Meal pattern analysis to investigate the satiating potential of fat, carbohydrate, and protein in rats

Britt Burton-Freeman, Britt, Dorothy W. Gietzen, and Barbara O. Schneeman. Meal pattern analysis to investigate the satiating potential of fat, carbohydrate, and protein in rats. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1916–R1922, 1997.—We examined meal patterns after isocaloric duodenal infusions of fat, carbohydrate (CHO), and protein by measuring meal size, intermeal interval (IMI) and total food intake (TFI). Wistar rats were adapted to normal feeding 6 h/day, with continuous computer monitoring of feeding patterns. One of five solutions (10 ml of 1 kcal/ml at 0.45 ml/min; 0, 20, 50, 80, or 100% of energy from fat) or saline (control) was infused 10 min after initiation of eating. Separate rats received casein or casein hydrolysate at 18.5 or 37% energy. Equivalent energy loads varying in fat, CHO, and protein content compared with saline resulted in similar reductions in first meal intakes. The second meal did not differ among fat and CHO treatments including saline; however, infusion with a protein-containing solution increased the size of meal 2. The IMI was doubled by protein infusion independently of dose or source but extended dose dependently by fat. TFI was lower after high fat and higher after protein than after saline infusion. The results indicate that the concentrations of fat, CHO, and protein differentially affect the qualitative and quantitative aspects of feeding in rats.

The intake of food is governed by physiological and psychological mechanisms in both the short term and long term. Among the physiological mechanisms, the short-term mechanisms are primarily associated with feeding and gastrointestinal (GI) activity, whereas the latter are related to maintaining overall energy status (28). Both temporal components are probably integrated to control food intake (FI); however, an understanding of the relationship between FI at a meal and control of body weight has not yet been achieved. Animal models that aid our understanding of the basic physiological controls of short-term FI should provide insight into the processes that control long-term energy status and thus the problems and pathogenic syndromes associated with energy balance, such as obesity and anorexia (13).

In its most parsimonious aspects, short-term FI encompasses the initiation and termination of feeding. Under usual conditions, food is ingested shortly after perceiving hunger and terminated when a feeling of satiation is recognized. Distinct systems, although subject to a multitude of influences, are believed to be responsible for the initiation and termination of FI. Each is finely regulated by feedback signals arising from central and peripheral sites, including the GI tract, liver, brain, and peripheral neural systems.

Whereas the stimulation to eat results from a combination of systemic or metabolic signals of hunger as well as sensory stimulation by food or the palatability of foods (6, 24), the termination of eating is governed by other mechanisms, including the physical and nutrient composition of the food(s) consumed. GI mechanisms in particular are responsible for the more "immediate" negative feedback inhibitions on FI and therefore influence FI from meal to meal (i.e., short-term FI). After a meal is consumed, the GI tract appears to have a major role in satiation, that is, the satisfaction of appetite that results in the cessation of a meal, as well as in the duration of satiety (29).

Satiation can be described behaviorally by the duration of a meal and/or the size of the meal. The shorter the duration and/or the smaller the meal, the greater the satiating response. Satiety is the state in which further eating is inhibited and generally occurs as a consequence of having eaten. It is presumed that satiety is the response after the generation of satiety or anorexigenic signals and/or the inhibition of meal-initiating signals. The intensity of the satiety response is described behaviorally by the duration of time between meals or eating occasions and/or the amount of food consumed at the next meal (3). Alterations in total FI will also provide insight into the potential influence of dietary manipulation on long-term energy balance because compensatory effects are considered. Collectively, the total pattern of the feeding response (qualitative aspects) along with quantitative measures provide information on mechanisms by which nutrients influence feeding activity (i.e., satiation and satiety).

