Prolonged effects of modified sham feeding on energy substrate mobilization[1–3]

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ABSTRACT

Background: Vagal stimulation in response to nutrients is reported to elicit an array of digestive and endocrine responses, including an alteration in postprandial lipid metabolism.

Objective: The objective of this study was to assess whether neural stimulation could alter hormone and substrate metabolism during the late postprandial phase, with implications for body fat mobilization.

Design: Vagal stimulation was achieved by using the modified sham feeding (MSF) technique, in which nutrients are chewed and tasted but not swallowed. Ten healthy subjects were studied on 3 separate occasions, 4 wk apart. Five hours after a high-fat breakfast (56 g fat), the subjects were given 1 of 3 test meals allocated in random order: water, a lunch containing a modest amount of fat (38 g), or MSF (38 g fat). Blood was collected for 3 h poststimulus for hormone and metabolite analyses.

Results: Plasma insulin and pancreatic polypeptide concentrations peaked at 250% and 209% of baseline concentrations within 15 min of MSF. The plasma glucose concentration increased significantly ($P = 0.038$) in parallel with the changes observed in the plasma insulin concentration. The nonesterified fatty acid concentration was significantly suppressed ($P = 0.006$); maximum suppression occurred at a mean time of 114 min after MSF. This fall in nonesterified fatty acid was accompanied by a fall in the plasma glucagon concentration from 122 to 85 pmol/L ($P = 0.018$) at a mean time of 113 min after MSF.

Conclusions: Effects on substrate metabolism after MSF in the postprandial state differ from those usually reported in the postabsorptive state. The effects of MSF were prolonged beyond the period of the cephalic response and these may be relevant for longer-term metabolic regulation. *Am J Clin Nutr* 2001; 73:111–7.

KEY WORDS Control group, modified sham feeding, nonesterified fatty acid, glucagon, insulin, pancreatic polypeptide, gastrin, glucose, postprandial period

INTRODUCTION

Gastrointestinal responses to nutrient intake can be subdivided into cephalic, enteric, and intestinal phase responses. Cephalic responses consist of vagal cholinergic activity initiated by the thought, sight, smell, and taste of food and are strategically placed to influence both the absorption and the utilization of incoming substrates.

Although vagal responses are transient, they may have longer-lasting metabolic effects. Vagal responses have been implicated in the improvement of postprandial glucose tolerance (1) and satiety (2). Elevations in postprandial lipemia over a period of several hours were reported when cephalic stimulation was combined with a gastric fat load compared with when the gastric load was given alone (3, 4), suggesting that there are also cephalic influences on the absorption and metabolic disposition of dietary fat.

Sham feeding, in which a gastric or esophageal fistula is fitted to an experimental animal and nutrients bypass the small intestine, may not be an appropriate model with which to study cephalic phase stimuli. Not only is it impossible to distinguish between oral taste receptors and pharyngeal and gastric mechano- or chemoreceptors stimulated before food exits through the fistula, but also the choice of food given to the animal is limited by the design of the fistula. Modified sham feeding (MSF), or the "chew-and-spit" technique, may provide a more selective test of vagal activity (5). One difficulty with studies of MSF is the incidental swallowing of nutrients. Although some authors weighed the expectorant as a crude index of swallowing (3), it may be preferable to use hormonal markers, eg, plasma cholecystokinin. Cholecystokinin is released via vagal mechanisms from cells of the duodenum by what is believed to be a fatty acid chain length–dependent mechanism (6); minimal evidence in the literature suggests release during MSF (7).

Most studies of MSF in humans were carried out in the postabsorptive (overnight fasted) state, when absorption of nutrients from the gastrointestinal tract has ceased and gastrointestinal effects therefore are not evident. In addition, metabolic patterns differ in the postabsorptive and postprandial states. Because humans in Western societies spend most of their day in

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TABLE 1
Fasting variables for the subjects

	Mean	Range
Age (y)	35.5	21–55
BMI (kg/m^2)	24.1	19.7–26.9
Plasma glucose (mmol/L)	4.98	4.24–5.39
Plasma triacylglycerol (mmol/L)	0.86	0.39–1.33
Total cholesterol (mmol/L)	4.98	3.4–6.21

a postprandial state, it is of interest to understand cephalic effects on nutrient handling and metabolic disposition during this period.

