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Pooled data analysis identifies pyloric pressures and plasma CCK concentrations as major determinants of acute energy intake in healthy lean males

Running title: GI determinants of energy intake

Radhika V Seimon [1,2], Kylie Lange [1,2], Tanya J Little [1,2] , Ixchel M Brennan [1,2], Amelia N Pilichiewicz [1,2], Kate L Feltrin [1,2], Astrid J Smeets [3], Michael Horowitz [1,2], Christine Feinle-Bisset [1,2]

[1]University of Adelaide Discipline of Medicine, Royal Adelaide Hospital, [2]NHMRC Centre of Clinical Research Excellence in Nutritional Physiology, Interactions and Outcomes, Adelaide, South Australia, Australia, and [3]Department of Human Biology, Maastricht University, Maastricht, Netherlands

Address for correspondence: A/Prof Christine Feinle-Bisset, PhD
NHMRC Senior Research Fellow
University of Adelaide Discipline of Medicine
Royal Adelaide Hospital
Adelaide SA 5000
Australia
Phone: +61 8 8222 5247
Fax: +61 8 8223 3870
Email: christine.feinle@adelaide.edu.au

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ABSTRACT

Background: The interaction of nutrients with the small intestine modulates gastropyloroduodenal motility, stimulates the release of gut hormones, and suppresses appetite and energy intake.

Objective: To evaluate in healthy, lean males, which, if any, of these parameters are independent determinants of acute energy intake.

Design: Data from eight published studies, involving a total of 67 healthy, lean males, in which antropyloroduodenal pressures, gastrointestinal hormones and perceptions were measured during intraduodenal nutrient, or intravenous hormone, infusions, were pooled. In all studies energy intake at a buffet lunch was quantified immediately after the infusions. To select specific motor, hormone or perception variables for inclusion in a multi-variable mixed-effects model for determination of independent predictors of energy intake, all variables were assessed for collinearity and, using bivariate analyses adjusted for repeated measures, within-subject correlations between energy intake and these variables were determined.

Results: While correlations were found between energy intake with antropyloroduodenal pressures, plasma hormone concentrations and gastrointestinal perceptions, only the peak number of isolated pyloric pressure waves, peak plasma cholecystokinin and AUC of nausea were identified as independent predictors of energy intake (all $P<0.05$), so that increases of 1 pressure wave, 1 pmol/L and 1 mm.min, respectively, were associated with reductions in energy intake by ~36 kJ, ~88 kJ and 0.4 kJ, respectively.

Conclusions: We have identified specific changes in gastrointestinal motor and hormone function, i.e. stimulation of pyloric pressures and plasma CCK, and nausea, that are associated with the acute suppression of energy intake.

Key words: Gastrointestinal motility; glucagon-like peptide-1; peptide YY; intraduodenal nutrient infusion; appetite perceptions

INTRODUCTION

In Western countries, the prevalence of obesity has more than doubled over the past three decades (1). Hence, there is an urgent need for effective prevention and treatment strategies. Numerous dietary and pharmacological treatments for obesity have been developed, however, most have limited efficacy and, in the case of drugs, adverse effects occur frequently (2). The available therapies have largely ignored the pivotal role of the gastrointestinal (GI) tract in the regulation of appetite and energy intake in humans (3-9).

The modulation of energy intake by the GI tract is likely to involve both motor and hormonal mechanisms. While distension of both the proximal and distal stomach increases fullness (10, 11) and suppresses energy intake (4, 12), the antrum may play the dominant role (4, 10). The presence of nutrients in the small intestine slows gastric emptying potently, by decreasing antral motility and stimulating phasic and tonic pyloric pressure waves, and stimulates the release of GI hormones, including cholecystokinin (CCK), peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) (13, 14), which may mediate the concomitant inhibition of appetite and subsequent energy intake. For example, studies using the CCK-1 receptor antagonist, loxiglumide, have established that endogenous CCK inhibits energy intake (6, 15, 16). Exogenous PYY(3-36) and GLP-1 have also been reported to decrease energy intake in some (5, 17), but not all (18-20), studies. We recently reported an inverse relationship between the suppression of energy intake and stimulation of pyloric pressures in response to intravenous CCK-8 infusion in healthy males (7), providing evidence of a link between specific changes in GI motor function and the suppression of energy intake in humans. Since modulations in antropyloroduodenal motility underlie the slowing of gastric emptying (21), with pyloric pressures playing the dominant role (22), this finding provides a rationale to account for the relationship between the slowing of gastric emptying and the suppression of energy intake reported previously (23). Changes in motility and hormone secretion occur concurrently with changes in appetite, and it is,

