Length of intestinal contact on nutrient-driven satiety

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Meyer, J. H., Y. Tabrizi, N. DiMaso, M. Hlinka, and H. E. Raybould. Length of intestinal contact on nutrient-driven satiety. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1308–R1319, 1998.—Chemosensors throughout small bowel and colon inhibit food intakes when contacted by monomeric nutrients. We postulated that calorie-dependent inhibition of food intakes depended on additions of feedbacks from sensors in proximal and distal bowel contacted after high intakes of nutrients. Therefore, we determined how feedback from sensors in proximal gut interacted with feedback from simultaneously contacted sensors in distal bowel and whether suppression of nutrient intakes by intestinally perfused nutrients depended on length of gut contacted. Suppression of food intakes by maltose simply added to that from dodecanoate when both were present together either in proximal or distal small bowel. When dodecanoate was infused into proximal gut while maltose was infused distally, suppression of intake was threefold higher and was thus potentiated. Limited contact of slowly absorbed lactose or oleate to 35 cm of jejunum nearly abolished the satiating potencies each exhibited during access to whole gut. The observations were consistent with our hypothesis.

FOOD INTAKES in rats are suppressed in a dose-responsive fashion by nutrients in intestines (21). Responses are mediated by intestinal sensory mechanisms that are selectively responsive to digestive products (21). Sensors for this feedback are distributed in small and large intestine and are about equally responsive in proximal and distal small gut (21). We observed (21) that suppression of food intake varied with load (amount/min) of nutrient entering gut whether the nutrients [oleate-monolein, dodecanoate, phenylalanine, tryptophan, or maltose (data for the latter not shown)] were infused at constant concentration, but varied flows, or at constant flow, but varied concentrations. Thus responses depended on nutrient loads (amount/min) infused, independent of concentrations over the ranges tested. We postulated that food intakes decreased with increasing loads of nutrients infused into the gut (21) or instilled into the stomach (20) because higher loads of nutrients contacted longer lengths of small intestine to excite additive feedbacks from more and more sensors along the bowel.

Load dependence of nutrient-driven feedback has been shown to relate to length of contact for several different nutrient-driven feedback responses for which chemosensors are distributed along most of the intestinal length, just as they are for intestinal satiety. The importance of length of contact has been verified repeatedly in dogs by showing that diverting incoming nutrients truncated the dose response for stimulation of pancreatic secretion or for signalling inhibition of gastric emptying. The fraction of maximal response in these truncations was lower when nutrient was diverted from shorter segments, higher when nutrient was allowed contact with longer segments, and maximal when nutrients had access to all intestine (5, 11–14, 23–25). Thus responses mounted with dose as more and more sensors were contacted along increasing lengths of bowel. Similarly, in rats (9), the secretion of the lipoprotein apolipoprotein (Apo) A-IV [a putative signal of hypothalamic satiety (20)] into intestinal lymph increased with the load of fat entering the duodenum, but this increase was the result of entraining each successive segment of small intestine, in turn, to synthesize and maximally secrete Apo A-IV, as increasing incoming loads saturated the more proximal segments to reach more and more distal gut.

In 10- to 45-cm segments of canine or human gut, the amount of monomeric nutrient absorbed within the segment depended on load entering the segment and was similar whether nutrient was infused at low concentration but at higher and higher flows or at constant flow but increasing concentrations (8, 23, 25, 26). There was a maximal rate of absorption along each centimeter within the segment, either because of saturation of a transport carrier or because of a rate-limiting process within the enterocyte (such as the export of fat into the lymph). As incoming loads increased, loads soon exceeded absorptive capacity within the more proximal centimeters of the segment. Eventually, the entire segment reached a maximal absorptive capacity so that further loading would result in spillage of more and more nutrient beyond the segment. Thus length contacted (cm) by incoming nutrient varied with the load entering the gut (1, 2, 11, 13, 23–25) and was an integration of load (g/min) entering divided by absorption (g·min⁻¹·cm⁻¹) of each succeeding centimeter of bowel, whether nutrient was infused at constant flows but increasing concentrations or at constant concentration but increasing flows.

Biological chemosensors exhibit concentration dependence in response to exciting molecules. When confined to short lengths of proximal intestine to prevent recruitment of more distal sensors (13), oleate-monolein inhibited canine gastric emptying in a concentration-dependent fashion at least over the range of 3–27 mM. If suppression of nutrient intake by duodenally perfused oleate was mediated by sensory mechanisms confined to a short segment of duodenum and if signals from such sensors were concentration dependent but independent of length of gut contacted beyond the duodenal sensory area, then suppression would increase with concentration of oleate infused, just as it did when 20–80 mM oleate was infused at 12 ml/h into rat duodenum (80 mM almost completely suppressed intake). However, infusing 80 mM oleate under these
circumstances should have produced a similar maximal response, whether the oleate was infused at 6 or 3 ml/h, because the relevant sensors within the duodenum would still be exposed to the slowly absorbed oleate and would still respond maximally to the 80 mM concentration. Instead, virtually the same dose response was observed when 80 mM oleate was infused at 3, 6, and 12 ml/h as when 20, 40, and 80 mM oleate was infused at 12 ml/h (21). Because luminal concentrations of fatty acids, even after high-fat meals, are <39 mM (27), the 80 mM concentration of oleate was probably supramaximal for fatty acid sensors. The various considerations suggested that receptors along a considerable length were maximally stimulated by the supraphysiologically high concentration of 80 mM oleate and that the response increased as successive feedback mechanisms were entrained and maximally stimulated along increasing lengths of gut contacted by the increasing loads of 80 mM oleate delivered with varied volume flows.

It was relatively easy to test length dependency for other types of feedback in dogs. Length dependency was demonstrated by utilizing chronic fistulas for reversible diversions at varying distances along canine bowel. Such easy reversibility allowed randomized comparisons between diversion at one point or another versus full access. Easy reversibility also allowed us to demonstrate, even in a few dogs, that satiety (evidenced by a decrease in food intake) significantly increased when the length of gut contacted by lipolytic products was extended from jejunum into ileum (3).