Therefore, we investigated substrate and hormonal responses to cephalic stimulation, using the MSF model, in subjects in the late postprandial phase, when nutrient absorption may still be occurring and when the metabolic pattern is distinct from that after an overnight fast. We compared these responses with those to ingestion of water and to real feeding and measured cholecystokinin and pancreatic polypeptide (PP) responses as indicators of successful sham feeding.

SUBJECTS AND METHODS

Subjects

Ten subjects (6 female) participated in this study. Baseline fasting values for the subjects are presented in **Table 1**. All the subjects were healthy and none were taking any medication likely to affect lipid metabolism or gastrointestinal motility. For the female subjects, the study days were standardized to the second half of the menstrual cycle. All subjects gave written informed consent and the study was approved by the Central Oxfordshire Research and the University of Reading Ethics Committees.

Study protocol

The study was conducted as a randomized trial. To standardize the nutritional state before the study, all the subjects consumed a low-fat evening meal and were then instructed to fast overnight (12–14 h). The next morning, an antecubital cannula was inserted under local anesthetic (1% lignocaine) and the subjects were provided with a standard high-fat breakfast ($t = -300$ min) and supervised until the beginning of the sampling period. At $t = 0$, subjects received, in random order, water alone, MSF, or a meal containing a modest amount of fat. Blood samples were taken 10, 20, 30, 60, 90, 120, and 180 min after the lunch stimulus. During the MSF study, additional blood samples were taken 2 and 5 min after the lunch stimulus. Details of the test meals provided are given in **Table 2**.

Modified sham feeding

All food for MSF was prepared in a room that was separate from the clinical area. The MSF involved the subjects chewing the test meal and then expectorating it from their mouths when they would normally swallow it. The subjects repeated the procedure until the meal had been fed completely (10–15 min). To aid in removal of the food, the subjects were provided with known volumes of water to rinse their mouths.

Blood analysis

Whole blood for measurement of metabolites and insulin was collected into heparin-containing syringes (Monovette; Sarstedt, Inc, Newton, NC). Plasma glucose and triacylglycerol (Instrumentation Laboratory, Warrington, United Kingdom) and nonesterified fatty acid (NEFA) concentrations (Wako NEFA C kit; Alpha Laboratories Ltd, Eastleigh, United Kingdom) were measured enzymatically by using an IL Monarch automated analyzer (Instrumentation Laboratory). Insulin was measured by radioimmunoassay with a commercially available kit (Pharmacia & Upjohn, Milton Keynes, United Kingdom). Metabolites were batch analyzed and had an intraassay CV of <2.5%. Whole blood for measurement of gastrin and PP was collected into plain tubes for serum radioimmunoassay (Eurodiagnostica, Boldon, United Kingdom). Blood for measurement of cholecystokinin and glucagon (DPC Ltd, Llanberis, United Kingdom) was collected into potassium EDTA–containing tubes with 200 kIU aprotinin/mL blood (Trasylol, Bayer PLC, Newbury, United Kingdom). All of the hormones were analyzed by using a double-antibody polyethylene glycol precipitation method. Except for cholecystokinin, which was extracted in ethanol before analysis (9), the assays were undertaken directly on unextracted plasma or serum. All samples for hormone analysis were frozen according to the instructions of the manufacturers of the kit and then batch analyzed; the inter- and intraassay CV was <10%.

Statistics

The time course of the postprandial metabolite and hormone responses between test situations was analyzed by a two-factor repeated-measures analysis of variance with interaction (SPSS, Chertsey, United Kingdom). The area under the curve (AUC) and the integrated AUC (IAUC) were calculated by using the trapezoidal method. Summary statistics (peak height, AUC, and IAUC) were computed by using paired Student's t tests (10). A P value ≤ 0.05 was considered significant. The significance levels of multiple comparisons were calculated by using a Bonferroni correction factor.

TABLE 2
Nutrient composition of the test meals[1]

Meal	Time of meal	Energy	Protein	Fat	Carbohydrate
	min	*kJ*	*g*	*g*	*g*
Breakfast[2]	−300	3474	6.1	56.0	72.6
MSF/modest-fat meal[3]	0	2733	26.1	38.3	53.2

[1] Determined from manufacturer's data and from food tables (8).
[2] High-fat milk shake, banana, and corn flakes.
[3] Cheese pizza served with a drink of full-fat milk and cream.