accordingly, not surprising that there is little information as to which, if any, of these factors are independent determinants of energy intake. For example, while CCK does have a role, this may potentially be mediated indirectly by its effect on motility (7, 19).

During the last few years, we have performed a series of studies in our laboratory in healthy males, relating to GI motor and hormonal function and appetite and energy intake in response to small intestinal nutrient (8, 24-29) or intravenous hormone (7, 19, 30) administration, accumulating a substantial body of data. A focus of this work has been on pyloric motility, given that the pylorus is of pivotal importance to the regulation of gastric emptying (22), but has hitherto received inappropriately little attention. Individually, such studies are often limited by small sample sizes, so that it is only possible to perform simple correlation or regression analyses between energy intake and physiological parameters, uncontrolled for other concurrent physiological changes. Pooling data from these studies has enabled us to generate a uniquely large set of data to examine the simultaneous relationships amongst multiple parameters and, thus, to determine independent predictors of acute energy intake.

METHODS

Subjects

A total of 67 subjects, with a mean age of 26 ± 1 years and normal body weight for their height (BMI 23.3 ± 0.3 kg/m^2), participated in the studies that were included in this analysis (7, 8, 19, 24-30). Of the 67 subjects, 6 subjects participated in 2, 4 subjects in 3, and 2 subjects in 5 studies. Information relating to the subjects in each study is provided in **Table 1**. All subjects were unrestrained eaters, as determined by a score of <12 on the eating restraint component of the three-factor eating questionnaire (31) and were questioned prior to the study to exclude significant GI symptoms or disease, current use of medication known to affect GI function or appetite, cigarette smoking, or intake of >20 g alcohol/day. The Royal Adelaide Hospital Research Ethics Committee approved the study protocols, and the studies were initiated between May 2003 - July 2008. All subjects provided informed, written, consent prior to their inclusion.

Study design

Data from eight published studies (7, 8, 19, 24-30), representing all studies conducted in our laboratory using identical methodologies and techniques and evaluating the same outcome measures, were pooled for analysis. The data were then analyzed employing the same statistical tests that would be appropriate for a full meta-analysis, although it is inappropriate to refer to the current study as such, given that the included studies were not identified through a systematic review (32).

Study protocols

Each study evaluated the effects of either intraduodenal nutrient (8, 24-29) or intravenous hormone (7, 19, 30) infusions on antropyloroduodenal motility, GI hormone release, appetite and energy intake. Intraduodenal or intravenous infusions, rather than oral ingestion, were used

in these studies to bypass 'orosensory' and 'gastric' influences on gut function and appetite. Energy intake was assessed at the end of the duodenal nutrient infusion period, or during the final 30 min of the intravenous hormone infusion, using a cold buffet-style meal. The treatments and infusion periods in each of the studies varied, and the protocol details are provided in **Table 1**.