The physiological mechanisms responsible for producing satiation and satiety are not clearly defined. However, compared with gastric mechanisms, the small intestine has a significant role in nutrient-induced satiation. As demonstrated by studies in which gastrectomized animals were used, meal size increases significantly in fasted animals when ingested liquid diet drains from an open gastric fistula (i.e., sham feeding), and, under the same experimental conditions, meal size decreases significantly when nutrients are infused into the duodenum concurrently (16, 17, 30, 36). Studies in which the sham feeding, duodenal infusion paradigm was used have further indicated that reductions in meal size vary by nutrient. Brenner et al. (5) showed that isocaloric (1.3 kcal) intestinal loads of oleate, maltose, L-phenylalanine, and unhydrolyzed casein (Cas) suppressed sham feeding by 61.5, 29.3, 45.7, and 5.36%, respectively. Under similar experimental conditions, Greenberg et al. (17) showed that intestinal fat loads ranging from 0.125 to 1 kcal/ml decreased FI as a function of concentration. This dose-related
inhibition of feeding reported by Greenberg et al. (17) is consistent with results obtained in real (nonsham)-feeding rats (35). However, questions about the satiating efficiency of nutrients such as fat have not been addressed independent of energy load. Therefore, the aim of the present study was to examine the process of satiation and satiety after isocaloric administration of macronutrients [fat, carbohydrate (CHO), and protein], as measured by meal size, intermeal interval (IMI), and total FI (TFI), in normal (nonsham)-feeding rats.

METHODS

The study was approved by the Animal Use and Welfare Committee at the University of California, Davis. The study was divided into two parts. The first part focused on the efficiency of fat and CHO to induce satiation and satiety, and the second part examined the satiety response to protein.

Part 1: Fat and CHO

Animals and surgical preparation. Six male Wistar rats (Simonsen Laboratories, Gilroy, CA) weighing 275–300 g were surgically equipped with duodenal cannulas. While rats were under anesthesia (ketamine-xylazine-acepromazine, 50: 5:0.75 mg/kg body wt, respectively), the abdomen was opened via a midline incision, and the cannula (Silastic medical-grade tubing, 0.025 in. ID; Dow Corning, Midland, MI) was placed in the duodenum distal to the pancreatic duct and proximal to the ligament of Trietz. We secured the tubing with a purse-string suture and collar, making sure that neither the lumen of the cannula nor the intestine was occluded. The abdominal incision was closed with sterile braided silk suture (5–0; Ethicon, Somerville, NJ). The tubing was exteriorized mid-scapularly and threaded through a coil spring that was attached to a swivel outside of the cage. This arrangement allowed rats relatively free movement in their cages and permitted us to administer daily nutrient infusions without handling or disturbing the animals. Rats were allowed at least 1 wk for postoperative recovery. During the recovery period, rats were infused daily with 3–5 ml saline to ensure that the cannulas remained patent. Rats were housed in hanging wire-bottom cages modified to allow computer analysis of FI and feeding patterns.

To determine if the implanted cannula, infusion rate or volume, or computer monitoring system had any effect on the feeding response, we monitored a separate group of intact rats (without cannulas) to obtain baseline feeding patterns. These rats were adapted to the same housing and feeding conditions as the cannulated rats (35). Therefore, the saline groups were combined and are referred to as saline in the text. To determine if differences existed between the baseline feeding patterns of intact rats and cannulated rats infused with saline, we analyzed meal size, IMI separating the first and second meal consumed, the IMI, and total (voluntary) energy intake for the 6-h feeding period. A meal was defined as ≥0.5 g of diet consumed, and the minimum IMI between two meals was defined as ≥10 min. These definitions for meals and IMI in the baseline pattern analysis are consistent with other reports (see Ref. 7).

Analysis. FI and feeding patterns were monitored and analyzed every day for 6 h/day. In addition, food cups were weighed before and after the feeding period. Variables measured to define the satiety response included the size of the first and second meal consumed, the IMI separating the first two meals, and total (voluntary) energy intake for the 6-h feeding period. A meal was defined as ≥0.5 g of diet consumed, and the minimum IMI between two meals was defined as ≥10 min. These definitions for meals and IMI in the baseline pattern analysis are consistent with other reports (see Ref. 7).

