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ORIGINAL ARTICLE

Ileal brake activation: macronutrient-specific effects on eating behavior?

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BACKGROUND: Activation of the ileal brake, by infusing lipid directly into the distal part of the small intestine, alters gastrointestinal (GI) motility and inhibits food intake. The ileal brake effect on eating behavior of the other macronutrients is currently unknown.
OBJECTIVE: The objective of this study was to investigate the effects of ileal infusion of sucrose and casein on food intake, release of GI peptides, gastric emptying rate and small-bowel transit time with safflower oil as positive control.
DESIGN: This randomized, single-blind, crossover study was performed in 13 healthy subjects (6 male; mean age 26.4 ± 2.9 years; mean body mass index 22.8 ± 0.4 kg m^{-2}) who were intubated with a naso–ileal catheter. Thirty minutes after the intake of a standardized breakfast, participants received an ileal infusion, containing control ((C) saline), safflower oil ((HL) 51.7 kcal), low-dose casein ((LP) 17.2 kcal) or high-dose casein ((HP) 51.7 kcal), low-dose sucrose ((LC) 17.2 kcal) and high-dose sucrose ((HC) 51.7 kcal), over a period of 90 min. Food intake was determined during an *ad libitum* meal. Visual analogue score questionnaires for hunger and satiety and blood samples were collected at regular intervals.
RESULTS: Ileal infusion of lipid, protein and carbohydrate resulted in a significant reduction in food intake compared with control (HL: 464.3 ± 90.7 kcal, $P < 0.001$; HP: 458.0 ± 78.6 kcal, $P < 0.005$; HC: 399.0 ± 57.0 kcal, $P < 0.0001$ vs control: 586.7 ± 70.2 kcal, $P < 0.001$, respectively). A reduction in energy intake was still apparent when the caloric amount of infused nutrients was added to the amount eaten during the *ad libitum* meal. Secretion of cholecystokinin and peptide YY but not of glucagon-like peptide-1 (7–36) was increased during ileal perfusion of fat, carbohydrates and protein. During ileal perfusion of all macronutrients, a delay in gastric emptying and intestinal transit was observed, but differences were not significant compared with control.
CONCLUSION: Apart from lipids, also sucrose and casein reduce food intake on ileal infusion, thereby activating the ileal brake. In addition to food intake, also satiety and GI peptide secretion were affected.

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INTRODUCTION

Worldwide, the incidence of overweight and obesity is rapidly expanding with tremendous negative impact on health and health care costs.[1,2] Up to now, various nutritional and pharmacological strategies for overweight have failed and new treatment modalities in the battle against obesity are urgently needed. An interesting but still poorly explored mechanism is to reduce caloric intake via activation of the so-called intestinal brake, in particular the ileal brake. The brake refers to an intestinal feedback mechanism that is triggered by nutrients at a specific location in the intestine, resulting not only in modulation of gastrointestinal (GI) secretions and motility but also of food intake and hunger.[3,4] This concept was first seen in ileal transposition studies performed in rats. Koopmans and Sclafani were the first to describe this model in 1981 and together with several others it was shown that ileal transposition in rats resulted in a reduction in food intake and weight loss on the long term.[5,6]

A few years later, Welch *et al.*[7,8] demonstrated in humans that ileal infusion of a high amount of lipid delayed gastric emptying, induced satiation and also reduced food intake. Recently, several studies have confirmed these findings with much smaller amounts of intact lipids.[9–12]

Although 'ileal brake'-inducing effects on satiety and food intake have been explored in more detail with respect to lipids, little is known about ileal brake-induced satiating effects of the other macronutrients, carbohydrates and proteins. Ileal infusion of carbohydrates is known to delay gastric emptying rate, decrease intestinal motility and enhance release of peptide YY (PYY) and glucagon-like peptide-1 (GLP-1),[13–15] gut peptides associated with induction of satiation and food intake. When administered intraduodenally, potent inhibitory effects of carbohydrates on energy intake have been demonstrated,[16–18] but effects of carbohydrates on food intake and satiety in humans on intraileal infusion have not been assessed.

Dietary proteins are commonly regarded as the most satiating macronutrients.[19] However, data on effects of intestinal protein infusion on satiety are scarce. It has been demonstrated that ileal protein infusion resulted in a delay in GI motility.[20] Intraduodenal administration of pea protein was shown to induce a more pronounced inhibitory effect on food intake compared with oral ingestion of the same amount of pea protein.[21]

Up to now, human data on effects of ileal exposure to carbohydrates and proteins on food intake and satiety are lacking. This study was undertaken to investigate effects of ileal infusion of

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different doses of carbohydrates and proteins on *ad libitum* food intake in comparison with placebo (control) and with ileal infusion of an equicaloric amount of lipids, as positive control. In addition to food intake, also satiety, gastric emptying, small intestinal transit time and GI peptide secretion were measured. Previous studies with duodenal infusion of macronutrients did not show major differences in satiety and food intake between macronutrients.[18,22] We therefore hypothesized that ileal infusion of carbohydrates and proteins will result in an equal, dose-dependent reduction of food intake, and in equally enhanced satiety compared with ileal infusion of equicaloric amounts of lipids.

MATERIALS AND METHODS

Participants

Healthy men and women, aged between 18 and 55 years with a body mass index between 18 and 25 kg m^{-2}, were recruited by local advertisement to participate in this study. Smoking, consumption of >100 g alcohol per week, medical history, active symptoms and medication use (apart from oral contraceptives) were exclusion criteria. All participants reported to be weight stable for at least 2 months before participation, to be unrestrained eaters (assessed by the Dutch eating behavior questionnaire) and were on a normal caloric diet.[23] Written informed consent was obtained from each individual before inclusion in the study. This study was approved by the Medical Ethics Committee of the Maastricht University Medical Center+, Maastricht, The Netherlands, and performed in full accordance with the Declaration of Helsinki. The study has been registered in the US National Library of Medicine (http://www.clinicaltrials.gov, NCT01509469).