In all studies, subjects arrived in the laboratory after an over-night fast. A 16-channel catheter (Dentsleeve International Ltd, Ontario, Canada), for the assessment of pressures in the antropyloroduodenal region, was inserted through an anesthetized nostril into the stomach, and allowed to pass into the duodenum by peristalsis (22). Six side-holes (channels 1 - 6) were positioned in the antrum, a 4.5 cm sleeve sensor (channel 7), with two channels present on the back of the sleeve (channels 8 and 9), was positioned across the pylorus, and seven side-holes (channels 10 - 16) were positioned in the duodenum. Side-holes were spaced at 1.5 cm intervals. An additional channel, positioned 11.75 cm distal to the pylorus, was used for intraduodenal infusion of nutrients or saline control (8, 24-29). Both the most distal antral (channel 6, ~ - 40mV), and the most proximal duodenal (channel 10, ~ 0mV), channels were perfused with degassed 0.9% saline so that the position of the catheter could be monitored continuously through measurement of the transmucosal potential difference (22). For this, an intravenous cannula was placed subcutaneously in the left forearm and filled with sterile saline as a reference electrode (22). All other channels were perfused with degassed, distilled water at 0.15 ml/min. For intravenous infusions of saline, CCK-8 or GLP-1, an intravenous cannula was placed in the right arm (7, 19, 30). A second intravenous cannula was inserted into a left forearm vein for blood sampling, and blood samples were obtained at regular intervals during studies. Gastrointestinal perceptions were assessed at regular intervals using a validated visual analogue scale questionnaire (VAS) (33). At the end of each infusion, subjects were extubated and offered a cold buffet-style meal to consume freely for up to 30 min, until comfortably full.

The meal consisted of white and whole-meal breads, cold meats, cheese, lettuce, tomato, cucumber, mayonnaise, butter, apple, banana, yoghurt, chocolate custard, fruit salad, iced coffee, orange juice and water, and the quantities of food offered were in excess of what the subjects were expected to eat (19).

Data analysis

The parameters assessed in each study are detailed in **Table 2**.

Manometric pressures were digitized and recorded on a computer-based system, running commercially available software (HAD, A/Prof GS Hebbard, Royal Melbourne Hospital, Australia), and stored for subsequent analysis. APD pressures were analyzed for (i) number and amplitude of antral and duodenal pressure waves (PWs), and (ii) basal pyloric pressure and number and amplitude of isolated pyloric pressure waves (IPPWs), using previously described criteria (34, 35). Antral and duodenal PWs were expressed as total numbers and mean amplitudes (mmHg). IPPWs, defined as pyloric pressure waves that occur in the absence of pressure waves on adjacent antral and duodenal channels, were characterized by the peak number during the infusion, time to peak number (min), number of IPPWs pre-meal (i.e. immediately before the buffet meal), total number and area under the curve (AUC; calculated using the trapezoidal rule, as a measure over the entire infusion period) (min), and AUC of the amplitude of IPPWs (mmHg.min). Basal pyloric pressure, or tone, was expressed as peak pressure (mmHg), time to peak pressure (min) and AUC (mmHg.min).

For subsequent analysis of CCK, PYY and GLP-1, venous blood samples were collected in ice-chilled EDTA-treated tubes containing 400 kIU aprotinin (Trasylol; Bayer Australia Ltd, Pymble, Australia) per ml blood. Plasma was obtained by centrifugation of blood samples at 3200 rpm for 15 min at 4^{o}C, and plasma was frozen at -70^{o}C for subsequent analysis of CCK

(7, 8, 19, 25-29), GLP-1 (8, 19, 26, 28) and PYY (7, 24, 25, 27, 29, 30) by radioimmunoassays. Plasma CCK, PYY and GLP-1 concentrations were expressed as AUC (pmol/L.min) and plasma concentrations pre-meal (pmol/L) and, for plasma CCK concentrations, peak concentration (pmol/L) and time to peak concentrations (min) were calculated. The latter were not calculated for plasma PYY and GLP-1, as these did not generally reach a peak, but continued to rise, throughout the infusion periods.

Appetite perceptions were rated using a validated VAS questionnaire (33). Nausea and bloating were also assessed. Each VAS consisted of a 100 mm horizontal line, where 0 represented 'sensation not felt at all' and 100 represented 'sensation felt the greatest'. Subjects placed a vertical mark along the line to indicate the strength of the sensation felt at that particular time point. All data were expressed as AUC (mm.min). Energy intake (kJ) was quantified by weighing the buffet meal before and after consumption and using the software programme Foodworks 3.01 (Xyris Software, Highgate Hill, QLD, Australia) (19).