Data analysis. The feeding patterns of rats infused with saline and Hsaline, as measured by meal size, IMI, and TFI, were analyzed using a repeated-measures analysis of variance (ANOVA) and were determined to be similar (P > 0.05); therefore, the saline groups were combined and are referred to as saline in the text. To determine if differences existed between the baseline feeding patterns of intact rats and cannulated rats infused with saline, we analyzed meal size, IMI, and TFI between the two groups, using Student’s unpaired t-test (27). The effect of saline or varying concentrations of fat and CHO on three variables, meal size, IMI, and TFI, was determined by analyzing the feeding response to each treatment per rat with repeated-measures ANOVA (27). Using treatment as the main effect and rat as a blocking variable, we analyzed significant differences among treatment means (adjusted) by pairwise t-tests for appropriate comparisons. To determine if dose-response relationships existed between the concentration of fat in the infusates and IMI, meal size, or TFI, we used a polynomial regression analysis (27). Statistical significance was assumed for the t-tests, ANOVA, and regression analysis when computed P values were <0.05. Statistical calculations were computed with the use of the PC-SAS generalized linear models (GLM) procedure.
Part 2: Protein and CHO

The protocol for the second part of the study was similar to the first part, with few exceptions. We describe briefly the methods for part 2, highlighting the differences between part 1 and part 2.

Animals and diet. An additional group of male Wistar rats (Simonsen Laboratories, Gilroy, CA) weighing 275–300 g was prepared with chronic duodenal cannulas. Surgical preparation, procedure, and postoperative care for this second group of rats did not differ from the group of rats in part 1. Rats were housed in the Food Intake Laboratory and were maintained on the same elemental diet.

Infusion treatments. Four isoenergetic intestinal infusates varying in the percent energy from protein and CHO were prepared in physiological saline. Two infusates were prepared with the use of sodium caseinate (Bio-Serve, Frenchtown, NJ) as the protein source, and the other two infusates were prepared with the use of hydrolyzed Cas (Dyets, Bethlehem, PA) as the source of protein. Food-grade dextrose (Dyets, Bethlehem, PA) served as the CHO source in all four infusion solutions. The experimental infusates were prepared in 10-ml aliquots and are described on the basis of their percent energy from protein either as Cas or Cas hydrolysate (Cas-H). The treatment infusates were 18.5% Cas-H, 18.5% Cas, 37% Cas-H, 37% Cas, and saline. The four protein-containing solutions were energy balanced with CHO to contain 1 kcal/ml for a total of 10 kcal (41.8 kJ)/infusion solution. Saline served as the non-energy-containing control. All solutions were infused duodenally at 0.45 ml/min.

Experimental protocol. The experimental protocol was similar to that described in part 1. Once the experimental period began, rats were infused with one of the four protein-containing solutions or saline, and F1 and feeding patterns were monitored and analyzed to determine alterations in meal size, IMI, and total energy intake. Criteria for defining meals and IMI concur with criteria in part 1. Issues of essential fatty acid deficiency were addressed by infusion of a high fat-containing solution (80 or 100% fat solution from part 1) every 3–4 days. In addition, on weekends on which no experiments were run, 10 ml of lipid was infused on Friday evenings after removal of food cups. No signs or symptoms of fatty acid deficiency were detected in any of the rats.

Data analysis. The effect of saline or different concentrations of protein and CHO on meal size, IMI, or TFI was determined with the use of a repeated-measures ANOVA similar to that described in part 1. Statistical calculations were computed with the use of the PC-SAS GLM procedure.

RESULTS

Animal model. Rats that had not been cannulated were adapted to the same feeding regimen as cannulated rats and were monitored by computer for meal pattern analysis to determine eating patterns of intact animals. Meal size, IMI, and TFI comparisons between intact rats and cannulated rats are displayed in Table 1. No significant differences in feeding patterns were detected between cannulated and intact rats, demonstrating that the saline infusion serves as an appropriate control treatment.