TABLE 3
Summary data for postprandial nonesterified fatty acid (NEFA) and glucose responses after water (control), modified sham feeding (MSF), and a meal containing a modest amount of fat[1]

	Control	MSF	Meal
NEFA (mmol/L)			
Basal	0.42 ± 0.04	0.51 ± 0.06	0.49 ± 0.03
Peak	0.61 ± 0.05[2]	0.60 ± 0.05	0.58 ± 0.05[3]
Nadir	0.37 ± 0.04	0.34 ± 0.04[2,4]	0.21 ± 0.03[2,5]
AUC	88.9 ± 8.3	81.6 ± 8.0	67.1 ± 5.7
IAUC	11.9 ± 6.1	−11.9 ± 9.8	−22.2 ± 6.3[5]
Glucose (mmol/L)			
Basal	5.2 ± 0.1	4.9 ± 0.1	5.2 ± 0.2
Peak	5.1 ± 0.1	5.9 ± 0.3[3,6]	6.6 ± 0.2[2,5]
AUC	867 ± 22	912 ± 31	1066 ± 32[5]
IAUC	−75 ± 26	14 ± 31	124 ± 47
Triacylglycerol (mmol/L)			
Basal	1.31 ± 0.21	1.68 ± 0.34	1.44 ± 0.27
Peak	1.33 ± 0.24	1.70 ± 0.34	1.62 ± 0.29[3]
AUC	196 ± 41	236 ± 50	254 ± 46[6]
IAUC	−40.6 ± 10.9	−65.6 ± 17.2	−5.1 ± 8.7

[1] $\bar{x}$ ± SEM; n = 10. Area under the curve (AUC) and integrated AUC (IAUC) are expressed as mmol·L^{-1}·180 min^{-1}.
[2,3] Significantly different from basal values (Student's t test): [2] $P \leq 0.01$, [3] $P \leq 0.05$.
[4] Significantly different from meal, $P \leq 0.05$ (Student's t test).
[5,6] Significantly different from control (Student's t test); [5] $P \leq 0.01$, [6] $P \leq 0.05$.

RESULTS

Plasma nonesterified fatty acid

The postprandial NEFA responses after the 3 test meals are summarized in **Table 3**. The ingestion of water resulted in a gradual increase in the circulating NEFA concentration (**Figure 1**). Feeding produced an initial NEFA peak 39 ± 9 min ($\bar{x}$ ± SD) after the meal (P = 0.01), followed by a rapid NEFA suppression that was sustained until the end of the sampling period. MSF also resulted in NEFA suppression, although significantly less than was observed with real feeding. The pattern of NEFA suppression during MSF differed significantly from both the fed and the water-ingestion pattern (Figure 1). Maximum suppression occurred 114 ± 16 min after MSF.

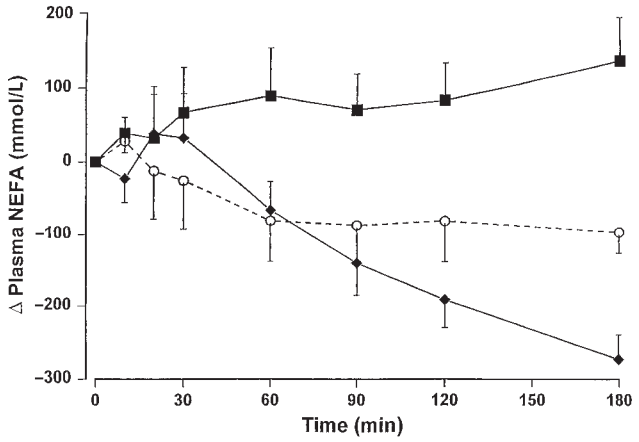

FIGURE 1. Mean (±SEM) change in plasma nonesterified fatty acid (NEFA) concentration after water (■), modified sham feeding (○), and a meal containing a modest amount of fat (◆). The subjects had consumed a high-fat breakfast 5 h previously. n = 10. Significant time effect ($P < 0.001$) and meal × time interaction ($P < 0.001$) by repeated-measures ANOVA.