Statistical analysis

Data are reported as means (± SEM). To assess the strength of the bivariate relationships between each motility, hormone, and perception variable with energy intake, within-subject correlations adjusted for repeated measures were performed (36). The independent effects of each motility, hormone and perception variable on energy intake were assessed by entering the variables simultaneously into a multi-variable maximum likelihood linear mixed-effect model, adjusted for repeated visits per subject and the clustering of subjects within studies (37). This is equivalent to the 'one-step' analysis approach in a meta-analysis of individual participant data (38). All variables were included in the multi-variable model, except when collinearity (defined as $r > 0.7$) was present. In this case, of the related variables from within the same underlying motility, hormone or perception parameter, only one was selected for inclusion into

the model, to ensure the robustness of the regression estimates. This variable was selected based on consistency across studies and the strength of bivariate associations with energy intake. As not all parameters were measured in all studies (see **Table 1**), the multi-variable analysis was conducted as three separate models. Model 1 included parameters that were measured in all eight studies. Model 2 included all parameters in Model 1 plus PYY (6 studies), while Model 3 included all parameters from Model 1 plus GLP-1 (4 studies). To test for potential selection effects, Model 1 was re-run on the 4 studies used in Model 3, to ensure that a particular variable was not identified as independent depending on which studies were included in the Model and to ensure that no variables were under-represented. Analyses were conducted using SPSS 17 software (SPSS Inc, 2008, Chicago, USA). Significance was determined at $P < 0.05$.

RESULTS

Bivariate correlation analyses

Within-subject correlations between energy intake and each of the measured parameters are presented in **Table 3**.

Collinearity was present amongst a number of variables, thus, only one could be entered into the multi-variate model to guarantee robust estimation of the regression effects. Within the variables characterizing IPPWs, peak number, total number and AUC of the number were strongly associated with each other (all $r > 0.74$). Of these, peak number was selected for inclusion in the multi-variable model, as it exhibited the strongest correlation with energy intake. Of the CCK parameters, peak concentration was strongly correlated with both AUC and pre-meal concentrations (both $r > 0.85$), and thus peak concentration was selected, as, of those three variables, it best characterized the CCK response. For PYY and GLP-1, pre-meal levels were strongly associated with the corresponding AUCs (both $r > 0.84$), thus, AUCs were included in the multi-variable model, as they best characterized these hormone profiles. Of the appetite-related scores, hunger, desire-to-eat and prospective consumption were strongly correlated with each other (all $r > 0.82$), thus, prospective consumption was included in the model, as it showed the strongest correlation with energy intake. All other variables were entered automatically into the multi-variable model, due to the absence of any multi-collinearity.

Multi-variable mixed-effects models

In all three models, the peak number of IPPWs, peak plasma CCK concentration and AUC for nausea were consistently identified as independent predictors of energy intake (all $P < 0.05$, **Table 4**), so that an increase in each of these variables by 1 pressure wave, 1 pmol/L and 1

mm.min, while controlling for all other parameters, was associated with a reduction in energy intake by ~36 kJ, ~88 kJ and 0.4 kJ, respectively.

In addition, models 1 and 2 indicated that the number of IPPWs pre-meal was independently associated with energy intake ($P < 0.05$). However, in contrast to the peak number of IPPWs, an increase in the number of IPPWs pre-meal by 1 pressure wave was associated with an increase in energy intake by ~19 kJ.

Model 2 further identified the time to peak number of IPPWs and peak basal pyloric pressure, but not plasma PYY concentration, as significantly associated with energy intake (all $P < 0.05$, **Table 4**). An increase in the time to peak number of IPPWs by 1 min, while controlling for all other parameters, was associated with a reduction in energy intake by ~10 kJ. In contrast, an increase in peak basal pyloric pressure by 1 mmHg increased energy intake by ~68 kJ.

Finally, model 3 indicated that the plasma GLP-1 concentration was not an independent predictor of energy intake.

The robustness of the above results was confirmed by re-running Model 1 (complete set of variables) on the subset of 4 studies that were used in Model 3 (data not shown). Despite the reduction in the number of studies included in the model from 8 to 4, peak number of pyloric pressures, plasma CCK concentrations and nausea were identified as independent determinants of energy intake, confirming the above results.