According to our power calculation, 13 subjects were needed to complete the study. This number was based on the difference in food intake observed in previous work.[7,10] Fifteen subjects met the inclusion criteria. Due to discomfort induced by the ileal catheter, two subjects did not complete all experiments and dropped out of the study. Thirteen healthy subjects (6 male, age 26.4 ± 2.9 years, body mass index of 22.8 ± 0.4 kg m^{-2}) completed the study.

Study outline

This single-blind randomized placebo controlled study compared the effects of six different interventions: (1) saline (control (C)); (2) lipid emulsion (6 g safflower oil (HL), 51.7 kcal); (3) protein low dose (5 g casein (LP), 17.2 kcal); (4) protein high dose (15 g casein (HP), 51.7 kcal); (5) carbohydrate low dose (4.3 g sucrose (LC), 17.2 kcal); and (6) carbohydrate high dose (12.9 g sucrose (HC), 51.7 kcal). Each of the substances was infused directly into the ileum over a 90-min period, on separate test days. Test days were randomly assigned (by using Research randomizer, www.randomizer.org) and subjects were tested in 2 consecutive weeks, with 3 test days planned in each week.

Catheter positioning

We used a 270-cm-long silicon 9-channel (8 channels, 1 balloon inflation channel, outer diameter 3.5 mm) custom-made catheter (Dentsleeve international, Mui scientific, Mississauga, Ontario, Canada). The catheter contained three sideholes per channel with 3-cm interspacing between consecutive side holes, and had an inflatable balloon (maximum inflation capacity 10 ml) integrated into the distal tip. Nutrients were directly infused into the ileum.

On the day of catheter introduction (monday), subjects were allowed to consume a light breakfast in the morning (ingested before 0800 hours). After local anesthesia of the nasal mucosa (xylocaine 10% spray; AstraZeneca, Zoetermeer, The Netherlands), the catheter was introduced transnasally into the stomach. Under intermittent fluoroscopic control, the catheter was positioned with the tube tip located in the proximal small intestine. Further progression of the catheter into the ileum was attained as described previously.[11] Participants returned to the department at 0800 hours the next 3 days for test days 1, 2 and 3 (tuesday, wednesday and thursday) and a week later for test days 4, 5 and 6, respectively. Before the start of each test day, the position of the catheter was checked by fluoroscopy. In all subjects, the tip of the catheter was placed at least 120 cm distal to the pylorus.[24]

Nutrient infusions

In this study, we infused protein, carbohydrate and lipid directly into the ileum. Casein (energy density: 3.45 kcal g^{-1}, Dutch Protein Services, Tiel, The Netherlands) was used as protein source. Sucrose (energy density: 4 kcal g^{-1}, van Gilse Automatensuiker, Oud Gastel, The Netherlands) was used as carbohydrate source. Safflower oil (6 g; energy density: 8.6 kcal g^{-1}, de Wit Specialty Oils, de Waal, The Netherlands) was used as positive control, as it was shown repeatedly that safflower oil appears to be the most potent lipid in ileal brake activation.[9] All nutrients were dissolved in a total volume of 180 ml water and administered at a rate of 2 ml min^{-1} (total infusion time 90 min).

Experimental design

Each subject participated in 6 test days. On all test days, an intravenous catheter was placed in a forearm vein for collection of blood samples. At 0830 hours, a basal blood sample, visual analogue scores for hunger and satiety and breath samples were obtained. Subsequently, a standardized breakfast meal, consisting of a sandwich and an egg (sunny side up, 210 kcal), was consumed. As intraileal infusion of nutrients is known to delay gastric emptying and intestinal transit, gastric emptying rate of the breakfast meal, determined by using the ^{13}C stable isotope breath test,[25] and duodenocecal transit time, measured by hydrogen breath testing, were included as GI transport parameters.[26] At the start of the ileal infusion (at $t=30$), 6 g of lactulose was administered directly into the duodenum to enable measurement of the small-bowel transit time (SBTT). Ileal substrate infusion was scheduled from $t=30$ to 120 min after breakfast ingestion. One hour after ending the infusion, the volunteer received a standardized *ad libitum* lunch meal (sandwiches with egg salad (energy density: 2.2 kcal g^{-1}), $t=180$). Sandwiches were randomly cut in different sized pieces, to mask the number of sandwiches eaten. After ingestion of the lunch meal, the test day was finished and subjects could return home.

GI peptides

Venous blood samples were drawn at regular intervals. For GLP-1 (7–36), PYY and cholecystokinin (CCK) measurements, blood was collected in ice-chilled aprotinin-coated tubes (Becton & Dickinson, Franklin Lakes, NJ, USA). Immediately after blood collection, 10 µl of dipeptidyl peptidase-4 inhibitor (Millipore, Billerica, MA, USA) per 1 ml of whole blood was added to the tubes to prevent proteolytic cleavage. Tubes were immediately centrifuged at a rate of 3000 r.p.m. and 4 °C for 15 min. Plasma was transferred into aliquots and stored on dry ice for the rest of the test day. At the end of the test day, samples were stored at − 80 °C.

Active GLP-1 (7–36) was determined using a Glucagon Like Peptide-1 (Active) ELISA kit (EGLP-35K, Millipore) with a range of 2–100 pM, an inter-assay coefficient variation (CV) of 11% and an intra-assay CV of 6% (EGLP-35K, Millipore, Linco Research). Total PYY (includes both peptide YY1–36 and peptide YY3–36) was measured using a Human PYY (Total) ELISA kit (EZHPYYT66K, Millipore) with an inter-assay CV of 6% and an intra-assay CV of 3% (EZHPYYT66K, Millipore, Linco Research). Plasma CCK-8 (CCK 26–33) concentrations were measured with an optimized and validated commercial human RIA kit (EURIA CCK, RB302, Euro-Diagnostica, Malmö, Sweden). This improved assay system has been optimized to reach a high sensitivity of 0.05 pmol l^{-1} and to have no cross-reactivity to gastrin-17 or sulfated gastrin. The intra-assay CV was 8.9% at a concentration of 0.84 pmol l^{-1} and 4.9% at a concentration of 1.98 pmol l^{-1}.

