the control (Figure 3B).

After protocol A, apolipoprotein B-100 concentrations were initially suppressed before they rose above fasting concentrations. With protocols B and C, this initial suppression was absent; the concentration increased immediately after the start of MSF. For both protocols B and C, the apolipoprotein B-100 concentration remained above baseline values for the duration of the study (Figure 4).

Other plasma metabolites

Plasma glucose concentrations remained close to fasting concentrations during all 3 protocols, although there was a small decrease immediately after ingestion of the fat load (Figure 5). MSF had no additional effect when compared with the control protocol.

Plasma fatty acid concentrations fell initially in all 3 protocols but returned to fasting concentrations by the end of the time period studied (data not shown). There was a greater degree of suppression after both of the MSF protocols, resulting in a lower integrated response. However, because of a high degree of interindividual variation, this effect was not significant.

Plasma hormones

Plasma PP concentrations rose significantly after fat ingestion in all 3 protocols (Figure 6), although the pattern of peptide release differed significantly (P = 0.018). The rise in PP was rapid after fat ingestion in both protocols A and C, with the combination of oral fat and MSF in protocol C appearing to be additive. The PP concentrations in all 3 protocols remained elevated for 120 min after fat ingestion.
Insulin concentrations increased after fat ingestion, although the pattern differed significantly ($P = 0.013$) between the protocols (Figure 7). Protocol B caused insulin to peak more rapidly ($P = 0.05$) and to reach a significantly higher peak ($P = 0.01$). The insulin response after protocol C was not significantly different from that after protocol A.

**DISCUSSION**

In this study, we confirmed earlier observations that orosensory stimulation enhances postprandial lipemia (10). We have now shown that this results from a more rapid appearance of chylomicron triacylglycerol in the plasma and significant elevations of both VLDL triacylglycerol and apolipoprotein B-100 particles. This is a novel effect that was not attributed to cephalic phase stimuli previously.

As with all orosensory studies in human subjects, it is important to verify whether the observed changes in physiology resulted from vagal stimulation or from the intestinal phase of digestion. PP is now known to be a reliable marker of parasympathetic vagal activity (18) and has been used frequently in sham feeding studies (19, 20). In our study, both of the MSF protocols resulted in elevations of circulating PP concentrations to values that were above baseline concentrations and were higher than values observed with the oral fat load alone (Figure 6).

Weighing the expectorated food provides only a crude measure of swallowing because of the additional weight of the saliva.
In this study, we attempted to improve on this measurement by analyzing the fat content of the expectorated food directly with gas chromatography and by comparing the results with expected values. The use of gas chromatography for assessing the swallowing of fatty food could become an additional research tool in such studies. Both of these recovery methods indicated that little (<1 g) of the modified sham-fed fat had been swallowed. This was further confirmed by assessing the plasma glucose data. There were no significant differences in the glycemic response between the meal protocols, which we would not have expected if significant amounts of the sham-fed meal (which contained 53 g carbohydrate) had been inadvertently swallowed. We can conclude, therefore, that the additional effects on the plasma triacylglycerol concentrations after orosensory stimulation resulted from increased parasympathetic activity and not from ingestion of additional nutrients.

When the oral fat load was preceded by MSF, the plasma chylomicron-triacylglycerol concentration rose immediately. This pattern is not normally observed after oral fat loads in fasted subjects (21, 22). The rapid appearance of plasma chylomicrons was reported in sequential meal studies in which one meal followed another 5–6 h later (17), although in such circumstances, preformed chylomicron particles from the earlier high-fat meal are released from an enteric store. It is unlikely that this pattern would explain our data, however, because our subjects consumed a low-fat evening meal ≥12 h previously and cephalic-phase stimuli alone were shown to be an inadequate stimulus for this so-called second-meal effect (14).

Vagally induced changes in gastric emptying of the fat load may be a more likely explanation. Recent studies showed that the vagus nerve plays an important role in the regulation of proximal gastric tone, gastric contractions, and pyloric tone (23) and that PP concentrations are inversely related to the gastric half-emptying time (24). Extrapolating this to our study, at \( t = 0 \), the PP concentrations were elevated in protocol B because of the MSF; this may indicate that fat consumed at this time would empty from the stomach more rapidly, resulting in the more rapid appearance of chylomicrons in the plasma. Gastric emptying was measured during MSF studies previously, although this was usually done by measuring the emptying of a simultaneously given meal (as in protocol C) (25) or water (26), or simply by measuring the motility of an empty stomach (27). The overall effects of MSF on gastric emptying, as assessed in such studies, are still unclear. However, the possibility that background parasympathetic activity could influence the gastric emptying of a sequential meal warrants further investigation.