Glucose

With water ingestion there was a gradual decline in the plasma glucose concentration (**Figure 2**). Feeding caused a significant increase in plasma glucose concentrations, which reached a peak 106 ± 19 min after the meal. This peak concentration was maintained for a further 1.5 h, approximately until the end of the sampling period. MSF produced a rapid glucose peak that occurred significantly earlier than with real feeding (39 compared with 106 min; P = 0.022), although this peak was not sustained and concentrations returned to baseline values.

Triacylglycerol

Feeding resulted in an increase in the mean triacylglycerol concentration 39 ± 6 min after the meal. Neither water inges-

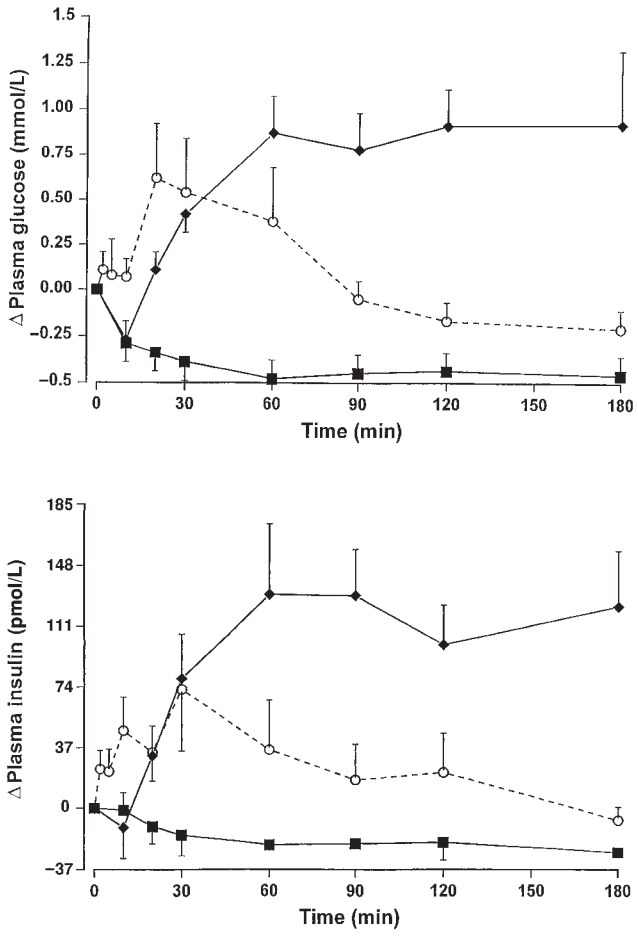

FIGURE 2. Mean (±SEM) changes in plasma glucose (top) and insulin (bottom) concentrations after water (■), modified sham feeding (○), and a meal containing a modest amount of fat (◆). The subjects had consumed a high-fat breakfast 5 h previously. n = 10. For plasma glucose, there was a main effect for meal ($P < 0.001$) and a meal × time interaction ($P < 0.001$); for plasma insulin, there was a significant time effect ($P = 0.001$), a main effect for meal ($P = 0.001$), and meal × time interaction ($P < 0.001$) by repeated-measures ANOVA.

TABLE 4
Summary data for postprandial insulin, glucagon, and cholecystokinin responses after water (control), modified sham feeding (MSF), and a meal containing a modest amount of fat[1]

	Control	MSF	Meal
Insulin (pmol/L)			
Basal	78.44 ± 20.72	58.46 ± 8.14	88.8 ± 23.68
Peak	88.06 ± 14.06	149.48 ± 23.02[2,3]	289.34 ± 23.68[4,5]
AUC	10715.2 ± 1746.4	14874 ± 3781.4[3]	33825.4 ± 4047.8[5]
IAUC	−3478 ± 2412.4	4351.2 ± 3581.6[3]	17774 ± 4040.4[5]
Glucagon (pmol/L)			
Basal	115.8 ± 11.0	121.6 ± 12.8	111.9 ± 7.3
Peak	130.3 ± 8.1[2]	135.8 ± 9.8	145 ± 9.4[4]
Nadir	91.1 ± 5.0	85.4 ± 6.3[2]	106.2 ± 8.2
AUC	19245 ± 954	19291 ± 1242	22082 ± 1400
IAUC	−1435 ± 1761	−2611 ± 1975	1930 ± 1116
Cholecystokinin (pmol/L)			
Basal	4.4 ± 1.2	3.2 ± 0.4	3.2 ± 0.4
Peak	6.7 ± 1.2	5.7 ± 1.2[3]	10.5 ± 2.0[4]
AUC	338 ± 59	346 ± 93	499 ± 91[6]
IAUC	−59 ± 100	55 ± 62[3]	206 ± 52
PP (pmol/L)			
Basal	54.6 ± 17.6	55.4 ± 14.5	46.3 ± 9.2
Peak	72.6 ± 20.1[2]	119.3 ± 26.8[3,4]	151.9 ± 25.8[4,5]
AUC	4854 ± 1531	6219 ± 1651	8063 ± 1728[5]
IAUC	−60 ± 492	1372 ± 1397	3891 ± 1214[6]