The effects of each intervention on peptide secretion were determined by analyzing the peptide levels at the onset of ileal infusion until ingestion of the *ad libitum* meal. All data were corrected for the values obtained at the onset of infusion.

Satiety and hunger scores

Scores for hunger and satiety feelings (for example, satiety, fullness, hunger, desire to eat, desire to snack) were measured using visual analogue scales (0–100 mm) anchored at the low end with the most negative or lowest intensity feelings (for example, extremely unpleasant, not at all), and with opposing terms at the high end (for example, extremely pleasant, very high, extreme).[27]

Gastric emptying

^{13}C-octanoic acid (100 mg, Campro Scientific bv, Veenendaal, The Netherlands) was mixed into the standardized breakfast meal ingested at $t=0$. Breath samples of $^{13}CO_2$ were obtained as described previously.[12] Samples

were analyzed by using isotope ratio mass spectometry (IRIS, Wagner, Bremen, Germany).

Small-bowel transit time

Duodenocecal transit time was determined by the lactulose hydrogen breath test, as described by Ledeboer *et al*.[26] Via an opening of the catheter located in the duodenum, 6 g of lactulose (Legendal, Inpharzam, Amersfoort, The Netherlands) was administered at the start of ileal infusion of the substrates. Breath samples were taken at 15 min intervals and analyzed using a handheld hydrogen breath test unit (Gastyrolyzer, Bedfont Scientific, Kent, UK). SBTT was defined as the time between lactulose administration and the onset of a sustained rise in breath hydrogen concentration of at least 10 p.p.m. above basal level.

Statistical analyses

Statistical analyses were performed using the SAS statistical software package (SAS version 9; SAS institute, Cary, NC, USA). Proc Gplots were used to test outcome variables for normality of distribution. If data were not normally distributed, log transformation was applied for further analysis of the data, as was the case for CCK, GLP-1 (7–36) and PYY.

Regarding food intake, statistical analysis was performed on the amount of food eaten in kcal. CCK, GLP-1 (7–36) and PYY are displayed from the start of the infusion ($t = 30$ min) until the last blood sample collected before the start of the *ad libitum* meal ($t = 180$ min). All variables were compared with a mixed analysis of variance model that included the fixed factors treatment (C, HL, LP, HP, LC and HC). For the plasma parameters, time and the interaction between treatment and time were added to the model. The factor subject was added to the model as random factor. A *post hoc* Tukey test was used to analyze differences between treatments. Data are presented as the mean ± s.e.m. (unless specified otherwise) and considered significant at $P < 0.05$.

RESULTS

Food intake

Ileal infusion of lipid, high-dose protein and high-dose carbohydrates resulted in a significantly lower energy intake during the *ad libitum* meal compared with control (HL: 464.3 ± 90.7 kcal, $P < 0.001$; HP: 458.0 ± 78.6 kcal, $P < 0.005$; HC: 399.0 ± 57.0 kcal, $P < 0.0001$ vs control: 586.7 ± 70.2 kcal, respectively, Figure 1). There were no statistically significant differences in food intake between the different nutrient infusions HL, HP and HC. No effect of LP and LC over control on food intake was observed (LP: 528.4 ± 86.1 and LC: 491.4 ± 77.5 kcal). Even after adding the caloric amount of infused nutrients to the amount eaten during the *ad libitum* meal (in kcal), the reduction in energy intake was significant (HL and HC vs C, $P < 0.05$ and $P < 0.005$, respectively).

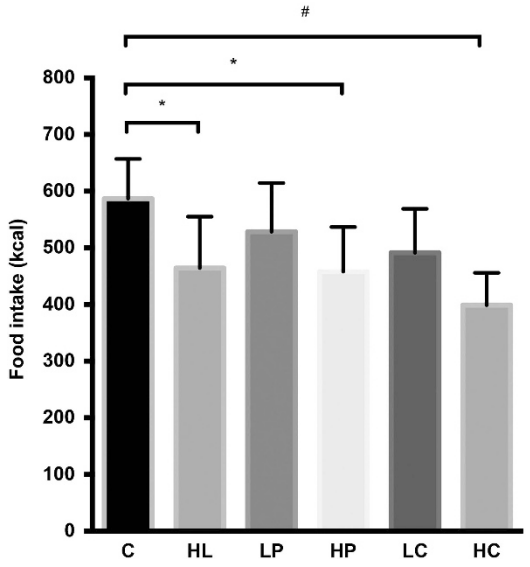

Figure 1. Food intake in kcal (mean+s.e.m.) of the *ad libitum* lunch ingested 60 min after ending the intraileal infusion of control (C), safflower oil (HL), low-dose casein (LP), high-dose casein (HP), low-dose sucrose (LC) and high-dose sucrose (HC). *$P < 0.005$ and #$P < 0.0001$.

Satiety and hunger scores

Fasting scores for hunger and fullness at the start of the experiments did not differ among the six interventions. Ingestion of the breakfast meal resulted in a significant decrease in hunger and an increase in fullness scores in all six treatments (data not shown). Significant differences in hunger scores were observed only after start of intraileal infusion of high-dose protein (from 30 to 180 min; $P < 0.0001$), but not of the other interventions compared with control (Figures 2a and b). After ingestion of the breakfast, fullness scores increased in all experiments. Figures 2c and d shows the integrated fullness scores from the start of ileal infusion up to the intake of the *ad libitum* meal. No significant differences were observed in fullness scores between the various treatments and control (Figures 2c and d).