An additional explanation for the rapid appearance of chylomicron triacylglycerol in the plasma may be local changes in the rate

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**FIGURE 5.** Mean (±SEM) plasma glucose concentration after ingestion of 50 g fat \( (t = 0) \) without modified sham feeding (MSF) (○), with MSF for 1 h before fat ingestion (●), and with MSF for 1 h after fat ingestion (■); \( n = 7 \). The time effect was significant, \( P < 0.001 \) (repeated-measures ANOVA).

**FIGURE 6.** Mean (±SEM) plasma pancreatic polypeptide concentration after ingestion of 50 g fat without modified sham feeding (MSF) (○), with MSF for 1 h before fat ingestion (●), and with MSF for 1 h after fat ingestion (■); \( n = 7 \). There was a significant time effect \( (P < 0.001) \), meal effect \( (P = 0.018) \), and meal × time interaction \( (P = 0.01) \) (repeated-measures ANOVA).

**FIGURE 7.** Mean (±SEM) plasma insulin concentration after ingestion of 50 g fat without modified sham feeding (MSF) (○), with MSF for 1 h before fat ingestion (●), and with MSF for 1 h after fat ingestion (■); \( n = 7 \). There was a significant time effect \( (P < 0.001) \), meal effect \( (P = 0.013) \), and meal × time interaction \( (P < 0.001) \) (repeated-measures ANOVA).
of mesenteric blood and lymph flow that could drain the fat away from the site of absorption to the peripheral circulation more rapidly. Plausible candidates for this effect during the cephalic period are insulin (28); vasoactive intestinal polypeptide, a neurotransmitter found in parasympathetic postsynaptic nerve fibres (29); and bile salts released vagally from the gallbladder (30). Although the splanchnic circulation is known to have parasympathetic innervation, mesenteric blood flow was not measured in previous MSF studies and so additional effects of these vasodilators released during the cephalic phase cannot be ruled out.

The most significant finding in this study was the increase in the VLDL-triacylglycerol response with MSF. Apolipoprotein B-100 particles are secreted solely by the liver (31), and so in this study the significant increase in particles containing apolipoprotein B-100 present in the triacylglycerol-rich fraction indicated that the main effects on triacylglycerol were endogenous in origin. It is now accepted that up to 80% of triglyceride-rich lipoprotein particles released during postprandial lipemia are of hepatic origin (32), and VLDL synthesis increases by up to 50% after fat intake (33). From our data, however, we cannot determine accurately whether the differences we observed were the result of enhanced synthesis or reduced clearance of VLDL, or a combination of both. Chylomicrons and VLDL share the same lipolytic pathway (31), but larger chylomicrons appear to be the preferred substrate for lipoprotein lipase, resulting in the postprandial accumulation of VLDL and apolipoprotein B-100 (34). One possibility in this study is that the more rapid appearance of chylomicron particles, and hence the increased initial clearance rate when the oral fat was preceded by MSF, may have inhibited the clearance of VLDL because of competition for lipoprotein lipase binding sites. VLDL synthesis may have increased also. Additional chylomicron remnants may have been taken up by the liver for increased VLDL production (35), although, because this was an oral fat tolerance test and not a mixed meal, it is unlikely that glucose was a precursor.

Another explanation is that MSF initiated direct vagal effects on the liver, causing enhanced secretion of both VLDL triacylglycerol and apolipoprotein B-100. The liver is highly innervated by both sympathetic and parasympathetic nerves (36), although liver metabolism during the cephalic phase has not been investigated previously. Hepatic membranes contain specific PP receptors (37); in vitro, PP was shown to enhance hepatic triacylglycerol synthesis and release in isolated rat hepatocytes (38). However, the in vivo effects on hepatic lipid metabolism are unknown.

Both MSF protocols led to increases in VLDL triacylglycerol and the number of apolipoprotein B-100-containing particles, although the metabolic challenge to the subject was identical in all 3 protocols. The magnitude of the response appeared to differ depending on whether the cephalic stimulation occurred in conjunction with the oral fat load (protocol C) or before the oral fat load (protocol B). Although the triacylglycerol concentrations in protocol C were well above those obtained when the fat load was given alone, this increase was not statistically significant. This is probably because of the small number of subjects studied. MSF before nutrient ingestion resulted in more dramatic effects on postprandial lipemia because of increased parasympathetic activity at the time of fat ingestion. This may be important with regard to patterns of eating behavior in societies where meals often consist of several courses eaten a few minutes apart or sequential meals taken close together.

REFERENCES