DISCUSSION

Our study provides persuasive evidence of a direct relationship between energy intake with specific changes in gastric motility and gut hormones. When controlling for all other variables, the peak number of isolated pyloric pressure waves, peak plasma CCK concentrations and AUC of nausea were consistently, i.e. in all three statistical models, identified as independent predictors of acute energy intake in healthy males.

It has long been assumed that acute changes in GI function in response to nutrient ingestion, which serve to optimize digestion and absorption of nutrients, also play a key role in the regulation of energy intake. For example, in dogs electrical stimulation of the pylorus, which increases both tonic and phasic pyloric pressures, is associated with suppression of energy intake (39), in line with our recent finding of an inverse relationship between the stimulation of pyloric pressures and subsequent energy intake (7). While the latter did not establish a causal association, the outcome of the extensive statistical analyses performed in the current study strongly supports this concept. Thus, the magnitude of stimulation of IPPWs (specifically the peak number) independently determines the degree of suppression of acute energy intake. Since pyloric stimulation is a major determinant of the slowing of gastric emptying (22), it could be argued, that prolongation of gastric filling underlies the 'pyloric' effects. However, as in all our studies the stomach was empty, it is clear that pyloric pressures may have a suppressant effect on energy intake, even in the absence of gastric filling. The number of IPPWs pre-meal and peak basal pyloric pressure indicated effects on energy intake in a counter-intuitive positive direction, and the reason(s) underlying this are unclear. It can be difficult to predict the direction of individual effects when controlling for other, inter-related, parameters and, as these parameters were not identified consistently in all three models, this may well represent a statistical anomaly.

There has been, and continues to be, substantial interest in the role of gut peptides in the regulation of energy intake, with the view to develop novel anti-obesity strategies. CCK is probably the best-studied of all gut peptides (7, 12, 40). Together with these previous data, our findings that peak plasma CCK concentration is an independent determinant of acute energy intake underlines the importance of CCK in the regulation of acute energy intake. Given that it is well established that CCK, when given intravenously, has a marked stimulatory effect on pyloric pressures (7, 41), by acting on CCK-1 receptors located on the pylorus (42), it could be argued that the effect of CCK on energy intake depends on its action on the pylorus. However, our statistical approach, which, when assessing one parameter, controls for all other variables, shows clearly that the two stimuli act independently. That the pylorus plays an independent role is supported by a recent study in dogs, in which electrical pyloric stimulation, in the absence of CCK, was associated with suppression of food intake (39). This said, it is important to recognize that while our statistical analysis indicated that these factors acted independently of each other, the information from both signals (as well as others) is transmitted to the brain and integrated within the central nervous system to result in the overall outcome, ie the magnitude of energy intake suppression.

The peak stimulation of both IPPWs and plasma CCK occurred 15 - 30 min after commencement of the intraduodenal nutrient or intravenous hormone infusions (8, 19, 24-30), and these responses had diminished by the time energy intake was assessed, consistent with the concept that the information was encoded in the brain and translated into a suppression in energy intake even after a temporal delay, yet still inversely proportionate to the maximum pyloric and CCK stimulation that occurred 60 - 90 min earlier. This relationship clearly warrants further investigation in prospective studies, but the finding offers initial insights as to how information on the extent of peripheral nutrient or hormonal stimulation may be conveyed to, and then used by, the brain to determine subsequent energy intake.

The effects of PYY(3-36), the active metabolite of PYY, on energy intake have been the subject of much debate, with a number of studies reporting profound suppressant effects of ~30 % (5, 43), in lean and obese humans, while extensive studies in rodents found no such effects (18). A study by Degen and colleagues provided a conceivable explanation for this major discrepancy by demonstrating that the suppressant effect of PYY(3-36) on energy intake in humans is only apparent at pharmacological doses and coincides with the induction of nausea (44). Thus, our finding that PYY is not an independent predictor of energy intake is not surprising, particularly since the vast majority of individuals did not experience overt nausea or other adverse effects during any of the treatment conditions. The fact that CCK stimulates the release of PYY (30), an action mediated by CCK-1 receptors (45), may, at least in part, explain why PYY was not identified as an independent predictor of energy intake. Data relating to the role of GLP-1 in the regulation of energy intake are also inconsistent. While many studies have demonstrated that intravenous infusion of GLP-1 suppresses energy intake (17, 46, 47), other studies found no effect (19, 20, 48, 49). We did not identify plasma GLP-1 concentration as an independent predictor of energy intake. Other gut peptides, including ghrelin and pancreatic polypeptide, have also been reported to modify energy intake in humans (50, 51). We were unable to investigate the potential contribution of these peptides.

