doi: 10.1111/j.1467-789X.2009.00707.x

ILSI Supplement

Gastrointestinal targets of appetite regulation in humans

N. Delzenne[1], J. Blundell[2], F. Brouns[3], K. Cunningham[4], K. De Graaf[5], A. Erkner[6], A. Lluch[7], M. Mars[8], H. P. F. Peters[9] and M. Westerterp-Plantenga[3]

[1]Louvain Drug Research Institute, Unit PMNT 7369, Université Catholique de Louvain, Brussels, Belgium; [2]Institute of Psychological Sciences, University of Leeds, Leeds, UK; [3]Department of Human Biology, School of Nutrition & Toxicology Research and Metabolism (NUTRIM), Maastricht University, Maastricht, The Netherlands; [4]The Coca-Cola Company, Europe Union Group, London, UK; [5]Division of Human Nutrition, Wageningen University, Wageningen, The Netherlands; [6]Nestlé Research Center, Lausanne, Switzerland; [7]Danone Research, Palaiseau, France; [8]Division of Human Nutrition, Wageningen University, Wageningen, The Netherlands & the Top Institute of Food and Nutrition, The Netherlands; [9]Unilever R & D Vlaardingen, Vlaardingen, The Netherlands

Received 14 September 2009; revised 18 November 2009; accepted 19 November 2009

Address for correspondence: ILSI Europe a.i.s.b.l., Avenue E. Mounier 83, Box 6, B – 1200 Brussels, Belgium. E-mail: publications@ilsieurope.be

Summary

The aim of this paper is to describe and discuss relevant aspects of the assessment of physiological functions – and related biomarkers – implicated in the regulation of appetite in humans. A short introduction provides the background and the present state of biomarker research as related to satiety and appetite. The main focus of the paper is on the gastrointestinal tract and its functions and biomarkers related to appetite for which sufficient data are available in human studies. The first section describes how gastric emptying, stomach distension and gut motility influence appetite; the second part describes how selected gastrointestinal peptides are involved in the control of satiety and appetite (ghrelin, cholecystokinin, glucagon-like peptide, peptide tyrosin-tyrosin) and can be used as potential biomarkers. For both sections, methodological aspects (adequacy, accuracy and limitation of the methods) are described. The last section focuses on new developments in techniques and methods for the assessment of physiological targets involved in appetite regulation (including brain imaging, interesting new experimental approaches, targets and markers). The conclusion estimates the relevance of selected biomarkers as representative markers of appetite regulation, in view of the current state of the art.

Keywords: Appetite, biomarker, gastric distension, gastrointestinal peptide.

obesity reviews (2010) **11**, 234–250

Introduction

The regulation of appetite is a complex process, involving the interaction of food with physiological targets in the short term, but also an adaptive process responding to and modulated by energy input and energy expenditure in the long term. Taken together, satiety is generated by sensory, post-ingestive and post-absorptive mechanisms that may be targets for sustaining or enhancing satiety and/or reducing energy intake. The aim of this paper is to identify the physiological processes that may be targeted to modulate appetite. The objective of this paper is to assess the relevance of the influence of ingredients, nutrients, specific foods and beverages on appetite in human studies.

The gastrointestinal tract is the site of origin for a wide range of signals that control hunger, food intake and satiety (1–3). For instance, the control of the gastrointestinal 'kinetics' function, upon eating, may play a key role in the context of regulation of satiation, satiety and feeding state. The functions of gastric distension and emptying will be the focus of the first part of this paper. The effect of the intestinal function (e.g. transit, motility) will also be discussed

Table 1 Overview of site of production, target function and target organs of the gastrointestinal peptides

Peptide	Site of production	Target functions	Target organs
Gastrointestinal peptides			
Ghrelin	Gastric cells	• Direct appetite stimulating effect. • Might increase hedonic and reward value of food.	CNS
CCK	Duodenum and proximal jejunum	• Stimulates gallbladder contraction. • Stimulates release of digestive enzymes. • Inhibits gastric emptying.	Gallbladder Exocrine pancreas Stomach
GLP-1	Ileum and colon	• Stimulates glucose dependent insulin secretion and inhibits glucagon release. • Inhibits gastric emptying. • Direct appetite suppressing effect on brain.	Endocrine pancreas Stomach CNS, e.g. hypothalamus
PYY	Ileum and colon	• Inhibition gastric emptying. • Inhibits bile acid secretion. • Inhibits secretion of digestive enzymes. • Direct appetite suppressing effect on brain.	Stomach Gallbladder Exocrine pancreas CNS

CCK, cholecystokinin; CNS, central nervous system; GLP, glucagon-like peptide; PYY, peptide tyrosin-tyrosin.

but less detailed, as the relationship with appetite is less clear.