[1] $\bar{x}$ ± SEM; n = 10. Area under the curve (AUC) and integrated area under the curve (IAUC) are expressed as pmol·L^{-1}·180 min^{-1} for insulin, pmol·L^{-1}·180 min^{-1} for glucagon, and pmol·L^{-1}·90 min^{-1} for cholecystokinin and pancreatic polypeptide (PP).
[2,4] Significantly different from basal value (Student's t test): [2] $P \leq 0.05$, [3] $P \leq 0.01$.
[3] Significantly different from meal, $P \leq 0.05$ (Student's t test).
[5,6] Significantly different from control (Student's t test): [5] $P \leq 0.01$, [6] $P \leq 0.05$.

tion nor MSF had any effect on the plasma triacylglycerol response (Table 3).

Insulin

The changes in plasma insulin followed those of plasma glucose (Figure 2). Feeding and MSF resulted in a peak in the insulin concentration; the increase was sustained only with actual feeding and not with MSF. As with glucose, the mean insulin peak with MSF occurred significantly earlier (16 compared with 98 min; P = 0.001). MSF and actual feeding resulted in a different pattern of hormone release (**Table 4**).

Glucagon

With water ingestion there was a gradual increase in the plasma concentration of glucagon through the sampling period. Feeding resulted in a significant increase in glucagon concentration, which reached its maximum concentration 74 ± 20 min after the meal. With feeding, the plasma glucagon concentration remained elevated above baseline values for the entire sampling period. MSF resulted in suppression of plasma glucagon concentrations (Table 4). Maximal suppression of glucagon occurred 113 ± 21 min after MSF.

Cholecystokinin

The postprandial cholecystokinin responses to the 3 test meals are summarized in Table 4. Feeding produced a significant (P = 0.003) peak in circulating cholecystokinin; the mean peak occurred 32 ± 9 min after the meal. The cholecystokinin response to MSF was not significantly different from that observed with water alone (**Figure 3**).

Pancreatic polypeptide

Water, feeding, and MSF all resulted in significant increases in circulating PP (**Figure 4**). MSF had a smaller effect than did real feeding on absolute PP concentrations, although maximum concentrations were attained at a similar time (14 ± 5 and 20 ± 7 min for MSF and real feeding, respectively).

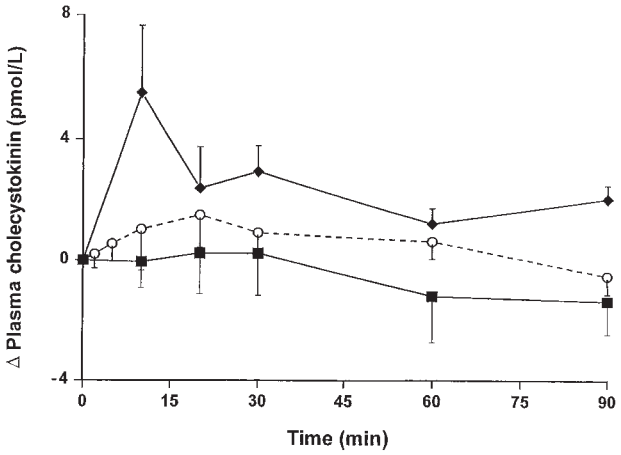

FIGURE 3. Mean (±SEM) change in cholecystokinin concentration after water (■), modified sham feeding (○), and a meal containing a modest amount of fat (◆) . The subjects had consumed a high-fat breakfast 5 h previously. n = 10. There was a significant meal effect (P = 0.017) and a meal × time interaction (P = 0.031) by repeated-measures ANOVA.