GI peptides

CCK. Plasma CCK levels were measured for the C, HL, HP and HC interventions but not for LP and LC. Baseline plasma CCK concentrations did not differ between interventions. The breakfast meal, ingested 30 min before starting the ileal infusions, induced an increase in CCK concentration in all four measured treatments from 0.30 ± 0.06 pmol l^{-1} at baseline to 0.74 ± 0.05 pmol l^{-1} at 30 min after breakfast intake ($P < 0.0001$; Supplementary Table 1). Data are corrected for the CCK levels at $t = 30$ min, when ileal infusions started. Figure 3a shows that, after an initial postprandial increase, the plasma CCK levels decline from 45 or 60 min after breakfast intake onwards. Ileal infusion of lipid, high-dose protein and high-dose carbohydrates all resulted in higher CCK levels following ileal infusions compared with control. Consequently, the negative area under the curve (AUC) of CCK concentrations over time, corrected for the CCK levels at the start of ileal infusions, was smaller after lipid and high-dose protein intervention ($P < 0.05$ and $P < 0.005$, respectively; Figure 3b), although this did not reach statistical significance for the high-dose carbohydrate treatment.

GLP-1 (7–36). Baseline plasma GLP-1 (7–36) concentrations did not differ between study days. The breakfast meal induced an increase in GLP-1 (7–36) concentration in all six treatments from 2.63 ± 0.26 pmol l^{-1} at baseline to 3.84 ± 0.25 pmol l^{-1} at 30 min after breakfast intake ($P < 0.005$; Supplementary Table 1).

The AUC GLP-1 (7–36; 30–180 min) from start of ileal infusion to onset of meal intake did not significantly differ between any of the treatments (Figure 4b).

Ileal infusion of high-dose protein resulted in a larger increase in plasma GLP-1 (7–36) when compared with low-dose carbohydrates (Figure 4a).

PYY. Baseline plasma PYY concentrations did not differ between study days. The breakfast meal induced an increase in PYY concentration in all six treatments from 51.36 ± 2.98 pmol l^{-1} at baseline to 60.66 ± 2.98 pmol l^{-1} at 30 min after breakfast intake ($P < 0.05$; Supplementary Table 1). The 30–180 min AUCs from the start of ileal infusion to onset of meal intake did not significantly differ between any of the treatments (Figure 5b).

Infusing high-dose carbohydrates and lipid into the ileum resulted in a significantly larger increase in plasma PYY when compared with control, and of high-dose carbohydrates versus low-dose carbohydrates, respectively (Figure 5a). The same was true for high-dose protein vs low dose of protein but not vs control.