On the other hand, the discovery of mediators produced outside the central nervous system (CNS) that communicate, directly or indirectly, with specific areas of the brain, allow a better understanding of the molecular control of hunger and satiety (1,4–6). The list of peptides that seem relevant in the control of appetite is presented in Table 1.

Certain peptides, which are relevant to appetite control, have been selected for inclusion in this paper as potential biomarkers, to be measured when assessing the influence of food/nutrients/ingredients on appetite. An important factor that determines the feasibility of a biomarker (as proposed in the Functional Food Science Project led by International Life Sciences Institute [ILSI] in 1999) (7) – is that it should be measurable in accessible or obtainable material using methodology that must be both ethical as well as minimally invasive. Therefore, the markers described in the second part of this paper are mainly gastrointestinal hormones that can be measured in plasma or serum in a short-term evaluation of appetite, namely cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), ghrelin and peptide tyrosin-tyrosin (PYY). Using some examples of human studies the relevance and efficacy of these peptides in relation to the modulation of appetite by specific nutrients or ingredients are explored.

Selection of biological targets and biomarkers

Emphasis on gastric distension and emptying

Potential role of gastric distension and emptying as physiological functions associated with appetite regulation

The gastrointestinal (GI) tract is host to a wide range of signals that ultimately act to influence and contribute towards the regulation of appetite and food intake (8). The response of the GI tract to the entry of nutrients is traditionally divided into cephalic, gastric and intestinal phases. The cephalic phase (primarily pre- and peri-ingestive influences of visual, and oro-nasal sensory stimulation) will not be addressed here.

The gastric phase involves a highly complex, multicompartmental process. The proximal part of the stomach (fundus) accommodates food by reduction of its tone (the process of gastric accommodation), followed by an increase in proximal and distal (antrum) stomach volume. The gastric motor function adapts to this fed state, in which it governs the gastric emptying behaviour, and mixing and digestion are initiated. In this state, the fasted motility pattern of Migrating Motor Complexes I, II and III turns into a fed pattern of phase II-like contractions, nutrient emptying starts with the liquid phase ingestion, while solids are initially retained in the fundus. After a lag phase, solids empty selectively from the stomach, propelled towards the pylorus when the particle size is sufficiently reduced (to about 1–2 mm) (9). Each of these gastric steps could potentially influence appetite and food intake. Indeed, a relationship between gastric parameters (e.g. gastric distension, emptying and accommodation) and appetite-related measures (e.g. reported sensations of fullness, hunger, etc.) has been shown many times (10–16).

Other possible gastric targets are gastric motility, pH and pyloric function (17,18), but these have rarely been studied in relation to appetite. For instance, the role of gastric pH and acid secretion on appetite is unknown, although this will affect the physical nature of certain food components (e.g. gelling or 'curd' formation of specific proteins, solubility of minerals) and it can be speculated that a different pH would affect the initial stages of

digestion via its effect on enzyme activity, and on the physical properties (3).

There are only sufficient human data available for gastric emptying and gastric distension to explore their potential role as key physiological functions associated with appetite regulation. The most potent gastric signals are probably those reflecting distension. Early work from Geliebter *et al.* (13) indicated that meal intake was lower when a gastric balloon was inflated to a volume of 400 mL or more, and hunger ratings also decreased accordingly. This was confirmed in several balloon studies (19–21).

Other types of research also show the role of gastric distension in relation to appetite. Benini *et al.* (22) and Jones *et al.* (15) both show with ultrasound that the intragastric volume is related to fullness, but not to hunger. Goetze *et al.* (23) have used magnetic resonance imaging (MRI) and have shown that perceptions of satiety and fullness were linearly associated with postprandial gastric volumes of meals (independent of macronutrient composition). Cecil *et al.* (24) have shown that intragastric infusion of tomato soup suppressed subjectively rated appetite, whereas intraduodenal infusions of soup did not. Gastric content measures could explain about 50–60% of the variance in the fullness ratings during intragastric soup delivery. Rolls and Roe (25) have demonstrated that increasing volume, but not the energy content, of gastrically infused food reduced hunger and food intake. However, this study measured the immediate impact of this meal and gave no information on subsequent effects. However, by using a meal both the gastric processes and duodenal feedback may play a role and the contribution of gastric distension could not be discriminated from gastric emptying and the intestinal processes.