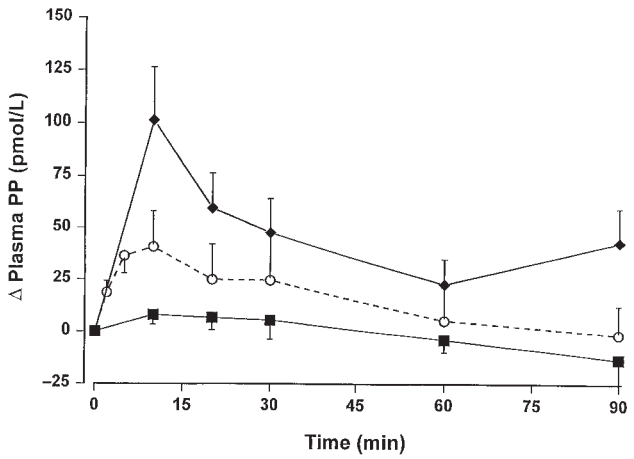

FIGURE 4. Mean (±SEM) change in plasma pancreatic polypeptide (PP) concentration after water (■), modified sham feeding (○), and a meal containing a modest amount of fat (◆). The subjects had consumed a high-fat breakfast 5 h previously. $n = 10$. There was a significant time effect ($P < 0.001$), meal effect ($P = 0.008$), and meal × time interaction ($P = 0.002$) by repeated-measures ANOVA.

Gastrin

Both MSF and real feeding resulted in a significant increase in the circulating gastrin concentrations (**Figure 5**), although the gastrin peak was significantly lower with MSF than with real feeding ($P = 0.012$).

DISCUSSION

MSF in these studies produced many of the effects expected: a brief increase in plasma insulin and PP concentrations and a modest increase in gastrin concentration. The effects were both quantitatively and qualitatively different from those following actual feeding and those following ingestion of water.

MSF produced a significant increase in plasma glucose concentration, which followed a pattern similar to the cephalic insulin response, suggesting a common stimulus. A change in plasma glucose is not normally attributed to MSF, although similar results were observed in sham-fed rats (11); these effects were suppressed with atropine. It is possible that liver glucose output may be under direct neural control, independent of both insulin and glucagon (12). This effect would be expected—that the cephalic phase of insulin release would lower blood glucose in the absence of a rise in pancreatic glucagon (Table 4), although this is rarely observed in sham feeding studies. The effect on plasma glucose may also be secondary to the sympathetic release of catecholamines, which were reported to be released during MSF (13). Epinephrine is capable of stimulating hepatic glucose production via the neuropeptide galanin (14), although we can only speculate as to why this was not observed previously with MSF. This study differed from other MSF studies in that the stimulus was given at lunchtime in the late postprandial state. Frape et al (15) observed that a fatty breakfast could influence the glucose tolerance of a meal eaten several hours later by impairing glucose oxidation and increasing hepatic gluconeogenesis (16). This hypothesis would help to explain the prolonged insulin and glucose responses observed during this study after the second high-fat meal (Figure 2) and may also have exaggerated the glucose and insulin concentrations observed after MSF.

In contrast with the observations made by Mattes (3) and Ramirez (4), cephalic phase stimuli alone, not combined with an enteral fat load, had no effect on circulating plasma triacylglycerol concentrations (Table 3). MSF may have caused the elevation in plasma triacylglycerol concentration in those studies by altering the rate of lipid absorption or clearance of the gastric fat load (17, 18). The release of local neurotansmitters, such as cholecystokinin (19) and somatostatin (20), triggered by fat absorption disrupting normal cephalic phase responses is also possible.

The regulation of plasma NEFA concentration is also part of the normal pattern of metabolic regulation during feeding and fasting. The postprandial increase in insulin secretion suppresses NEFA by suppressing hormone-sensitive lipase and increases the rate of fatty acid re-esterification (21). After the fatty meal, NEFA was suppressed significantly, corresponding with an increase in insulin without an associated drop in the plasma glucagon concentration. MSF also resulted in NEFA suppression; however, we can only speculate as to whether this was due to a change in adipose tissue lipolysis or to reesterification. The decrease in NEFA during MSF could be attributable to the cephalic insulin response, although the maximum suppression of NEFA occurred 114 min after the start of MSF compared with the mean insulin peak at 14 min. This temporal difference in the pattern of the NEFA and insulin response may indicate that they were independent events; recent results do suggest that the acute regulation of hormone-sensitive lipase and hence NEFA concentrations may occur by insulin-independent mechanisms (22). The timing of the NEFA nadir coincided with a decrease in plasma glucagon concentration. Glucagon has been shown to increase the rate of lipolysis in isolated adipocytes (23); however, its effects on fat mobilization in vivo remain controversial (24). Another possible mechanism is the suppression of NEFA secondary to the cephalic phase release of PP, which was reported to reduce NEFA concentrations by inhibiting cyclic AMP–directed lipolysis in adipocytes in vitro (25).