In contrast to canine gut, rat intestine is less muscular and much smaller in diameter, making it impossible to surgically implant diverting cannulas without obstructing the small bowel. Consequently, we were forced in this work to test length dependency in rats with less direct methods. Three different techniques were utilized to test our hypothesis that suppression of food intakes by intestinal nutrients varies with length of gut (i.e., number of sensors) contacted.

Our first approach was to divide the delivery of a dose between duodenum and midgut. We were never completely certain in our previous study (21) how much of various loads of duodenally infused nutrients contacted distal small intestine or even colon, but our observations suggested that, on duodenal infusion, the middle and high doses of slowly absorbed oleate or the highest dose of somewhat more rapidly absorbed dodecanoleate spread into the colon. If so, the high satiety from the highest doses of oleate or dodecanoleate might have resulted from addition of feedbacks from jejunal, ileal, and colonic sensors. In the first experiments, we wanted to see whether inputs from proximal sensors would somehow add together with feedback from sensors in distal gut. The general idea was to determine whether simultaneous infusions of half-doses into duodenum and into midgut would evoke responses similar to or even greater than suppressions of intake during infusion of a full dose alone entirely into duodenum.

In the second set of experiments, we took advantage of the known regional distributions of three mucosal disaccharidases (maltase, trehalase, and lactase) to vary the length over which glucoytic products were released to contact sensors along small intestine. We assumed that only hydrolytic products in gut were capable of signalling a suppression of food intakes (21). Substrate-specific, brush-border glucosidases have differing distributions along rat small intestine (1, 2, 30). Maltase abounds in proximal, middle, and distal thirds (1, 30). In the proximal third of small bowel, trehalase (α-1,1-glucosidase) is 25% as abundant as maltase; in the middle third, it is 11% as abundant; and in the distal third, it is nearly absent (1). Even if higher and higher loads of duodenal maltose saturated jejunal maltase, undigested maltose spilling even into ileum would be readily hydrolyzed and could thus signal satiety there. By contrast, trehalase is almost completely absent from the distal third of rat small intestine (1). In other words, because of the limited distribution of trehalase, the release of glucose from trehalose (α-1,1-glucosylglucose) entering duodenum is pretty much confined to the proximal two-thirds of small bowel, regardless of load. Therefore, the potency of trehalose should be significantly less than that of maltose if the latter derived its full potency at high dose from additive feedback along the entire small gut. Lactase has a broader and much different distribution than trehalase, as it is ~10% as abundant as maltase in each third of rat small intestine (2, 30). In addition, proximal colonic content is rich in lactase of bacterial origin (2). Because lactase is even less abundant than trehalase in proximal small intestinal mucosa, an even larger fraction of moderate and even lower duodenal loads of lactose (β-1,4-galactosylglucose) spill undigested into distal bowel, but there is enough lactase in the middle and lower thirds of small bowel and, especially, in colon to ensure a length of contact by released glucose and galactose over the entire small and large intestine (2). Therefore, if the total length of intestinal contact with glucose (and galactose) is an important determinant of intestinal satiety, small to moderate loads of duodenal lactose should prove to be more satiating than similar loads of maltose, as there is known to be a greater spread of glucoytic products along the bowel during digestion of the former (2).

Thus our second approach compared dose responses of duodenal trehalose with duodenal maltose and duodenal lactose with duodenal maltose.

Our third experimental test was to determine whether dose-responsive satiety would be lessened if perfused nutrients were confined to 35 cm of proximal small bowel in a Thiry Vella loop instead of being given access to whole small intestine. We first studied how confinement to 35 cm of bowel would affect satiation from perfused lactose compared with maltose. If perfused lactose indeed signalled from longer lengths of bowel than more rapidly hydrolyzed and absorbed maltose, then its ability to suppress nutrient intake should be more profoundly reduced than that of maltose. We also examined suppression of intakes by jejunally perfused oleate-monolein vs. maltose. Because oleate-monolein is more slowly absorbed than glucose or maltose [80
mM oleate was absorbed from 65-cm segments of canine proximal small intestine ~1/10th as fast as 1.0 M glucose (13)]. We predicted that confining perfusate to a 35-cm loop would reduce whole gut suppression of intake more for oleate than for maltose if length of intestinal contact were important in these responses.

METHODS

We used the same experimental techniques in sham-feeding or naturally feeding rat models that we employed in our earlier study. More complete information on how these animals were prepared and how they were tested can be found in the preceding paper (21). Both models had infusion catheters placed in the duodenum (~2 cm from the pylorus) and at mid small intestine (55 cm from the pylorus) through which we could instill nutrients or control solutions in desired amounts. The two models were previously shown to give similar information, but the ability to test daily in the sham-feeding model allowed more rapid completion of protocols, whereas the much greater experimental life of the freely feeding model allowed repeated testings to be averaged across day-to-day variations.

Animals were housed in individual cages in a room with a natural light cycle. After recovery from surgery, sham-fed rats were trained to eat their day's ration from 1200–1700 and were then deprived of food but not water from 1700–0900 the next day. These animals were allowed to sham feed for 90 min some time during 0900–1200. Naturally feeding rats were trained to eat daily from 0900–1200 and were studied during this feeding period. For the remaining 21 h, the naturally feeding rats were deprived of food but not water. In either model, constant intestinal perfusions with nutrient or control solutions began at the start of the ingestive period and continued for its full duration.