In subsequent research it is becoming clear that only during, but not after distension, hunger scores are reduced (26) and that distension in the (less compliant) antral area is probably more important than in the fundal region (14,27–30). For instance, Marciani *et al.* (28) show, by using agar beads differing in strength, that fullness increases with increasing gel strength of the beads and that the antral grinding forces are important determinants in this.

In addition to distension, gastric emptying might be an important determinant of ingestion behaviour. Indeed, gastric emptying correlates to ingestion behaviour in many studies (e.g. (28,31–37). However, some studies do not find such a relationship (38,39), either because gastric distension is more important than gastric emptying (14,15,40), the intestinal effects are more important (39), or because not the meal itself but one of its meal components is a more important determinant of gastric emptying or satiety (41,42) (see also below).

The magnitude and timing of each gastric step, and its effect on satiety and food intake, are dependent on meal composition parameters such as volume, consistency, energy value and macronutrient (e.g. fat) content (25,32,43–48). Furthermore, a meal might consist of many components that behave and might be handled differently in the stomach, and consequently leave the stomach at different rates (selective emptying) (49). This can have an impact on postprandial plasma glucose and insulin profiles (49). For example, in the intragastric environment separation of fat content can occur with different meals or emulsions, whereby fat and water undergo phase separation into distinct upper (fat) and lower (water) layers (50). This separated meal leads to less reported satiety than an emulsified meal that did not separate into two distinct phases (43). Another example of the importance of gastric distribution for appetite is described by Jones *et al.* (42). They have found that the gastric emptying of the aqueous phase of a soup is inversely correlated to fullness, while the oil component is related to hunger. In any case, it seems best not to assume that meals will be emptied as a homogenous mass. Thus, relationships with behavioural parameters may be improved when the selective emptying of aqueous and non-aqueous phases or different nutrients is considered and measured, and not just the total meal.

Some authors suggest that the antral distension is more important than the overall rate of gastric emptying (14,51), but this remains to be confirmed and is dependent on the meal composition. It is likely that both are important. Gastric emptying affects not only the magnitude and duration of gastric distension, but also the rate of nutrient delivery to the small intestine and how that interacts with the stomach. Increasing the volume of a meal can increase gastric emptying but the main influence on gastric emptying is the feedback received from the intestine in response to the presence of nutrients in the intestinal lumen and the extent that they are able to travel down the lumen stimulating receptors as they pass down the intestinal lumen. Habitual diet can have an effect on the responsiveness of the intestine and hence the extent at which gastric emptying is inhibited (52,53).

In conclusion, there appears to be a direct, inverse, and causal relation between gastric distension and appetite (mainly satiation). Also numerous studies have shown a relationship between gastric emptying and appetite (mainly satiety), although here the relationship is somewhat weaker and not necessarily causal and direct. Nevertheless, measures of gastric distension and gastric emptying may serve as sensible functions that are related to feelings of satiation (fullness) and satiety, respectively.

While the effect of gastric emptying and distension on appetite is rather clear, the relationship between the intestinal function and appetite is less clear.

As discussed before, there is a feedback signal from the small intestine to the stomach and as such gastric emptying over time cannot be judged as solely a gastric phenomenon

but more as an intestine mediated response. Exposing the small intestine to nutrients leads to release of gut peptides and neurotransmitters that induce a reduction in hunger levels and food intake (54). During the process of meal ingestion and subsequent gastric handling, some nutrients will already begin to enter the small intestine, and gastric and intestinal satiety signals interact in order to limit meal size, to increase satiation and to increase satiety between meals (3).

Possible intestinal functions associated with appetite regulation are gallbladder emptying and motility (45), intestinal motility and transport (17,18,34,55), intestinal absorption, colonic motility and transport (56,57), colonic distension (58,59) and fermentation (e.g. related to the production of short chain fatty acids or satiety hormones (57,60–62)), but data on their relationship with appetite are scarce. Upon entry of nutrients into the small intestine, motility changes from the propagative contractions of peristalsis of the fasted state to the non-propagative pattern that slows intestinal transit in the fed state (63) and vice versa when fed changes to fasted state. Sepple and Read (34) have found that the postprandial onset of a small intestinal fasting motility pattern (phase-III-like activity) always occurs when the stomach empties more than 80% of its contents and after hunger has increased. They interpret those findings by the return of hunger as being directly related to a decline in the exposure of the upper small intestine to nutrient stimuli. Manometry has been used to examine various motility parameters that may be associated with ingestion behaviour (17,18,55), but the relationships between the various aspects of gastrointestinal motility and appetite appeared variable and unclear. There is a need for more extensive study of the motor function governing gastric emptying, mixing and digestion in relation to ingestion behaviour. As manometry is invasive, alternatives like MRI should be used.