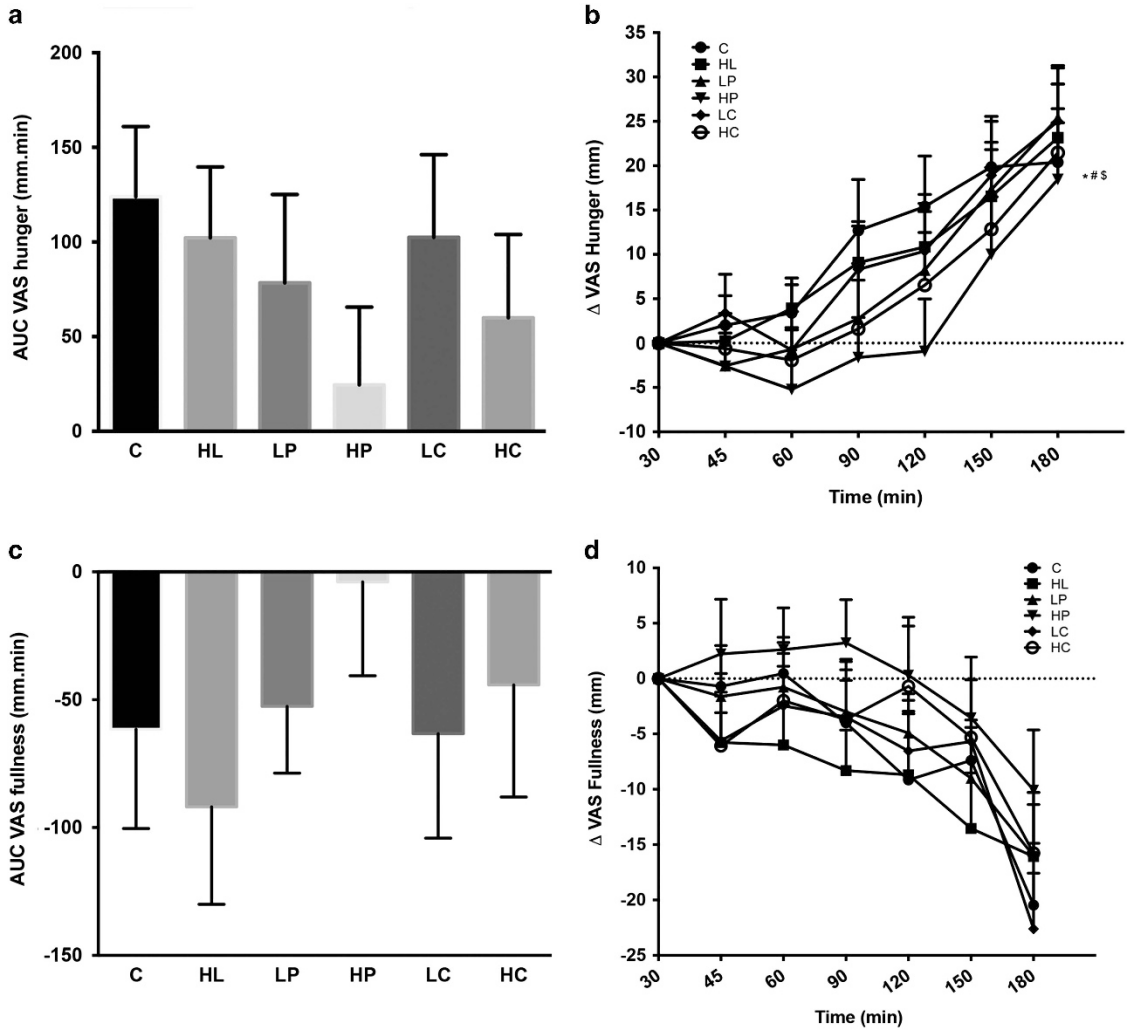

Figure 2. Hunger (mean+s.e.m.; **a** and **b**) and fullness (mean+s.e.m.; **c** and **d**). Visual analogue scores (VAS) hunger concentrations (**b**); AUCs (30–180 min, **a**) and VAS fullness concentrations (**d**); and AUCs (30–180 min, **c**) during intraileal infusion of control (C), safflower oil (HL), low-dose casein (LP), high-dose casein (HP), low-dose sucrose (LC) or high-dose sucrose (HC). Ileal infusion was started at $t = 30$ (30 min after breakfast consumption) and continued for 90 min. An *ad libitum* lunch was offered at $t = 180$ min. AUCs were calculated by using the trapezoid rule. $^{*}P < 0.005$, significantly different from C; $^{\#}P < 0.05$, significantly different from HL; $^{\$}P < 0.05$, significantly different from LC.

Gut peptides and food intake

Energy intake during the *ad libitum* meal intake (in addition to the amount of kcal infused) was inversely related to plasma GLP-1 (7–36) AUC ($r = -0.4$, $P < 0.0005$) and CCK AUC ($r = -0.4$, $P < 0.005$). No significant correlation between food intake and PYY was found.

GI transport

Ileal nutrient infusion resulted in a tendency to a slower gastric emptying half time ($t^1/_2$); however, differences were not statistically significant vs control. This was also true for SBTT compared with control (saline infusion); nonsignificant differences (Table 1).

DISCUSSION

We have shown that ileal infusion of proteins and carbohydrates in healthy volunteers suppresses food intake to the same extent as an equicaloric amount of lipids. Lower doses of proteins and carbohydrates did not affect food intake or satiety/satiation. Thus, the three macronutrients all affected the ileal brake and associated eating behavior to the same extent, whereas low concentrations may not reach the sensing threshold to induce such effects.

Macronutrients and its metabolites are sensed by various receptors in the GI tract. Each macronutrient activates different receptors, mainly present on I- and L cells. Lipids trigger the release of CCK, GLP-1 and PYY primarily via activation of several fatty acid receptors (FFARs, GPR120), whereas recent evidence suggests that carbohydrates exert their effects on GI peptide release via a possible interaction between the sweet taste receptor (T1R2-T1R3) and the sodium-glucose cotransporter 1 (SGLT1).[28,29] Proteins and their metabolites are able to trigger the peptone (LPAR5) or the umami receptor (T1R1-T1R3). Activation of these receptors by macronutrients results in the release of a variety of GI peptides, which exert their actions through endocrine, paracrine and neurocrine pathways.[30]

Welch *et al.*[7] were the first to demonstrate that infusion of lipid in the form of corn oil in the ileum caused a significant reduction in food intake in healthy volunteers. We infused a much smaller amount of lipid but still observed a significant decrease in food intake, confirming results of previous studies from Welch *et al.*[7] and others.[10] However, in other studies, applying ileal intubations, no effects of safflower oil on food intake could be demonstrated.[9,11,12] This lack of effect may have been caused by differences in study