Fat and CHO. The size of the first two meals consumed by the rats in the 6-h feeding period is shown in Table 2. The mean grams of diet eaten in meal 1 did not differ among rats infused with an energy-containing solution [all infusates contained 10 kcal (41.8 kJ)] except saline. However, the size of the first meal was significantly smaller after infusion with any one of the solutions containing energy than when the same rats were infused with saline [F(5,59) = 5.61, P < 0.0003]. In contrast, the size of the second meal did not differ among any of the infusion treatments, whether the infusion was an energy-containing solution or a saline solution.

As shown in Table 2, the length of the IMI, the time between meal 1 and meal 2, ranged from 40 ± 11 min for the saline infusions to 168 ± 9 min for the 20% CHO:80% fat infusion. The IMI for the 80 and 100% fat solutions was significantly longer than the IMI for saline and treatment infusates containing lower fat concentrations [F(5,59) = 25.31, P < 0.00001]. The IMI for the lowest lipid-containing solution (i.e., 80% CHO:20% fat) did not differ from the 100% CHO or saline solution. Infusion of the 50% CHO:50% fat solution resulted in an IMI that was intermediate; this IMI was different from the IMI with saline infusion but not different from the IMI produced with the infusion of the 80% CHO:20% fat or the 100% CHO solution. Regression analysis of these data revealed a significant dose-response correlation between the percent energy from fat in the infusate and the length of the IMI [F(3,49) = 25.32, P < 0.00001, r² = 0.64; cubic relationship, P < 0.006] (Fig. 1).

Table 2. Effect of fat and carbohydrate infusion on meal size and IMI

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Meal 1, g</th>
<th>Meal 2, g</th>
<th>IMI, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>4.72 ± 0.47*</td>
<td>3.12 ± 0.69</td>
<td>40.2 ± 11.6†</td>
</tr>
<tr>
<td>100% CHO</td>
<td>2.04 ± 0.50‡</td>
<td>3.46 ± 0.73</td>
<td>76.5 ± 12.2‡</td>
</tr>
<tr>
<td>80% CHO:20% fat</td>
<td>3.93 ± 0.50‡</td>
<td>3.63 ± 0.72</td>
<td>57.6 ± 12.1‡</td>
</tr>
<tr>
<td>50% CHO:50% fat</td>
<td>2.70 ± 0.65‡</td>
<td>3.60 ± 0.94</td>
<td>86.5 ± 15.9‡</td>
</tr>
<tr>
<td>20% CHO:80% fat</td>
<td>1.98 ± 0.38‡</td>
<td>2.66 ± 0.55</td>
<td>168 ± 9.20*</td>
</tr>
<tr>
<td>100% fat</td>
<td>2.34 ± 0.68‡</td>
<td>3.50 ± 0.99</td>
<td>154 ± 16.6*</td>
</tr>
</tbody>
</table>

Meals values are adjusted means ± SE of meal size. IMI values represent adjusted mean ± SE of time (min) between meal 1 and meal 2. CHO, carbohydrate. Means with different symbols indicate significant differences (P < 0.05) among treatments for that meal and IMI.
infused with 100% CHO, 80% CHO:20% fat, or 50% CHO:50% fat. In contrast, rats consumed an average of 7.55 g of diet when the two higher-fat treatments were infused.

Table 3 presents total energy intake, which includes the energy consumed orally as well as the energy provided by the infusion treatments. Total energy intake in rats infused with low to intermediate fat levels (80% CHO:20% fat or 50% CHO:50% fat treatments) was ~100% of the saline control, indicating that rats compensated for the energy provided by the infusates by reducing oral intake during the 6-h experimental feeding period. In the case of the nonfat, 100% CHO treatment, rats did not reduce oral intake during the 6-h period to compensate for the infusate energy, and, consequently, energy intake was ~20% higher than that of saline controls. When higher fat levels (i.e., 80 and 100% fat) were infused, rats did not fully compensate and total energy intake was ~72% of the saline control group [F(5,60) = 27.46, P < 0.00001].