Methodological aspects of the measurement of gastric distension and gastric emptying

Gastric distension and emptying should be measured by methods that are feasible, valid, reproducible, sensitive and specific. A large number of methods have become available to measure these parameters and numerous reviews have discussed them (63–65). The choice of methods should be directed by the study population (type of subjects, but also number of subjects), the type of meal (liquid or solid), the meal constituents and how they are anticipated to act in the stomach (e.g. will they separate?) and the availability and feasibility of the methodology.

Some methods are more specifically used for either gastric emptying, or gastric distension. The techniques currently most widely applied for gastric emptying are ultrasonography, the paracetamol absorption test, stable isotope breath test and MRI, while gastric distension is most often measured by ultrasonography and MRI. Scintigraphy is regarded as the 'gold standard', but requires radionucleides and a gamma camera, and is presently only used in some specific research centres. Another method, only applied for liquid emptying, is gastric aspiration. This sampling technique allows measurements of the volume and composition in real life situations (e.g. during exercise), but is rather invasive (some subjects report some discomfort) and requires volumes larger than 150 ml for accuracy. Other techniques are available, e.g. tomography methods like SPECT, PET or CAT (single photon emission computed, positron emission or computerized [axial] tomography), but they require very expensive equipment and a large ionising radiation dose.

Ultrasound is a relatively cheap, non-invasive and safe technique, and 3D methods can be used to estimate gastric volume (66). Several studies showed a good correlation with scintigraphy; this method is reliable and valid (67). However, the ultrasound signal is disrupted by air/liquid interfaces and imaging of complete gastric contents is not possible. The method also requires a skilled technician and is time-consuming (65,68).

The paracetamol absorption test is a well-tolerated and relatively easy test. Like the other tracer methods it is an indirect method, which requires repeated blood sampling, and assumes the rate of gastric emptying to be the rate-limiting step and not the absorption and subsequent metabolism. Several investigators found a significant correlation between paracetamol absorption and scintigraphy parameters (for a review see (69), recent papers (70–72), and a reasonable reproducibility (69), although others found moderate or no correlation (69)). The paracetamol method may have a number of limitations, however, that may lead to outcomes that are less reliable: (i) it is focused on liquids only and will mostly measure water-soluble compounds like carbohydrates and proteins, but will underestimate lipid emptying (although a recent paper suggests semi-solid application (71)); (ii) the metabolism and elimination of paracetamol might show large individual differences (73) and (iii) although it is an indirect method, the paracetamol itself (1–2 g is recommended) may effect physiology.

The ^{13}C breath test is a very easy test, requiring only repeated breath sampling. The disadvantage, though, is the rather high costs of the measurement and isotopes. Excellent correlations between gastric emptying parameters in breath test and scintigraphy/sampling technique have been obtained (e.g. (63,74–77)) and the test was found to be reproducible (78,79), although others found variable results (e.g. (70,78,80)), probably because of individual differences in substrate metabolism (which appear the rate-limiting step rather than gastric emptying). Current discussions are around the proper method for analysis (65,74,81). But again, when only changes after different nutrients are important within subjects, this technique can

be applied for measuring gastric emptying of liquids (using ^{13}C-acetate or ^{13}C-octanoate depending on the nutrient of interest) and solids (using ^{13}C-octanoate). The ^{13}C-acetate is more water-soluble and will mostly represent the water-soluble compounds of the meal, while ^{13}C-octanoate is less water-soluble and will mostly represent the fat-soluble compounds of the meal.

Magnetic Resonance Imaging is a safe, non-invasive, but very costly technique, which has been validated against scintigraphy and can measure liquid and solid meals (82,83). The technique used to have some disadvantages such as motion artefacts and less contrast of the stomach for proper visualization of the gastric lining, but fast MRI techniques (82,84) and contrast agents have now overcome these problems (85). Further studies have successfully evaluated the additional use of MRI for gastric accommodation (regional) motility, and intragastric (fat) distribution (84,86,87). The latter is especially important when the meal inside the stomach does not behave like one homogeneous mixture of components. However, MRI is mostly performed in the supine position, which can be a disadvantage when gravity (e.g. for fats) plays a role in (liquid) emptying and satiety (33). However, assessment of the subject in the left lateral decubitus position might prevent this (84).