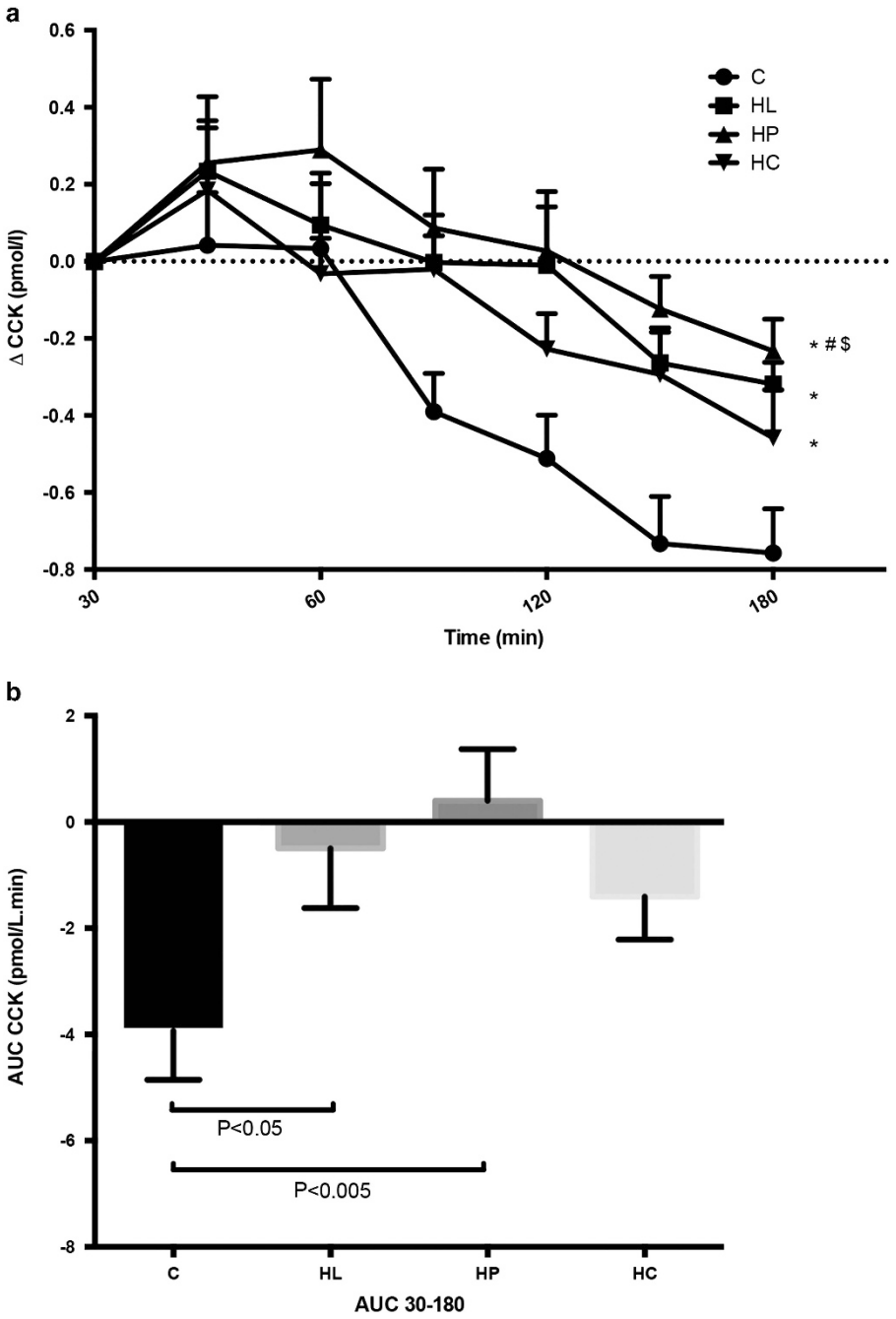

Figure 3. CCK (mean+s.e.m.). ΔCCK concentrations (**a**) and AUCs (**b**). Intraileal infusion of control (C), safflower oil (HL), high-dose casein (HP) or high-dose sucrose (HC) was scheduled from 30 to 120 min. AUCs were calculated by using the trapezoid rule. $^{*}P < 0.0005$, significantly different from C; $^{\#}P < 0.05$, significantly different from HL; $^{\$}P < 0.0005$, significantly different from HC.

design, leading to a longer time interval between the end of the infusion and start of the *ad libitum* test meal.[31]

Effects of intraileal infusion of carbohydrates or proteins on food intake and appetite have not been studied previously. Intraduodenal glucose infusion was shown to induce a reduction in food intake.[16–18,32] The caloric content of infused carbohydrates varied from 180 to 480 kcal in these studies and was not added to the total energy intake during the *ad libitum* meal. None of these studies showed a significant reduction in energy intake when intraduodenal infused calories were added to the ingested calories during the *ad libitum* meal. With regard to intraduodenal protein infusion, comparable amounts of calories, 180–210 kcal, were used. It was shown that activation of the duodenal brake by pea or whey protein infusion resulted in a significant decrease in food intake.[21,22,33] Compared with these previously mentioned studies, we have infused far less calories (52 vs 180–210 kcal) but still found a significant decrease in food intake. Furthermore, we showed that adding the amount of ileal-delivered calories to the calories ingested during the *ad libitum* meal after ileal brake induction still resulted in a significant decrease in overall energy intake. This decrease in overall net energy intake may seem rather small (HL: 70 kcal; HC: 135 kcal). However, it should be noted that this acute effect was achieved with a single infusion. Furthermore, it was shown that a positive energy balance may become negative already with very small daily reductions in energy intake of ~100 kcal per day.[34]