To study sham-feeding rats with Thiry Vella loops, the following procedure was adopted. After conditioning to body restraint, rats were prepared as standard sham-fed models but only with one infusion catheter, 15 cm distal to the pylorus. After recovery, the animals were trained for 14 days to sham feed and then underwent 4–6 days of sham feeding for determination on successive days of response to controls, to oleate or lactate, and to maltose. Immediately thereafter, the rats were fasted 20 h and then operated on again. The jejunum was transected ~3 cm above the infusion catheter, and the distal margin was closed with an inverting suture of 6.0 Ethicon and buttressed with a patch of Marlex (Bard Marlex mesh; Bard, Cranston, RI) sewn over the surface of the closure. A second transection was made 2 cm from the pylorus (Bard Marlex mesh; Bard, Cranston, RI) sown below the first to form a Thiry Vella loop of proximal jejunum. The distal stoma of this loop was brought out through a stab wound in the abdominal wall where it was sutured to the skin. Intestinal continuity was reestablished by anastomosing the proximal margin at the first transection to the distal margin of bowel from the second transection. When used, perfusates entered the proximal end of the loop through the polyethylene catheter and exited from the distal end out the cutaneous stoma so that perfusates had access limited to 35 cm of gut. Rats were allowed to eat again one day later, and sham feeding resumed on the fourth postoperative day. For 2 days, rats sham fed during perfusions of the Thiry Vella loops with saline. Provided that each animal 1) continued to appear healthy, 2) had resumed eating ~70% of preoperative daily intakes, and 3) had sham intakes >70% of preloop intakes, the animals again underwent 2 or 3 consecutive days of testing with oleate or lactose controls and oleate or lactose, followed by another 2 days of maltose control and maltose.

Thus apparently healthy animals were retested over postoperative days 6–11. In these experiments, maltose was always given after oleate or lactose as a positive control.

In some animals with Thiry Vella loops, we measured the absorption of oleate from oleate-monolein when infused at loads of 0.24, 0.48, and 0.96 mmol/h of oleate, at either fixed concentration (80 mM) or fixed volume flow (12 ml/h). Nonfasting animals were perfused in restraint cages for 120 min; they were perfused with oleate-monolein during the first 90 min and then with a wash of 0.15 M NaCl at 12 ml/h for the last 30 min. Effluents from the loops were collected in catch basins beneath the restraining cages, acidified with HCl, and extracted two times with equal volumes of 5:4 petroleum ether-ethanol. Extracts were evaporated to dryness, and redissolved in absolute ethanol, so that their content of recovered fatty acid could be determined by titration with 0.1 N NaOH to pH 9.50.

Most perfusates were made isosmolar by adding NaCl as needed to achieve 300 mosmol/kgH2O. For solutions of carboxylates, osmolalities were restricted to a maximum of 400 mosmol/kgH2O for duodenal instillates and 800 mosmol/kgH2O for midgut infusions, because we found in other experiments (21) that solutions of NaCl alone markedly inhibited intakes at osmolalities above, but not below, 500 mosmol/kgH2O in duodenum and above, but not below, 800 mosmol/kgH2O in ileum. Oleate plus monolein were emulsified with 10 mM taurocholate at pH 7 in a 2:1 molar ratio (oleatemonolein). Control solutions were NaCl plus 10 mM taurocholate. The source of crude taurocholate was desiccated ox bile (Sigma Chemical, St. Louis, MO), which also contains lecithin. Most solutions at 21°C were instilled at pH 7, but solutions of dodecanoate (C-12) were at pH 8.1. In tests of maltose alone vs. maltose plus C-12, control solutions for tests of maltose plus C-12, control solutions (no maltose) containing C-12 were NaCl plus NaHCO3 (pH 8.1); for tests of maltose plus C-12, control solutions (no maltose) containing C-12 were NaCl plus C-12.

Except in animals having Thiry Vella loops, doses or treatments were scheduled in Latin square designs. In most tests, two-way ANOVAs were used to determine whether there was a significant treatment effect. If so, we used linear contrasts to compare individual treatments. If full dose-response curves were generated, dose-responsive effects were examined by linear regression of values in each animal, with 0 dose = control and successive doses (given in geometric progression) arrayed arithmetically as doses 1, 2, and 3. Significance (difference from 0 by t-test) of slopes (g ingested/unit dose) was then computed from the mean and SE of individual slope values from all animals. Sometimes the slopes obviously differed between one set of perfusates and another. At other times, slopes were similar, but one dose response was obviously much above the other. To avoid possibly spurious statistical significance from multiple, point-by-point comparisons (4), we first made global comparisons of slope and additionally of sums of responses from all doses of each perfusate. These comparisons were made by paired t-tests (for 2 treatments) or by ANOVAs (for 3 or more treatments). If significant differences in global comparisons were detected by these means, then individual doses along the dose response to one perfusate were compared with the corresponding doses of the other perfusate to determine the most likely origin of the global differences.

Because of the possibility that Thiry Vella loops would lose responsiveness over time, experiments were scheduled in these animals so that high-dose maltose was always given after oleate-monolein or lactose as a kind of positive control. These experiments were designed in couples so that oleate-monolein (or lactose) was first alternated among animals.
with its corresponding control. After the first 2 days, when every animal had received nutrient and control perfusions, each animal was perfused for two more days with maltose or maltose controls in an order that alternated among animals.

RESULTS

Interactions within or between segments. The first experiment was designed to determine whether suppression of food intakes from 0.48 mmol/h of dodecanoate (C-12) infused into duodenum of naturally feeding rats would add to suppression from dodecanoate infused simultaneously into midgut. Six naturally feeding rats were studied, and the order of perfusions (Table 1) was varied among the rats. Simultaneous infusion of 0.48 mmol/h into duodenum and midgut resulted in significantly (P < 0.001, ANOVA) less food intake than during 0.48 mmol/h singly into duodenum or midgut, but intakes during dual 0.48 mmol/h simultaneously into the two sites were not different from 0.96 mmol/h alone into duodenum or midgut.

We wished to determine whether two different nutrients (maltose and C-12) would add their suppressive effects when perfused together into the same segment of bowel, whether jejunum or ileum. Two groups of sham-feeding rats were used: one for duodenal infusions and the other for infusions into mid intestine. In each experiment, one-half of the animals received maltose alone (50–400 mM at 12 ml/h) first and then maltose plus C-12, whereas the other one-half was perfused with maltose plus C-12 first and then with maltose alone. Doses of maltose were randomized in a Latin square design.