Our findings of correlations between appetite perceptions and subsequent energy intake confirm data from a previous study in young and older subjects (33) and are not surprising. In contrast, our analysis indicated that appetite perceptions are not determinants of energy intake. Perhaps this can be explained by our study design - intraduodenal infusion of nutrients or intravenous administration of gut peptides may not elicit the same feelings of fullness and satisfaction compared with oral meal ingestion, as both orosensory and gastric mechanisms are bypassed. Alternatively, it may suggest that the degree of hunger preceding a meal is not a good predictor

of the amount consumed at that meal. Interestingly, nausea was identified as an independent predictor of energy intake. We cannot entirely exclude the possibility that nausea occurred as a result of the direct intraduodenal nutrient, or intravenous hormone administrations utilized in all our studies, but it is important to emphasize that, on average, nausea scores did not increase by more than ~10 %. Hence, the statistical outcome of nausea as an independent predictor of energy intake is based on very modest changes, suggesting that energy intake may, at least in part, be regulated by subtle feelings of nausea, only perceived subconsciously by the subjects. That said, our analysis also indicated that the contribution of nausea to the suppression of energy intake is very small, particularly when compared with the effect of pyloric stimulation and CCK. More research is required to determine how nausea may be part of the spectrum of appetite perception.

Some limitations of the study need to be recognized. All studies were performed in healthy, lean males, accordingly, we cannot draw any firm conclusions with regards to outcomes in females, with increasing body weight or age. Only sub-sets of studies evaluated plasma PYY and GLP-1 concentrations, which may have influenced the statistical outcomes, however, the standard errors for these parameters remained within reasonable limits, indicating sufficient statistical power, and the main outcomes were confirmed when model 1 was repeated including only the four studies included in model 3. While the studies were performed over a number of years, the techniques, equipment and calibration methods used were identical and the within-subject reproducibility of our techniques is very good (52). Moreover, inter-individual variations in responses were taken into account by employing a multi-variable mixed effects model appropriate for this type of data analysis.

In conclusion, our findings provide strong evidence that pyloric pressures, plasma CCK and nausea are independent predictors of acute energy intake in healthy males. Evaluation of these

parameters as determinants of energy intake, and their potential as screening tools for the appetite-suppressant potency of novel, gut-focused therapeutic agents, in prospective studies would be of interest. Strategies modulating these GI functions to regulate energy intake have the potential to lead to novel approaches to the prevention and management of obesity.

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None of the authors have any personal or financial conflict of interest to declare.

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520 **Table 1:** Subject and protocol details for each study included in the data analyses.

Study	Publication (reference)	Subject criteria			Protocol
		n	Age (yr)	BMI (kg/m^2)	
1	**Feltrin (2004)** (8) **Feltrin (2006)** (24)	8 7*	24 ± 4	22.0 ± 1.6	Intraduodenal saline (control); dodecanoic acid (C12) or decanoic acid **(**C10) at 0.375 kcal/min for 90 min. Buffet meal at 90 min.
2	**Little (2005)** (28)	13	23 ± 2	23.6 ± 0.5	Intraduodenal saline; C12 at 0.1, 0.2 or 0.4 kcal/min for 90 min. Buffet meal at 90 min.
3	**Brennan (2005)** (19) **Brennan (2007)** (30)	9	22 ± 1	23.0 ± 0.5	Intravenous saline; CCK-8 (1.8 pmol/kg/min), GLP-1 (0.9 pmol/kg/min) or CCK-8+GLP-1 for 150 min. Buffet meal at 120 min.
4	**Pilichiewicz (2007)** (26)	10	32 ± 4	25.1 ± 0.4	Intraduodenal saline; 25% glucose at 1, 2 or 4 kcal/min for 120 min. Buffet meal at 120 min.
5	**Pilichiewicz (2007)** (27)	16	31 ± 3	23.8 ± 0.5	Intraduodenal saline; lipid at 0.25, 1.5 or 4 kcal/min for 50 min. Buffet meal at 50 min.
6	**Brennan (2008)** (7)	10	26 ± 2	23.0 ± 0.5	Intravenous saline; CCK-8 at 0.3, 0.6 or 1.8 pmol/kg/min for 120 min. Buffet meal at 90 min.