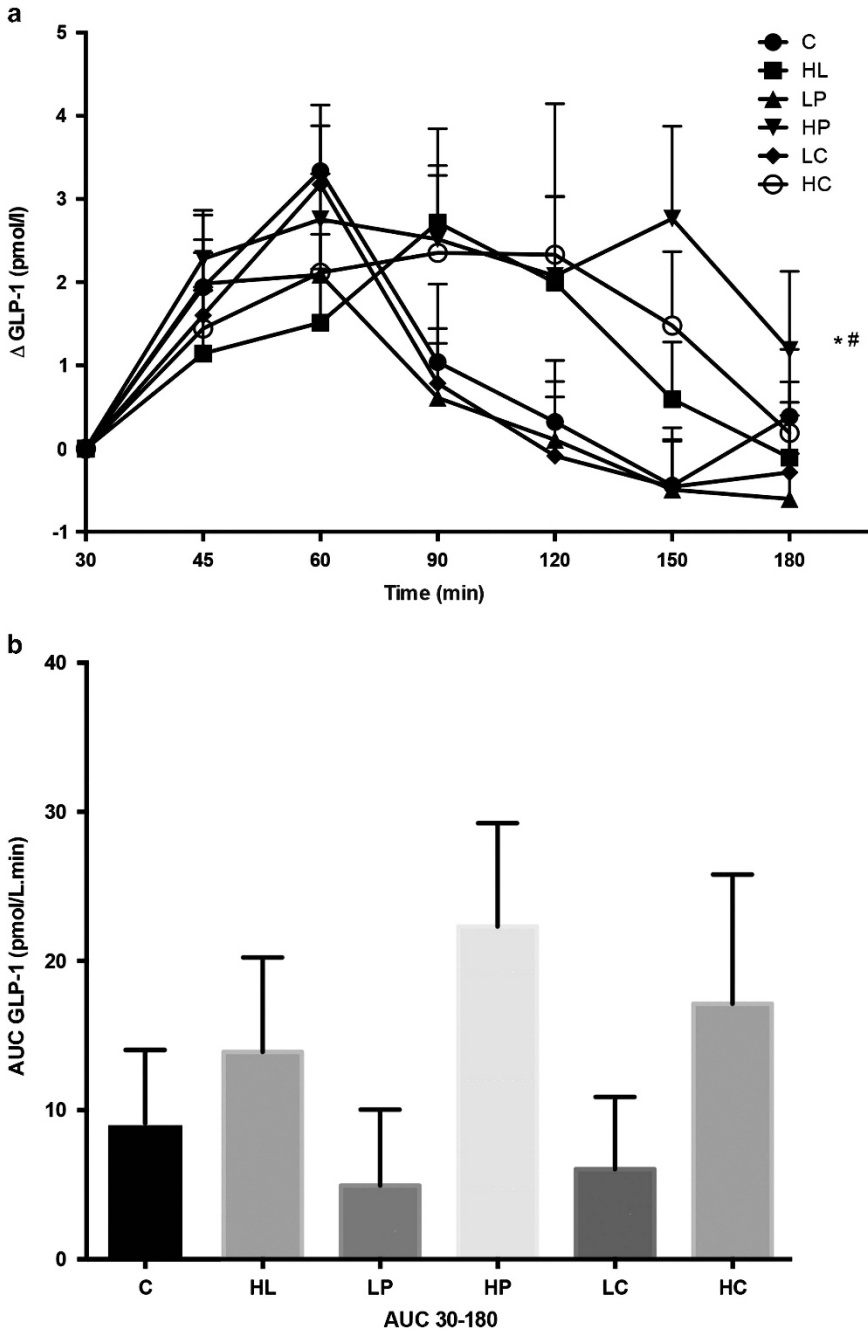

Figure 4. GLP-1 (7–36; mean+s.e.m.). ΔGLP-1 (7–36) concentrations (**a**) and AUCs (**b**). Intraileal infusion of control (C), safflower oil (HL), low-dose casein (LP), high-dose casein (HP), low-dose sucrose (LC) or high-dose sucrose (HC) was scheduled from 30 to 120 min. AUCs were calculated by using the trapezoid rule. $^{*}P < 0.005$, significantly different from LP; $^{\#}P < 0.005$, significantly different from LC.

With respect to satiety feelings, only infusion of high-dose protein resulted in a significant decrease in hunger. Infusions of lipids or high-dose carbohydrates did not significantly affect feelings of hunger and satiety. The absence of such effects may have been caused by certain aspects in our study design, such as feeding status before intestinal brake induction and timing of substrate infusion. However, a significant effect was found in the high-dose protein treatment. This was not unexpected as it is well established that protein is more satiating than carbohydrates or lipid.[35] Furthermore, it is possible that certain amino acids contribute to the perception of satiety.[36]

We observed a significant increase in the release of GI peptides to the systemic circulation after ileal infusion with safflower oil, casein and sucrose. Although release of CCK after intraduodenal protein, carbohydrates and lipid infusion has been well documented,[18,22,33] less is known on CCK release in response to ileal infusion of macronutrients. CCK was regarded as proximal GI peptide.[37] We and others have shown that CCK is also released on distal, ileal nutrient infusion. We cannot differentiate between a direct effect of ileal nutrient on I cells or an indirect effect via paracrine or neurocrine mechanisms, by feedback signaling to the more proximal parts of the small intestine.[38]

GLP-1 release by distal L cells after ileal infusion of triolein, sodium oleate, starch and maltose, but not after peptone infusion, was previously reported.[14] Here we demonstrate a clear increase in GLP-1 (7–36) release after infusion of casein. In fact, we confirm