When maltose (50–400 mM) or maltose (50–400 mM) plus C-12 (30 mM) was infused into duodenum, feeding decreased as the dose of maltose increased (Fig. 1A). Sham-feeding responses to maltose plus C-12 were numerically, but not statistically, greater than to maltose alone at the lower doses, but at the highest dose of maltose, the responses to the two solutions were the same. Neither the slopes nor the sums of the responses were significantly different (paired t-tests).

The second group of nine rats was infused at midgut, with the concentration of C-12 increased to 40 mM to offset a slightly weaker effect of low doses of C-12 at midgut compared with duodenum (21). Each type of nutrient solution (those with maltose alone or those with maltose plus C-12) was infused at 12 ml/h and was

Table 1. Redistribution of the load of dodecanoate on food intakes

<table>
<thead>
<tr>
<th>Site</th>
<th>Infusion</th>
<th>Load</th>
<th>Amount Eaten, g</th>
<th>Difference From Control, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td>80 mM @ 6 ml/h</td>
<td>0.48</td>
<td>15.1 ± 1.2</td>
<td>1.0 ± 1.5</td>
</tr>
<tr>
<td>Midgut</td>
<td>80 mM @ 6 ml/h</td>
<td>0.48</td>
<td>15.2 ± 0.8</td>
<td>0.9 ± 1.1</td>
</tr>
<tr>
<td>Duodenum + midgut</td>
<td>80 mM @ 6 ml/h</td>
<td>0.96</td>
<td>7.5 ± 2.0*</td>
<td>8.6 ± 2.0</td>
</tr>
<tr>
<td>Duodenum</td>
<td>80 mM @ 12 ml/h</td>
<td>0.96</td>
<td>5.9 ± 1.2</td>
<td>10.3 ± 1.3</td>
</tr>
<tr>
<td>Midgut</td>
<td>80 mM @ 12 ml/h</td>
<td>0.96</td>
<td>7.1 ± 0.4</td>
<td>9.0 ± 0.7</td>
</tr>
</tbody>
</table>

*Simultaneous infusions of 0.48 mmol/h into duodenum and midgut resulted in significantly (P < 0.001, ANOVA) less food intake than during 0.48 mmol/h singly into duodenum or into midgut, but intakes during dual 0.48 mmol/h simultaneously into the two sites were not different from 0.96 mmol/h alone into duodenum or midgut.

Data are means ± SE from 6 animals. Effect of redistributing a full load (0.96 mmol/h) of dodecanoate (C-12) at duodenum or midgut alone to one-half load each at duodenum and midgut simultaneously.

Fig. 1. Effect of combinations of dodecanoate (C-12) with maltose vs. maltose alone on sham feeding intakes (means ± SE) in three different groups of rats. A: duodenal perfusions of maltose vs. maltose + 30 mM C-12 at 12 ml/h in 10 animals. B: midgut infusions of maltose or maltose + 40 mM C-12 at 12 ml/h in 9 animals. C: duodenal infusion of NaCl-NaHCO3 or 60 mM C-12 at 6 ml/h while saline or 0.1–0.8 M maltose was infused into midgut of 9 rats at 6 ml/h into each site.
found to inhibit sham feeding in a dose-related fashion, but the inhibitions were higher at all doses of maltose plus C-12 than with maltose alone (Fig. 1B). The sum of the responses (Table 2) to maltose plus C-12 was higher than to maltose alone (P < 0.05).

In the preceding experiments, we tested interactions between maltose and C-12 when they were distributed in the same segment of either proximal or distal small bowel. In a third set of nine sham-feeding rats, we wished to determine how maltose and C-12 would interact when they were distributed separately, with C-12 in proximal bowel and maltose in distal small intestine. Therefore, we instilled 0.36 mmol/h of C-12 (that is, 60 mM C-12 at 6 ml/h) or NaCl-NaHCO₃ control solution at pH 8.1) at 6 ml/h into duodenum while perfusing at midgut at 6 ml/h with 0.4-8.8 mmol/h of maltose. Four animals first received maltose alone into midgut plus saline bicarbonate into duodenum and then maltose into midgut plus C-12 into duodenum. The other five animals were perfused in the reverse order.

There was a much greater interaction (Fig. 1C) than in the previous two tests. Even the weak satiety stimulus (6) of ileal 800 mosmol/kgH₂O NaCl (control) was markedly increased by the duodenal C-12 (vs. the control duodenal perfusions with bicarbonate), and, similarly, all responses to ileal maltose were markedly enhanced by the duodenal C-12 when compared with ileal maltose plus duodenal bicarbonate. Despite the markedly increased interactions between stimuli during recruitment of sensors along the entire intestinal length, the slope of the dose response to the ileal maltose was not affected (Fig. 1 and Table 2). A one-way ANOVA of the sums of responses (Table 2) indicated that the three groups of rats responded similarly (P > 0.25) to maltose plus bicarbonate but differently (P < 0.025) to maltose plus C-12, almost entirely because of the much greater satiety with the duodenal C-12 plus ileal maltose.