Table 4 illustrates the effect of isocaloric protein-containing and CHO-containing infusates on the first two meals consumed by rats in the 6-h feeding period. The size of meal 1 did not differ

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Food Intake, g</th>
<th>Total Energy Intake, kcal (kJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>14.7 ± 0.63*</td>
<td>48.7 (204) ± 2.09*</td>
</tr>
<tr>
<td>100% CHO</td>
<td>15.3 ± 0.67*</td>
<td>60.4 (252) ± 2.21*</td>
</tr>
<tr>
<td>80% CHO:20% fat</td>
<td>12.7 ± 0.66*</td>
<td>51.9 (217) ± 2.19*</td>
</tr>
<tr>
<td>50% CHO:50% fat</td>
<td>13.4 ± 0.83*</td>
<td>54.2 (227) ± 2.74*</td>
</tr>
<tr>
<td>20% CHO:80% fat</td>
<td>7.61 ± 0.50†</td>
<td>35.1 (147) ± 1.66†</td>
</tr>
<tr>
<td>100% fat</td>
<td>7.53 ± 0.91†</td>
<td>34.8 (145) ± 3.00†</td>
</tr>
</tbody>
</table>

Total food intake values are adjusted mean ± SE of g of diet consumed in a 6-h feeding period. Total energy intake represents mean ± SE energy (kcal or kJ) provided by diet + infusion. Means with different symbols indicate significant differences (P < 0.05) among treatments.

Table 4. Effect of protein and carbohydrate infusion on meal size and IMI

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Meal 1, g</th>
<th>Meal 2, g</th>
<th>IMI, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>4.41 ± 0.48*</td>
<td>2.65 ± 0.99</td>
<td>35.7 ± 8.63†</td>
</tr>
<tr>
<td>18.5% Cas-H</td>
<td>1.72 ± 0.31†</td>
<td>5.26 ± 0.64</td>
<td>86.9 ± 5.57*</td>
</tr>
<tr>
<td>18.5% Cas</td>
<td>2.65 ± 0.33†</td>
<td>6.05 ± 0.70</td>
<td>71.2 ± 5.91*</td>
</tr>
<tr>
<td>37% Cas-H</td>
<td>2.06 ± 0.39†</td>
<td>5.33 ± 0.80</td>
<td>71.2 ± 6.93*</td>
</tr>
<tr>
<td>37% Cas</td>
<td>1.93 ± 0.36†</td>
<td>6.13 ± 0.74</td>
<td>73.2 ± 6.41*</td>
</tr>
</tbody>
</table>

Meal values are adjusted means ± SE of meal size. IMI values represent adjusted mean ± SE of time (min) between meal 1 and meal 2. Cas, casein; Cas-H, Cas hydrolysate. Percentages represent % protein energy in the infusate; corresponding CHO energy percentages are 81.5 and 63%, respectively. Means with different symbols indicate significant differences (P < 0.05) among treatments.

among rats infused with an energy-containing solution, regardless of the protein-CHO combination tested. However, compared with saline, a non-energy-containing solution, first meal intakes were 39–60% smaller after infusion with the protein-containing solutions [F(4,43) = 6.22, P < 0.0005].

The size of meal 2 tended to be larger after protein-CHO infusion than after saline infusion: however, statistical significance was marginal [F(4,42) = 2.23, P < 0.08]. Among the protein treatments, no differences were observed resulting from the level of protein in the infusates. Infusion of Cas tended to result in a larger second meal than that of Cas-H; however, no significant difference was observed.

Table 5 also shows the length of the IMI separating meal 1 and meal 2 after infusate treatment. Infusion with any one of the four protein-containing solutions lengthened the IMI to approximately twice that observed after saline infusion [F(4,43) = 6.00, P < 0.01]. No differences in IMI were detected between Cas and Cas-H or among the different protein concentrations.