7	**Feltrin (2008)** (25)	13	26 ± 2	22.9 ± 0.6	Intraduodenal saline; C12 or oleic acid (C18:1) at 0.4 kcal/min for 60 min. Buffet meal at 60 min.
8	**Seimon (2009)** (29)	10	25 ± 3	22.8 ± 0.4	Intraduodenal saline; fat emulsions with droplet sizes of 0.26 μm, 30 μm or 170 μm at 2.8 kcal/min for 120 min. Buffet meal at 120 min.

Part of the hormone data in studies 1 and 3 were analyzed and published separately, resulting in 2 publications for these studies.

* In Study 1, sufficient plasma for the additional hormone analyses was available from only 7 of the 8 subjects.

523 **Table 2**: Parameters measured in each study

Study	Publication (reference)	Antral pressure waves	Isolated pyloric pressure waves	Basal pyloric pressure	Duodenal pressure waves	Plasma CCK	Plasma PYY	Plasma GLP-1	Hunger	Desire -to-eat	Prospective consumption	Fullness	Nausea	Bloating
1	**Feltrin (2004)** (8)	X	X	X	X	X		X	X	X	X	X	X	X
	Feltrin (2006) (24)						X							
2	**Little (2005)** (28)	X	X	X	X	X		X	X	X	X	X	X	X
3	**Brennan (2005)** (19)	X	X	X	X	X		X	X	X	X	X	X	X
	Brennan (2007) (30)						X							
4	**Pilichiewicz (2007)** (26)	X	X	X	X	X		X	X	X	X	X	X	X
5	**Pilichiewicz (2007)** (27)	X	X	X	X	X	X		X	X	X	X	X	X
6	**Brennan (2008)** (7)	X	X	X	X	X	X		X	X	X	X	X	X
7	**Feltrin (2008)** (25)	X	X	X	X	X	X		X	X	X	X	X	X
8	**Seimon (2009)** (29)	X	X	X	X	X	X		X	X	X	X	X	X

524 CCK, cholecystokinin, PYY, peptide YY, GLP-1, glucagon-like peptide-1, X, parameter measured.

525 **Table 3:** Within-subject correlations between energy intake and gastrointestinal motor,
526 hormone and perception variables

Parameter	n*	mean	SD	r	P
Antral pressure waves					
Number	88	44.2	79.7	0.12	0.068
Amplitude (mmHg)	88	31.0	26.8	0.23	<0.001
Isolated pyloric pressure waves					
Number pre-meal (/15 min)	84	7.5	10.3	-0.06	0.366
Peak number (/15 min)	84	20.7	12.9	-0.30	<0.001
Time to peak number (min)	84	25.8	20.0	-0.10	0.166
Total number	88	43.6	60.6	-0.12	0.052
AUC of number (min)	84	793	833	-0.25	<0.001
AUC of amplitude (mmHg.min)	84	2296	1622	-0.16	0.015
Basal pyloric pressures					
Peak pressures (mmHg)	85	4.5	5.2	-0.09	0.197
Time to peak pressures (min)	85	30.2	24.7	0.20	0.005
AUC (mmHg.min)	86	86	236	-0.23	<0.001
Duodenal pressure waves					
Number	88	450	396	0.29	<0.001
Amplitude (mmHg)	88	27.0	7.9	0.14	0.029
Plasma CCK					
Pre-meal (pmol/L)	82	6.9	6.5	-0.42	<0.001
Peak concentration (pmol/L)	82	8.5	8.1	-0.33	<0.001
Time to peak (min)	82	29.8	28.3	0.02	0.784
AUC (pmol/L.min)	76	647	8000	-0.38	<0.001