Because our sham-feeding animals had useful experimental lives of only ~3 wk, we were forced to use three different sets of rats to complete the above experiments (Fig. 1). Consequently, there was the possibility that the third set of rats somehow differed from the others to give a spurious impression of potentiation between proximal and distal segments. To eliminate this possibility, we performed an analogous experiment in one set of 11 naturally feeding animals. Over four experimental days, these animals were perfused with a low dose of maltose alone into midgut, a low dose of dodecanoate alone into duodenum, the dodecanoate plus the maltose together into duodenum, or the dodecanoate into duodenum simultaneously with the maltose into midgut. When dodecanoate or dodecanoate plus maltose was infused into duodenum, the midgut was perfused with 400 mosmol/kgH₂O NaCl, and when maltose alone was infused into midgut, the duodenum was perfused with 0.1 M NaHCO₃ plus 0.5 M NaCl (Table 3). In the four tests, duodenum and midgut were thus perfused simultaneously each at 6 ml/h with nutrient or saline. The low doses of maltose alone or C-12 alone only slightly suppressed daily intakes of rat chow. The maltose plus C-12 together into duodenum suppressed food intakes somewhat (but insignificantly) more than maltose alone into midgut or C-12 alone into duodenum. However, there was a more marked and significant (P < 0.01) suppression of intake when C-12 was perfused into duodenum while maltose was instilled at midgut, compared with maltose plus C-12 together into duodenum (Table 3), even though the caloric loads were the same during these last two treatments.

Dose responses to three disaccharides. This series of experiments began with a comparison of dose responses to duodenal trehalose vs. duodenal maltose.
Five naturally feeding rats received duodenal maltose (100–400 mM at 12 ml/h) first and then duodenal trehalose (100–400 mM), and four rats received the reverse. Doses of each sugar were randomized in a Latin square design. There was significantly \((P < 0.005)\) less dose-responsive suppression of food intakes by duodenal trehalose (Fig. 2A). The slope of this dose response \((-0.99 \pm 0.21)\) was significantly \((P < 0.0005, \text{paired } \text{t-test})\) less negative than that from maltose \((-2.37 \pm 0.21)\) in the same animals, and, similarly, the sum of responses to trehalose differed \((P < 0.025)\) from that to maltose. Both differences derived from a much lower suppression after the 4.8 mmol/h dose of trehalose compared with maltose, as the low and moderate loads of trehalose gave responses similar to isocaloric loads of maltose.

Lactose is hydrolyzed in the intestine to glucose plus galactose, whereas maltose yields two glucoses. Before comparing lactose with maltose, we had to establish that glucose and galactose were equipotent in inhibiting food intakes. The dose responses to each duodenally infused sugar were compared in 12 naturally feeding animals, with one-half of the animals receiving the glucose (100–400 mM at 24 ml/h) first and the other one-half the galactose (100–400 mM at 24 ml/h) first (Fig. 2B). The two hydrolytic products of lactose proved to be equally satiating. Next, we compared dose-response curves from duodenal lactose with those of duodenal maltose in 10 naturally feeding rats. One-half of the animals received lactose (100–400 mM) first, and the other one-half received maltose (100–400 mM) first. Doses were given to individual animals under a Latin square design. Both disaccharides (Fig. 2C) inhibited intakes in a dose-responsive fashion \((P < 0.0005)\). The slopes of the two dose-response curves \((-2.13 \pm 0.28 \text{ for maltose vs. } -2.39 \pm 0.22 \text{ for lactose})\) did not significantly differ because the responses to the highest doses of each disaccharide were the same, but the sum of responses was significantly \((P < 0.025)\) lower for lactose because each of the lower loads of lactose, but especially the 2.4 mmol/h dose \((P < 0.005)\), inhibited food intakes more than the corresponding loads of maltose.

The experiment was repeated to compare lactose with maltose when each was infused into midgut in another group of nine rats. The low and middle loads of midintestinal lactose were no longer more effective than isocaloric loads of maltose (Fig. 2D). The differences between the two sugars observed on duodenal infusion disappeared on infusion into midgut, when contact with the proximal one-half of small gut was bypassed by the midintestinal infusion. The result was consistent with the idea that lower loads of duodenal lactose were more effective than isocaloric maltose because more slowly hydrolyzed products from the duodenal lactose contacted a longer total length of intestine.
proximal plus distal gut than did glucose released from rapidly hydrolyzed maltose. This idea was tested further in limiting access to 35 cm of jejunum.

Limiting access to 35 cm of jejunum. Eleven sham-feeding animals survived to be tested with lactose and with maltose before and after creation of a jejunal Thiry Vella loop (each was instilled at 4.8 mmol/h or 6.9 kcal/h at 12 ml/h; Fig. 3). When given access to the whole gut (before creation of the Thiry Vella loops), the isocaloric loads of lactose and of maltose potently (** P < 0.01) inhibited sham feeding intakes compared with control perfusions with 400 mosmol/kgH₂O saline, and each sugar inhibited about equally, just as it had at this high dose in the naturally feeding rats described above. When confined to 35 cm of jejunum, the inhibition from each sugar (difference from control) dropped, significantly with lactose (** P < 0.05 ANOVA) but not significantly with maltose.

Another nine animals survived in good health after the creation of the Thiry Vella loops to be tested with oleate-monolein and with maltose. When given access to whole gut, the oleate-monolein (0.96 mmol/h or 4.0 kcal/h at 12 ml/h) reduced intakes (compared with its 300 mosmol/kgH₂O saline plus taurocholate control) at least as much as maltose did (4.8 mmol/h or 6.9 kcal/h) when compared with its 400 mosmol/kgH₂O saline control (Fig. 4), and each nutrient inhibited significantly (** P < 0.01, paired t-test). When the same perfusates were later confined to 35 cm of the previously perfused jejunum in the Thiry Vella loops, the oleate-monolein no longer significantly inhibited intakes, whereas the maltose still inhibited (** P < 0.01) nearly as well as it did when given access to the whole gut.

To confirm that oleate was in fact absorbed, we measured how much perfused oleate we could recover in the effluents from loops of 12 animals (Fig. 5) when three loads of oleate-monolein were infused at varying concentrations, constant flow, or constant concentration, or varied flow. It was apparent that the oleate was absorbed about as well when infused at varying concentrations vs. varying flows (Fig. 5). Thus the sums of absorbencies (926 ± 109 µmol/h absorbed during varying concentrations vs. varying flows) were not significantly different (paired t-test). If, however, the common dose (960 µmol/h) was removed from these sums, then the sum of absorbencies during the lower two loads was significantly (** P < 0.005) higher during perfusions with 80 mM oleate at varied flows.