TFI represents the total amount of elemental dry diet consumed during the 6-h experimental period. As shown in Table 5, TFI after infusion with either Cas or Cas-H at the 37% protein energy level was similar to that of saline but greater than intakes after infusion with 18.5% Cas-H [F(4,40) = 5.93, P < 0.0008]. The higher-protein infusates were only marginally different from the 18.5% Cas (P = 0.04). Infusion with 18.5% Cas-H solution resulted in the lowest TFI, which was 86% of

Table 5. Effect of protein and carbohydrate infusion on food intake

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Food Intake, g</th>
<th>Total Energy Intake, kcal (kJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>15.7 ± 0.75*</td>
<td>51.9 (217) ± 3.71†</td>
</tr>
<tr>
<td>18.5% Cas-H</td>
<td>13.5 ± 0.52†</td>
<td>51.5 (215) ± 2.50†</td>
</tr>
<tr>
<td>18.5% Cas</td>
<td>14.6 ± 0.53†</td>
<td>58.4 (244) ± 2.63†</td>
</tr>
<tr>
<td>37% Cas-H</td>
<td>16.3 ± 0.60*</td>
<td>64.0 (268) ± 2.98*</td>
</tr>
<tr>
<td>37% Cas</td>
<td>16.8 ± 0.56*</td>
<td>65.3 (274) ± 2.75*</td>
</tr>
</tbody>
</table>

Total food intake values are adjusted mean ± SE of grams (g) of diet consumed in 6-h feeding period. Total energy intake values are adjusted mean ± SE of energy (kcal or kJ) provided by diet + infusion. Percentages represent % protein energy in infusate; corresponding CHO energy percentages are 81.5 and 63%, respectively. Means with different symbols indicate significant differences (P < 0.05) among treatments.
FI after saline infusion. TFI tended to be affected by protein type at the lower protein energy level but not at the higher protein level (37%).

Table 5 illustrates the effects of saline and protein infusions on total energy intake. Total energy intake includes the energy consumed orally as well as the energy contributed by the infusate. Total energy intake was significantly higher after 37% Cas-H and 37% Cas than after saline infusion [F (4,41) = 5.28, P < 0.002]. No differences in total energy intake were observed that resulted from the protein source (Cas vs. Cas-H); however, total energy intake was higher after the 37% protein energy infusions than after the 18.5% infusions. In general, compensation for protein-CHO energy in the infusates was more accurate at lower protein levels and when hydrolyzed protein was infused.

**DISCUSSION**

FI typically refers to the quantitative aspects of feeding and focuses on the mass of nutrients consumed to reveal certain features of energy balance. Feeding behavior, on the other hand, represents the qualitative features of feeding characterized by the sequence or structure of actions taken to meet nutritional requirements (2). Analysis of the structure of feeding behavior after treatment provides information on the way in which physiological processes exert control over feeding activity. The animal model developed for the present study combined measures of FI with patterns of feeding behavior to assess the relationship between specific nutrients and the processes involved in satiation and satiety. Our goal was to determine the role of macronutrients acting as satiety factors in the control of short-term FI. Three important features of this study contributed to the accomplishment of this goal: 1) Rats were allowed to eat normally in a 6-h meal-fed paradigm, 2) implanted cannulas were sufficiently innocuous that typical feeding behavior was undisturbed, and 3) the use of a computerized continuous monitoring system permitted continuous recording and analysis of changes in feeding activity.

The results of this study demonstrate that fat has a significant role in satiation and satiety. The degree of satiation can be described by a reduction in meal size and/or the extension of the postmeal period (7). The former is considered a within-meal effect that occurs as a consequence of feeding, and the latter pertains to the postprandial effect of the meal (9). In the present study, both meal size and the postmeal period were altered by infusing energy-containing solutions into the intestine. All of the energy-containing infusions, whether derived from fat, CHO, or protein, resulted in a significantly smaller first meal than saline infusion, but the degree of reduction did not differ as a result of macronutrient composition. Thus, in short-term FI control, the infusion of nutrients that provide energy had a greater influence than the proportion of fat and CHO or protein and CHO.