Plasma PYY					
Pre-meal (pmol/L)	59	114.7	136.4	-0.23	0.005
AUC (pmol/L.min)	59	6933	10458	-0.22	0.006
Plasma GLP-1					
Pre-meal (pmol/L)	38	21.3	17.3	-0.24	0.012
AUC (pmol/L.min)	38	1936	1424	-0.20	0.041
Gastrointestinal perceptions					
AUC Hunger (mm.min)	84	-853	1838	0.21	0.001
AUC Desire-to-eat (mm.min)	84	1044	1910	0.24	<0.001
AUC Prospective consumption (mm.min)	84	1065	1777	0.31	<0.001
AUC Fullness (mm.min)	84	1745	1838	-0.12	0.080
AUC Nausea (mm.min)	84	297	968	-0.38	<0.001
AUC Bloating (mm.min)	84	838	1447	-0.28	<0.001

527 AUC, area under the curve; SD, standard deviation; r, correlation coefficient; P, significance
528 level.
529 * Variations in the number of subjects (n) for the various parameters are due to missing data.

530 **Table 4:** Results of mixed-effects multivariable models for determination of independent predictors of energy intake
531

	Model 1			Model 2			Model 3		
Parameter	**Estimate**	**SE**	**P**	**Estimate**	**SE**	**P**	**Estimate**	**SE**	**P**
Antral pressure waves									
Number, total	-2.40	1.4	0.092	-2.07	1.6	0.195	3.10	4.0	0.442
Amplitude, mean (mmHg)	3.75	4.0	0.354	6.10	5.5	0.268	1.19	6.2	0.850
Isolated pyloric pressure waves									
Number pre-meal	20.09	8.5	**0.019**	17.70	8.4	**0.037**	19.80	21.7	0.367
Peak number	-36.85	7.4	**<0.001**	-39.36	8.5	**<0.001**	-32.32	13.9	**0.025**
Time to peak number (min)	-3.29	3.4	0.341	-10.30	3.9	**0.009**	-4.27	5.4	0.436
AUC amplitude (mmHg.min)	-0.03	0.1	0.619	-0.08	0.1	0.262	0.04	0.1	0.669
Basal pyloric pressures									
Peak pressure (mmHg)	27.47	21.6	0.206	67.91	22.4	**0.003**	31.63	32.5	0.334
Time to peak pressure (min)	-2.74	3.3	0.403	-2.00	3.7	0.588	-2.55	6.2	0.682
AUC (mmHg.min)	0.11	0.4	0.799	-0.41	0.5	0.395	0.19	0.6	0.763
Duodenal pressure waves									
Number, total	0.40	0.3	0.146	0.12	0.4	0.785	0.28	0.4	0.506
Amplitude, mean (mmHg)	-5.17	8.5	0.555	-19.52	10.3	0.062	16.62	14.9	0.269

Plasma CCK									
Peak concentration (pmol/L)	-70.39	16	**<0.001**	-78.09	15.6	**<0.001**	-115.5	31.3	**0.001**
Time to peak conc (min)	1.55	2.6	0.551	1.37	3.1	0.657	1.73	3.9	0.656
Plasma PYY									
AUC (pmol/L.min)	-	-	-	0.01	0.0	0.317	-	-	-
Plasma GLP-1									
AUC (pmol/L.min)	-	-	-	-	-	-	0.03	0.1	0.744
Gastrointestinal perceptions									
AUC Prospective consumption (mm.min)	0.04	0.1	0.548	0.06	0.1	0.480	-0.01	0.1	0.918
AUC Fullness (mm.min)	0.04	0.1	0.516	0.12	0.1	0.151	-0.01	0.1	0.931
AUC Nausea (mm.min)	-0.38	0.1	**<0.001**	-0.39	0.1	**<0.001**	-0.45	0.1	**<0.001**
AUC Bloating (mm.min)	-0.05	0.1	0.388	-0.05	0.1	0.472	-0.13	0.1	0.179

532 AUC, area under the curve; SE, standard error of the mean; P, significance level

533