In these experiments, sham feeding was measured in a total of 28 rats in which perfusions of maltose vs.
saline were carried out before and after creation of 35-cm loops (11 animals for experiments with lactose, 9 with oleate, plus 8 more for testing absorption of oleate). Although there was quite a bit of variation among animals, it was, nevertheless, clear that limiting the maltose to the loops also reduced the suppressive effect that the maltose had during its access to the whole gut (Table 4).

**DISCUSSION**

Intestinally perfused nutrients inhibited food intake (21, 32) whether infused into proximal duodenum (within 2 cm from the pylorus), proximal jejunum (15 cm from the pylorus in the present studies), mid small intestine [55 cm from the pylorus (6, 21)], distal ileum [95 cm from the pylorus (32)], or cecum [125 cm from the pylorus (21)]. The cecal infusion site was not tested [95 cm from the pylorus (32)], or cecum [125 cm from the pylorus (6, 21)]. The cecum infusion site was not tested (21). Whether infused into proximal duodenum or was confined to the loops. All perfusions were delivered at 12 ml/h.

![Graph](image)

**Fig. 5. Absorption of oleate by 35-cm loops of jejunum in 12 rats during infusions of oleate-monolein at 0.24, 0.48, and 0.96 mmol/h given at constant flow (12 ml/h), variable concentration or at constant concentration (80 mM), variable flow. Oleate was infused for 90 min. Per hour absorptive rates were calculated by subtracting oleate recovered from oleate infused over the 90 min and dividing this difference by 1.5.**

Table 4. Sham feeding intakes during access of perfusates to whole gut vs. 35-cm Thiry Vella loops

<table>
<thead>
<tr>
<th></th>
<th>Whole Gut</th>
<th>Thiry Vella Loop</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline controls</td>
<td>39.6 ± 3.5</td>
<td>43.9 ± 3.7</td>
</tr>
<tr>
<td>Maltose (4.8 mmol/h)</td>
<td>16.5 ± 2.3</td>
<td>26.0 ± 3.8*</td>
</tr>
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</table>

Data are means ± SE. Units are ml drunk in 90 min in 28 rats. Intakes during control perfusions with 400 mosmol/kg H2O saline did not differ statistically, whether the saline had access to the whole gut or was confined to the loops. All perfusions were delivered at 12 ml/h.

*Different from maltose during access to the whole gut (P < 0.05, 2-way ANOVA).
sensors because the C-12 was readily absorbed from all intestinal sites exposed to it.

There are several arguments against the second explanation, but the most revealing is our observation (Fig. 1 and Tables 2 and 3) that simultaneous perfusions of C-12 into duodenum and maltose into midgut produced a significantly (P < 0.01) greater inhibition of nutrient intake than did perfusion of C-12 together with maltose into the same segment of duodenum (or midgut). If signalling were predominantly postabsorptive, then it should make no difference where along the gut these absorbable nutrients were infused, but it did. The other arguments against postabsorptive signalling are less direct but still compelling: intravenous nutrients are not as potent and/or as rapidly effective as the same amount of nutrients instilled directly into gut [discussed more extensively elsewhere (21, 20)]; some nonmetabolized or poorly metabolized glucose analogs are nevertheless highly potent (21) on instillation into gut. That slowly absorbed lactose was as potent as maltose is a new argument against the third explanation (Fig. 1 and Tables 2 and 3) that simultaneous perfusion of C-12 together with maltose into the same segment of duodenum (or midgut). If signalling were predominantly postabsorptive, then it should make no difference where along the gut these absorbable nutrients were infused, but it did. The other arguments against postabsorptive signalling are less direct but still compelling: intravenous nutrients are not as potent and/or as rapidly effective as the same amount of nutrients instilled directly into gut [discussed more extensively elsewhere (21, 20)]; some nonmetabolized or poorly metabolized glucose analogs are nevertheless highly potent (21) on instillation into gut. That slowly absorbed lactose was as potent as maltose is a new argument against explanation 2 (discussed further, below). The sum of observations makes explanation 2 implausible, so we are left to conclude under explanation 1 that sensors along the gut directly signal satiety, that the magnitude of the satiety response is some sort of integration of inputs from sensors along the length of bowel contacted by the satiating nutrient, and that these combined inputs can be potentiating if sensors are contacted simultaneously in both jejunum and ileum (Fig. 1 and Tables 1–3).

Comparison of satiating efficacies of three disaccharides. These experiments were conducted under the assumption that only glucolytic products released from each disaccharide triggered the suppression of food intakes, an assumption based on our previous observation (21) that acarbose, a competitive inhibitor of intestinal glucosidases, significantly reduced the satiating effects of maltose infused at duodenum (not shown) or at midgut. In other experiments (20), the noncompetitive inhibitor of lipase, orlistat, completely abolished lipolysis and satiation from premeals of triglyceride, so we reasoned that the statistically significant but only partial blockade of the satiating effects of maltose was the outcome of an only partial, competitive inhibition of maltase by the acarbose. An alternative interpretation might be that suppression of intakes by maltose was signalled both by sensors triggered by maltose itself and additional sensors triggered by glucose released from the maltose.

Nevertheless, on the basis of this assumption [the known distributions of the three disaccharidases in the brush borders of enterocytes along the gut (1, 2, 30), the known (1, 2) differences of spread of glucolytic products from these three disaccharides along the gut (in turn, the outcome of the content per cm of disaccharidase activities), and the idea that suppression of nutrient intakes varied with the length of gut contacted by glucolytic products], we made three predictions (Fig. 2) that proved to be correct: 1) duodenal trehalose was less potent than duodenal maltose (presumably because the intestinal distribution of trehalase limited release of glucose to the proximal small intestine); 2) low and moderate loads of duodenal lactose were more effective than isocaloric loads of duodenal maltose (presumably because even low loads of duodenal lactose were known to spread glucolytic products farther along the gut than isocaloric loads of maltose); and 3) by contrast, lactose infused into midgut was not more effective than maltose (presumably because the greater spread of lactose on duodenal infusion was eliminated by removing one-half of the small intestinal length from the perfusion).