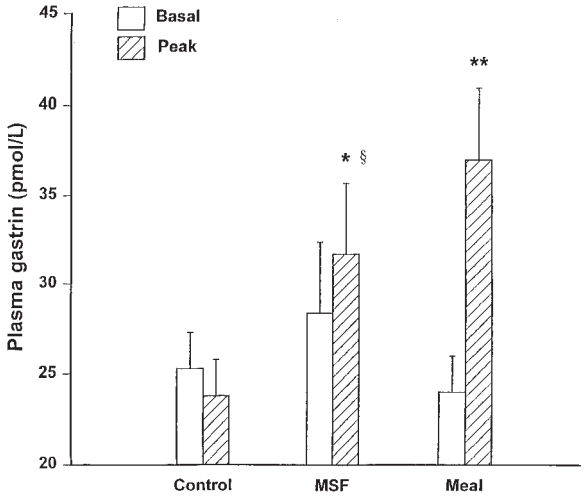

FIGURE 5. Mean (±SEM) plasma gastrin concentration at baseline ($t = 0$) and maximal concentration attained. The subjects had consumed a high-fat breakfast 5 h previously. $n = 10$. MSF, modified sham feeding. *,**Significantly different from baseline (Student's t test): $^{*}P \leq 0.05$, $^{**}P \leq 0.01$. §Significantly different from the meal, $P \leq 0.05$ (Student's t test).

An important question is whether the responses to MSF that we observed were due to actual cephalic stimulation or could reflect inadvertent swallowing. Although there appeared to have been a small peak in plasma cholecystokinin after MSF, this was late and nonsignificant, in contrast with the clear peak seen after feeding, which would have been triggered by the absorption of fat or protein (26). PP release from the pancreatic F cells is biphasic; the initial phase of release is dependent on vagal cholinergic activity (27). Water, MSF, and real feeding all resulted in increases in circulating PP. We would not normally expect an increase in PP without the presence of food-related stimuli but it is likely that the ingestion of water initiated vagal activity due to the combination of both swallowing and gastric distension (28). However, natural diurnal variations in the plasma PP concentrations are also a possible explanation for this observation (29). MSF of actual nutrients is likely to have produced more vagal stimulation than did swallowing alone, and the combination of cephalic stimulation with ingestion resulted in the highest PP concentrations that were still within the first phase of PP release. Tease feeding, in which food is presented but not masticated, is reported to have no effect on PP release (13), so it appears that the magnitude of the initial PP response is "dose-dependent" on the degree of cephalic stimuli. There was also a modest gastrin response after MSF, strengthening the results of an earlier study (30); however, water alone had no effect, indicating that gastric distension and swallowing alone in the absence of cephalic stimulation by nutrients could not stimulate gastrin release (31). The release of both PP and gastrin to concentrations greater than those achieved with water alone, but lower than those achieved with ingestion, provides us with evidence for the degree of vagal cholinergic activity achieved during MSF.

In summary, we observed effects on substrate metabolism after MSF that differed from those usually reported (eg, an increase in plasma glucose concentration) and this may reflect different effects of cephalic stimulation in the postabsorptive and postprandial states. We also observed metabolic effects that were prolonged beyond the period of cephalic responses. It is interesting to speculate whether these may be relevant to longer-term energy balance. However, there is mounting evidence to suggest that obesity is characterized by a reduction in vagal catecholamine (13) and PP release (32) combined with an elevated release of insulin (33) and gastric acid (34) during the cephalic phase period. It is assumed that the major signals for the control of energy balance are adiposity-related changes in insulin (35) and leptin (36) concentrations. It could be speculated, however, that repeated meal-related signals that alter adipose tissue metabolism and substrate utilization could also be implicated in the long-term regulation of adiposity.

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