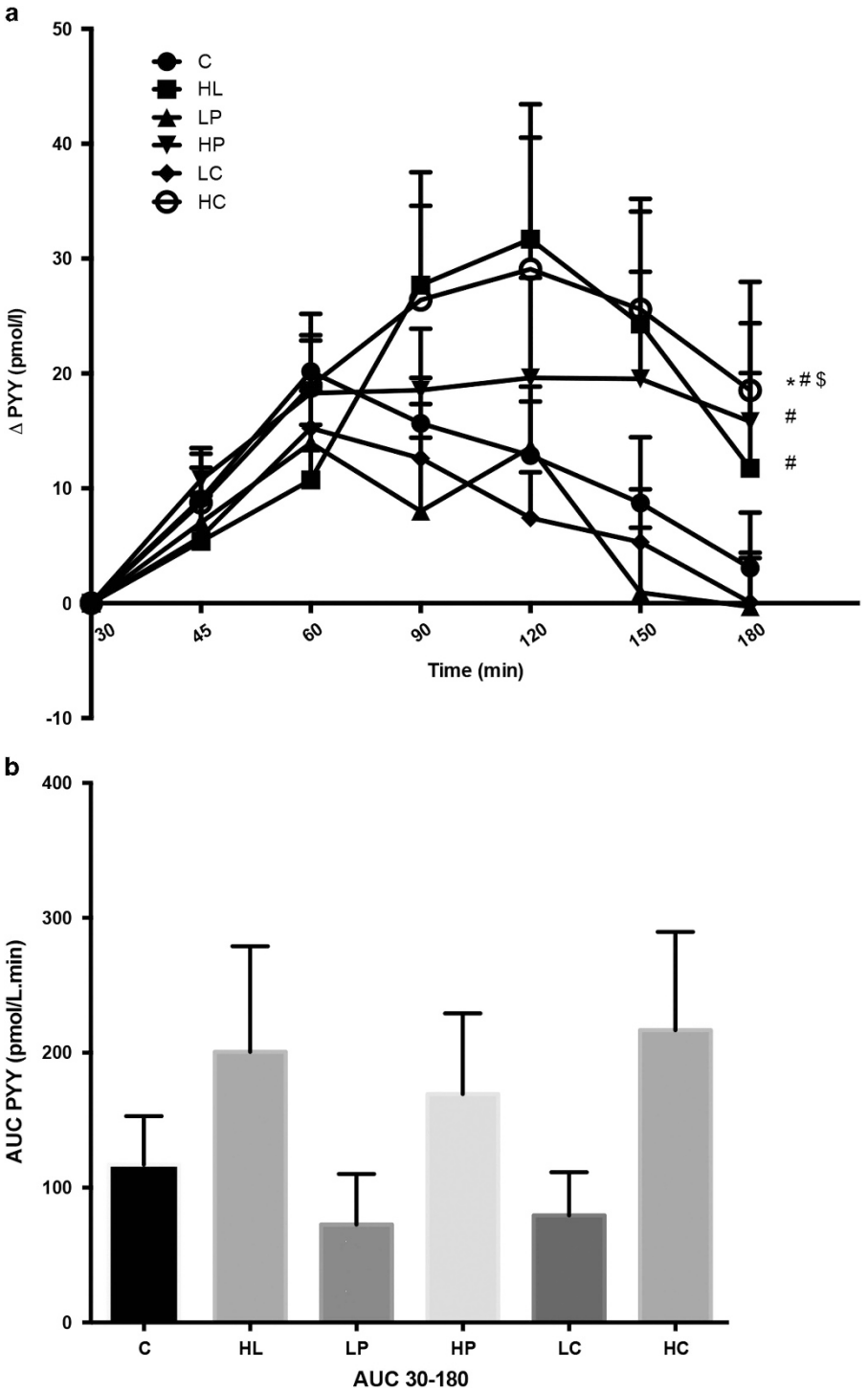

Figure 5. PYY (mean+s.e.m.). ΔPYY concentrations (**a**) and AUCs (**b**). Intraileal infusion of control (C), safflower oil (HL), low-dose casein (LP), high-dose casein (HP), low-dose sucrose (LC) and high-dose sucrose (HC) was scheduled from 30 to 120 min. AUCs were calculated by using the trapezoid rule. $^{*}P < 0.005$, significantly different from C; $^{\#}P < 0.005$, significantly different from LP; $^{\$}P < 0.005$, significantly different from LC.

previous data, as it has been reported that intestinal exposure to intact proteins induces a stronger effect on GLP-1 release compared with protein hydrolysates.[39] In our study, food intake was inversely correlated to both CCK and GLP-1 (7–36) plasma levels, confirming the hypothesis that indeed these GI peptides are involved in the regulation of food intake.[22]

We also observed an increase in PYY secretion following lipid and carbohydrate infusion, which is in line with previous observations on ileal exposure to lipid or rice starch with glucose infusion.[40,41] Infusion of the low dosages of casein and sucrose did not result in enhanced GLP-1 (7–36) or PYY release.

Ileal infusion of lipids and carbohydrates is known to delay gastric emptying and SBTT.[8,11,15,20,24] The nutrient-dependent delays in GI transit data found in this study was of the same magnitude as found in other studies but was not statistically significant, due to study design with activation of ileal brake

Table 1. GI transport

	C	*L*	*LP*	*HP*	*LC*	*HC*	P-*value*
GE 1/2 (min)	155.5 ± 13.8	179.4 ± 20.0	139.9 ± 8.3	166.1 ± 9.7	137.3 ± 9.9	156.2 ± 7.9	NS
SBTT (min)	154.6 ± 13.5	173.1 ± 11.2	180.0 ± 12.4	186.9 ± 12.8	184.6 ± 12.2	186.9 ± 13.8	NS

Abbreviations: GE 1/2, gastric emptying half time; SBTT, small-intestinal transit time. All values are means ± s.e.m. GE 1/2 of the breakfast meal ingested 30 min before, and SBTT during intraileal infusion of control (C), safflower oil (HL), low-dose casein (LP), high-dose casein (HP), low-dose sucrose (LC) and high-dose sucrose (HC).

30 min after onset of gastric emptying and transit time measurement.[42]

Some limitations of our study should be acknowledged. First, effects of macronutrients were studied applying naso–intestinal intubations in healthy individuals. The intubation with a naso–ileal catheter for several consecutive days may have induced discomfort and changes in total well-being, thus affecting study outcome parameters. However, sequence of test days did not influence food intake during the *ad libitum* meal. Second, the various nutrients were infused during 3 consecutive days, resulting in a possible carry-over effect between infusions, although the randomized control design prevented that this effect did influence study outcome. Third, only healthy lean men and women were included in this study. Therefore, these results cannot directly be applied to overweight or obese individuals, as some studies showed a less pronounced suppression of food intake after intraduodenal lipid infusion in obese subjects compared with lean individuals.[43,44] However, it is not clear whether this reduced GI sensitivity also applies for different infusion locations and more importantly other macronutrients.

We are the first to demonstrate that ileal infusion of all three macronutrients induces a decrease in food intake. Furthermore, we showed that this effect was dose dependent. The reduction in food intake confirms the findings in ileal transposition in rats and shows the potential of the ileal brake as a target for food-based strategies in the prevention or treatment of overweight and obesity.[3,45] Conducting a proof of principle study in overweight/obese individuals would contribute to a better understanding of the effect of ileal brake activation on food intake in obese subjects. Therefore, reliable dietary encapsulation or slow release strategies are needed to investigate the application of ileal brake activation in weight management strategies.

In conclusion, we have shown that an ileal brake-satiating effect leading to a decrease in food intake is obtained with small amounts of lipid, protein and carbohydrates. Ileal infusion of equicaloric amounts of these macronutrients modulates food intake, GI peptide release (CCK, GLP-1 (7–36) or PYY) and feelings of hunger.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

FJT, HFH and AAMM designed the research; MvA and FJT conducted the research; MvA and DR analyzed data and performed the statistical analyses; DR performed the hormone analyses; MvA, FJT, HFH and AAMM contributed to interpretation of the results; MvA, FJT and AAMM wrote the manuscript; and AAMM had primary responsibility for the final content of the manuscript.

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