Because of the underlying assumption that the sugars signalled only via released glucolytic products, the fact that the three predictions proved to be correct was only weakly supportive of the idea that intensity of feedback varied with length of gut contacted by glucolytic products. However, the argument was considerably strengthened by the additional observation (Fig. 3) that confining lactose to a 35-cm loop of jejunum abolished its suppressive effects observed previously in the same animals when the lactose had access to virtually the entire gut. That the efficacy of lactose was more reduced by this confinement than the correspondingly efficacious effects in the same animals is more consistent with the idea that both disaccharidases saturated through the release of their constituent sugars but not consistent with the alternative, that satiation was mediated by sensors specific to dimeric lactose and dimeric maltose. If the alternative was correct, then sensors specific to duodenal lactose must have been either more responsive and/or more numerous in proximal bowel than putative sensors to maltose in order to explain the higher efficacy of duodenal, but not midintestinal, lactose over maltose (Fig. 2, C and D). If that were so, then confining both perfusates to proximal bowel in the Thiry Vella loops should have diminished the response to maltose more than to lactose; but, in fact, the reverse was the case. Therefore, the higher efficacy of duodenal lactose over maltose was the result of a longer spread of lactose products along the gut.

Because we previously (21) observed good correlations between percent inhibition of intakes in sham-feeding vs. naturally feeding rats during intestinal perfusions of various nutrients, we felt that it would be informative to compare percentage (of control) intakes during jejunal perfusion with maltose in sham-feeding rats with Thiry Vella loops with percentage (of control) intakes during duodenal trehalose in naturally fed animals. A total of 28 animals received the high dose of maltose before and after creation of Thiry Vella loops. When the 4.8 mmol/h of maltose were given access to the entire gut, sham-feeding intakes were 44 ± 5% of those during control perfusions with saline, but when the maltose was later confined to the 35-cm Thiry Vella loops, intakes during the maltose were 71 ± 12% of control intakes. These percentages before vs. after creation of the loops differed significantly (P < 0.05, paired t-test) from each other, although each was nevertheless significantly lower than 100%. In comparison, during 4.8 mmol/h of duodenal trehalose given access to the entire gut, food intakes in naturally...
feeding animals were 75 ± 4% of control intakes, whereas intakes during isocaloric, duodenal maltose were 40 ± 4% of control in the same animals. Release of glucose from trehalose is functionally confined by the distribution of mucosal trehalase (1) to the proximal 67% of gut, but trehalase activity declines rapidly along the middle third. Thus there was a similar order of satiation by maltose confined to the proximal one-third of gut and by trehalose functionally confined to about the proximal one-half of bowel.

In our earlier study (21), comparisons of duodenal to midintestinal maltose were conflicting; in 9 sham-feeding animals there were no significant differences between duodenal and midintestinal maltose, whereas in 10 naturally feeding animals, the slope of the duodenal dose response was slightly but significantly (P < 0.025) more negative. In the present experiments, there were seven additional naturally feeding animals that received both duodenal and midintestinal maltose. Among all 17 animals the slope of the dose response to duodenal maltose was significantly more negative than the slope for midintestinal maltose, mainly because intakes during the 4.8 mmol/h of duodenal maltose were much lower (P < 0.005) than those during the same dose delivered at midgut (as in Fig. 2, B vs. D). Therefore, either 1) proximal sensors were more potent or more numerous than distal sensors or 2) the duodenal maltose gave a larger response because the whole gut participated, whereas the midintestinal perfusions bypassed the jejunum to diminish the total number of additive sensors contacted. Because restricting maltose to 35-cm loops of jejunum in 28 sham-feeding animals significantly reduced inhibition of intakes by the maltose, finding 2 appears to be the correct alternative.

In comparison with isocaloric loads of duodenal maltose, duodenal lactose given access to the whole gut was more effective at lower loads and as potent at the highest load (Fig. 2). Yet the absorption of component glucose and galactose from lactose is known to be very slow in comparison with absorption of glucose from maltose because of the rate-limiting, slow hydrolysis of the lactose by low amounts of mucosal lactase. Thus overall absorption of lactose from the entire gut may have been only one-tenth as fast as overall absorption of maltose (2). That such slowly absorbed duodenal lactose was as effective or more effective than isocaloric maltose on inhibiting food intakes indicates that lactose signalled at the level of the gut and not from putative postabsorptive mechanisms, which might depend on quantitative metabolism of absorbed sugars. This idea differs from, but is analogous to, previous observations that partially metabolized sugars (o-xylate) or nonmetabolized sugars (<-methylglucose, 3-O-methylglucose) with affinities for the glucose transporter were as potent as fully metabolizable, isocaloric glucose (21). Both observations (with dimeric lactose vs. maltose and with monomeric glucose analogs vs. glucose) reenforce the notion that short-term signaling of satiety by these carbohydrates is an intestinal, not a postabsorptive, phenomenon.

On the other hand, lactose confined to 35 cm of jejunum was less satiating than maltose similarly confined (Fig. 3) in a situation in which each undoubtedly contacted the full length of the 35-cm loop. This observation suggests that the 10-fold higher (2) rate of hydrolysis and absorption of monomeric sugars from maltose resulted in a slightly stronger (but not statistically significant and certainly much less than 10-fold) stimulus from this fixed, 35-cm segment. Alternatively, the mix of glucose and galactose from the lactose could have been less potent than the two glucose released from the maltose, but this second idea seems unlikely because duodenal glucose and galactose individually were about equipotent (Fig. 2B). In other nutrient-driven, intestinal feedbacks, responses varied with loads and/or concentrations of signalling nutrients even when nutrients were confined to short, submaximal lengths of gut (5, 13, 23, 25). From all of these considerations, we think it is likely that intestinal satiety varies with an integration by mucosal sensors of both total length of gut contacted and the intensity of stimulus along each centimeter of contact.