In contrast to the apparent effects of energy on meal size, postprandial satiety as measured by IMI was sensitive to the macronutrient composition of the energy load. Meal pattern analysis demonstrated that the IMI lengthened as the percent energy from fat increased in the isocaloric infusates. Sensitivity to the fat load became more apparent at the higher fat concentrations, and regression analysis of these data illustrates a significant dose-response relationship between the percent energy from fat and the length of the IMI. As shown in Table 4, infusion with protein extended the IMI significantly (~2-fold) compared with saline infusion; however, unlike fat, protein infusion did not appear to influence the IMI in a dose-related manner. The lack of detection of a dose-response relationship between protein and IMI may be partly explained by the fact that only two levels of protein were tested. Infusions of higher concentrations of protein would not flow through the duodenal cannulas; therefore, measurement of feeding patterns after infusion of >37% energy from protein was not possible in the experimental model used in this study. Furthermore, infusing animals with a solution containing >3.7 kcal (15.5 kJ) from protein while they were ingesting a protein-inadequate diet (18% wt/wt) would result in supraphysiological protein loads; thus precipitation of unusual feeding patterns was a concern. The IMI response to protein differed from fat in that the IMI was longer after protein infusion than after saline; however, differences resulting from the percent energy from protein did not occur.

The observation that reductions in meal size occur in response to the energy load, whereas postprandial satiety (i.e., IMI) is predominately responsive to the macronutrient composition of the energy load, is interesting in light of a publication by Greenberg and co-workers (17). They reported a dose-response relationship between fat infused into the duodenum and percent inhibition of sham feeding (changes in 30-min liquid intake). Their observation of a dose-response relationship between duodenal fat and meal size during a 30-min interval, which is in contrast to the present study, may have been a result of the increasing energy load associated with the increasing fat infusion. Woltman et al. (35) reported a similar dose-response relationship between meal size and duodenal protein infusion, but, again, as protein increased, so did the energy content of the infusate. These data taken together with our data suggest that meal size is more responsive to the total energy load, whereas postprandial satiety is more responsive to macronutrient composition.

In addition to meal size and IMI, the feeding response is further defined by nutrient effects on subsequent and total energy intake. Fat was more effective than CHO in producing satiety in an equicaloric comparison; however, alterations in short-term FI may influence subsequent FI and, consequently, total energy intake. In the present study, subsequent and total intake were examined by recording FI for a 6-h period. Complete, over-, or undercompensation for the infused energy tended to depend on the macronutrient composition of the energy load. Infusion of the nonfat, 100% CHO solution resulted in an increase in subsequent intake (under compensation), whereas replacement of...
CHO energy with fat energy at the lower fat levels (i.e., 20 and 50% fat) resulted in total energy intakes that were similar to saline control (i.e., complete compensation). At the higher fat levels (80 and 100% fat), overcompensation, which resulted in undereating, was observed. A decrease in subsequent FI resulted in a total energy intake that was 30% lower than that of the saline control. Unlike the data of some studies in humans (23, 25), these data suggest that the control of energy intake by fat is not less precise than the control by CHO. Protein, on the other hand, has differential effects on subsequent FI. In studies of humans and in other studies in which rats were used, protein has been shown to suppress subsequent FI (1, 4, 12, 18, 19, 31). However, in our experimental model, subsequent FI (meal 2) was higher after infusion with a protein-containing solution than after saline infusion. This energy surplus was not corrected at subsequent meals, and percent consumption greater than saline control rats during the 6-h postprandial period ranged from 9 to 38%, such that undercompensation (i.e., overeating) was observed. These data suggest that the satiating capacity of protein is limiting in this model when energy is maintained constant. Moreover, it can be argued from the data that, in rats, intestinal mechanisms exist that enable them to recognize macronutrient composition and then modify the degree of compensation in FI according to the energy infusion. These mechanisms may be important for regulating fat as well as total energy intake.