Limiting access of oleate to 35 cm of jejunum. Our studies on the absorption of oleate from perfusates of oleate-monolein (Fig. 5) confirmed 1) that oleate was slowly absorbed (5, 13) and 2) that its absorption was similar (i.e., no significant difference between sums of responses) whether varied loads were introduced at high or low concentrations, respectively, at low or high flows (8, 23, 25, 26). Although more oleate may have been absorbed from the lower two loads when delivered as 80 mM oleate at 3 or 6 ml/h compared with 20 or 40 mM oleate at 12 ml/h, it is probable that our inability to collect small volumes of effluent adhering to body fur and restraint cages resulted in a lower percentage recovery of the smaller volume perfusates, giving, to some degree, a distorted impression of higher absorption from the 80 mM oleate. On the other hand, it may also be that the 80 mM concentration actually enhanced absorption by speeding diffusion into absorptive cells.

Sixty to seventy percent of the oleate was absorbed from each of the two lower doses, but only 50% of the 960 µmol/h dose was absorbed by the jejunal loop that was one-third of gut length. Because ileum absorbs fat at one-half the rate of jejunum (33), about one-sixth of the 0.96 mmol/h of duodenal oleate probably escaped small intestinal absorption to spill over into colon, a conclusion that well accounts for patterns of diarrhea seen during perfusions with oleate-monolein. Our rather crude data suggest that the loops absorbed approximately 10% of the 480 and 960 mmol/h loads because absorption dropped from 70 to 50% of load between these two doses. It is known that the entire rat small intestine can transport triglyceride (resynthesized after absorption of fatty acids and monoglycerides) at a maximum rate of 200 µmol/h (29) or ~400 µmol/h of fatty acid for the proximal one-half of small intestine. During the 960 µmol/h loads, absorption of ~480 µmol/h of oleate was of this order of magnitude. Thus we would conclude that absorption of
oleate by the jejunal Thiry Vella loops was completely normal and, therefore, that the loss of inhibition of sham feeding on confining oleate to these loops was not an artifact of disordered absorption.

We observed nearly a complete loss of inhibition of food intakes when perfused oleate-monolein was confined to 35-cm Thiry Vella loops in sham-feeding rats compared with potent inhibition observed in the same animals when the perfused oleate had access to the full intestinal length (Fig. 4). However, in the same animals, maltose was as effective when confined to the loop as it had been when given access to the entire gut. Thus suppression of food intakes by intestinally perfused, slowly absorbed oleate appeared to be much more dependent on full intestinal access than was maltose. This result with oleate-monolein was consistent with three previous observations. 1) We observed in dogs (3) that promoting (via reversible exclusion of bile) the spread of digested, but unabsorbed, fat from an initial meal into ileum significantly suppressed intake of a second meal taken 4 h later. 2) Duodenally perfused oleate-monolein (21) produced an inhibition of food intake in naturally feeding rats with a significantly more negative slope to the dose response than did the same solutions infused at midgut, that is, the inhibition was significantly less when the most proximal 50% of the small intestinal length was removed from contact with the oleate during midintestinal perfusions. The principal reason for the greater negative slope of the duodenally perfused oleate-monolein was that inhibition from the highest dose (0.96 mmol/h) was significantly greater on duodenal perfusion. 3) Similarly, resecting the distal 50% of small bowel significantly reduced inhibition of intakes of naturally feeding rats by duodenally perfused oleate. Loss of inhibition from oleate-monolein after removal of 50% of distal bowel was greater than loss of inhibition after bypassing the proximal 50% of bowel during perfusions into midintesti-
tine, but the smaller difference during midintestinal perfusions, with bypass of proximal bowel, probably resulted from the fact that some ingested rat chow emptied into the bypassed jejunum in unresected ani-
imals where it also inhibited intake. The present result (that confining oleate-monolein to 35 cm of jejunum nearly abolished its satiating properties) is entirely consistent with all three previous observations; all indicate that high satiation from fat depends on entrain-
ing additive inputs from sensors of fatty acids along the full intestinal length. Complete loss of efficacy on confinement to 35-cm loops simply reflected a more severe shortening of total length of contact with small and large bowel than encountered when jejunum was bypassed or ileum was resected.

Monomeric and dimeric nutrients were used to demonstrate how intestinal distributions of nutrients may affect satiety. With polymeric foods, intestinal distribu-
tions of satiating hydrolytic products are additionally determined by digestion. Thus daily intakes of chow could be significantly reduced in dogs just by reversibly shifting entry of pancreatic enzymes to ileum (17). Normally, triglycerides enter duodenum at rates that supersaturate pancreatic lipase. As duodenal loads increase, lipolysis does not keep pace and is completed farther and farther downstream (18, 20). Thus the ratio of fat to lipase entering the duodenum, in turn related to the load of fat ingested (14, 16, 19, 20), may profoundly affect the intestinal distribution of and satiation from lipolytic products (14, 20). By contrast, amylase-
sis is a very rapid process that is carried out jointly by luminal amyloses and by saccharidases in enterocytes. For many dietary saccharides, hydrolysis is so rapid that the distribution of released glucose is determined by transport maxima of cells along the gut (1, 2, 7). That intestinal distributions of products stem from absorptive, rather than hydrolytic, capacities probably accounts for the fact that easily digestible starch, Polycose, maltose, and glucose were about equally satiating, calorie for calorie, (15, 21), in a system in which feedback inhibition of food intake depends heavily on length of sensory contact with released glucose. Nevertheless, some polymeric carbohydrates (10, 31) may satiate differently because their hydrolysis is rate limiting (as with lactose or trehalose). Satiating poten-
tials of polymeric nutrients may therefore depend on the specifics of their digestion and on their digestibility (10, 28, 31), as well as on their caloric content.

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