Experimental evidence supports the concept that fat has a significant role in satiety and that the presence of fat in the small intestine induces the signals that terminate feeding and maintain postprandial satiety. Because intraluminal fat delays gastric emptying, one hypothesis is that the effects of intestinal fat on satiety may be explained by secondary increases in gastric distension. Although gastric distension is an obvious physical sign of fullness, the gastric emptying-distension theory has been challenged by several studies (8, 11, 15, 26, 29). Results from these studies indicate that the importance of gastric mechanisms in fat-induced satiety are minimal compared with intestinal mechanisms. In addition to the studies noted above, we found that the satiating effects of fat on meal size, IMI, and total energy intake were dependent on mechanisms specific to the upper one-third of the small intestine (data not shown). In a separate group of animals, we infused the 80% fat solution directly into the ileum and measured FI and feeding patterns according to the protocol outlined in the present study. The feeding patterns after fat infusion into the ileum were indistinguishable from duodenal infusions with saline. This observation indicates that the proximal small intestine contains a site for the mediation of fat-induced satiety. Computer-assisted meal pattern analysis coupled with nutrient infusions demonstrated that animals typically stopped eating within 10 min after the start of an energy-containing infusion. This early meal termination compared with saline infusion has been documented by others (17) and further supports preabsorptive mechanisms for the induction of satiation and satiety. Several hypotheses have been proposed to account for the initiation of satiety at the level of the intestine, but one hypothesis in particular suggests that the brain-gut peptide cholecystokinin (CCK) is a mediator of satiety. The pancreatic secretion literature indicates that fat and intact but not hydrolyzed protein stimulate the release of endogenous CCK (10, 14). Therefore, in the second part of this study we used two sources of protein, hydrolyzed and unhydrolyzed Cas, to determine if the feeding response, specifically the IMI, to the two forms of protein would differ on the basis of the potency and/or effectiveness of the treatment to stimulate CCK release. Contrary to our expectations, on the basis of the CCK hypothesis, no differences were detected between the hydrolyzed and unhydrolyzed Cas with regard to IMI. However, an explanation for this observation is that the Cas-H used in this study may not have been completely hydrolyzed to single amino acids. Thus peptide fragments could provide ample stimuli for activation of the protein-trypsin feedback loop, which is responsible for protein-stimulated CCK release (33, 34). As a result, plasma CCK concentrations may not have differed sufficiently between the two protein sources to cause significant differences in the postprandial feeding response (i.e., IMI). Differences between Cas and Cas-H treatments as measured by other feeding variables (i.e., meal size and total energy intake) may be the result of differences in sensitivity or to separate control mechanisms.

The feeding response after duodenal infusions with varying concentrations of protein and CHO compared with the saline controls indicates that protein influences FI and feeding patterns by reducing the size of meal 1, increasing the size of meal 2, doubling the length of the IMI, and increasing total energy intake. Equivalent energy loads varying in fat and CHO content reduced the size of the first meal, extended the IMI dose dependently, and reduced total energy intake when higher fat concentrations were infused.

The mechanisms underlying nutrient-induced satiety are multifaceted, and it is the interaction of these regulatory mechanisms that fully develops the satiety-inducing effects of intestinal fat, CHO, and protein. The experimental approach taken to investigate the effect of macronutrients on satiety-related mechanisms under normal (nonsham) feeding conditions has clearly demonstrated a role for fat in the modulation of short-term FI by acting as a satiety factor. It appears that fat and protein have similar effects on within-meal satiety (meal 1), and both can prolong postprandial satiety (IMI); however, the postprandial response invoked by protein and the effects on subsequent FI are dissimilar. These findings support the concept that the efficiency of certain foods to suppress hunger and subsequent FI may be a function of the macronutrient composition of the foods (21).

**Perspectives**

Dietary-induced signals of satiation and satiety have been investigated for many years; however, the role of each macronutrient (fat, CHO, protein) in either satiation or satiety is still unclear. This study has demon-
strated that, in an isocaloric experimental design in which FI and feeding patterns were monitored, fat, CHO, and protein differentially affect mechanisms governing satiation and satiety. Furthermore, the effects of energy load vs. macronutrient composition on FI control became evident. For example, the recognition of fat as a satiety signal has been associated with its energy contribution to the diet. This study showed that fat has a dose-related effect, independent of energy, on postprandial satiety. From a physiological perspective, it may be that the intestine has fat-specific sensors that are associated with mechanisms involved with relaying satiety to the brain. Because fat has over twice the energy value of CHO or protein, a fat-sensitive satiety signal initiated at the level of the intestine (i.e., preabsorptive) could be useful to discourage consumption of excess energy.

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