In conclusion, gastric emptying and distension changes within subjects can be determined using a number of different techniques, each having its specific advantages and disadvantages. The selection of method should be very carefully considered for the particular test materials and subjects (as outlined above), and potential shortcomings of a given method understood. A comparison of results from one to another technique appears problematic. This is partly inherent to differences in the techniques, but also to the large inter- and intra-individual differences in gastric emptying.

Gastrointestinal peptides as biomarkers

Various properties of food stimulate entero-endocrine cells present at different levels of the gastrointestinal tract. These cells secrete several peptides, mentioned below, that diffuse across the subepithelial lamina propria to activate vagal-, enteric- and spinal afferent nerves. Some peptides have direct access to the arcuate nucleus of the hypothalamus and to the area postrema, brain regions involved in the regulation of food intake. These peptides may also work outside the CNS to influence the activity of neurons such as the vagal nerve, which projects to the nucleus of the solitary tract in the brain stem (88). Primarily, nutrients stimulate the release of these peptides, but also intestinal satiation and entero-endocrine cells activation can occur without nutrient uptake or intracellular metabolism, by mechanisms resembling oral taste sensation (for reviews (2,6,89)). We will describe sequentially the role of ghrelin, secreted mostly by the stomach, in the modulation of food intake; thereafter, we will focus on the first peptide – CCK- shown to be involved in the 'upper' intestinal satiation and we will give a brief overview of the other anorexigenic peptides secreted throughout the intestine, including GLP-1 and PYY (Table 2).

Focus on ghrelin

Ghrelin and appetite regulation. Ghrelin is a peptide released primarily by the stomach, but also from the duode num, ileum, caecum and colon (90,91). Identified first as an endogenous ligand for growth hormone secretagogue receptor (92), ghrelin is a 28-amino acid peptide which has two major molecular forms: acylated ghrelin (n-octanoic acid on serine 3) and non-acylated ghrelin. The acylated conformation of the peptide has been previously described

Table 2 Overview of relevance of the markers/target for appetite regulation

Marker	Hypothetic mechanism	Positive points	Negative points
Gastrointestinal peptides			
Ghrelin	• Direct effect on CNS. • (Anticipated) meal initiation. • Increased palatability of the food.	• Appetite stimulating. • Causal relation with appetite. • Correlation of serum levels with reported appetite.	• Anticipated learned effects. • Active vs. non-active form.
CCK	• Stomach distension.	• Causal relation with appetite.	• Difficult to measure.
GLP-1	• Glucose homeostasis. • Ileal break mechanism. • Direct effect on CNS.	• Causal relation with appetite. • Correlation of levels with reported appetite.	• Biphasic response.
PYY	• Stomach distention. • Direct effect on CNS.	• Causal relation with appetite.	• Only high exogenous dosages effect on food intake. • Limited studies on effects of foods on PYY concentrations.

CCK, cholecystokinin; CNS, central nervous system; GLP, glucagon-like peptide; PYY, peptide tyrosin-tyrosin.

Table 3 Selection of arguments supporting a role for ghrelin as a key factor in preprandial hunger

Action of ghrelin
1. Produced by organs recently exposed to food (stomach and duodenum).
2. Triggers eating when administered at times of minimal spontaneous food intake.
3. Extremely rapid and short-lived orexigenic actions, as required for a signal influencing individual meal-related behaviour.
4. Modulates meal patterns: decreases the latency to feed, increases meal number without affecting meal size.
5. Primarily increases motivation to seek out food and initiate feeding.
6. Contributes to an interoceptive hunger cue.
7. Stimulates gastrointestinal motility, gastric acid secretion and pancreatic exocrine secretion, all of which increase in anticipation of meals, preparing the gastrointestinal tract for effective transport and processing of food.
8. Stimulates hydrolysis of nutrients.
9. Targets in the brain are hypothalamic neurons that co-secrete the well-known orexigens, neuropeptide Y and agouti-related protein, both implicated in the central control of meal initiation as well as in anticipation of regularly scheduled meals.

as essential for its orexigenic action (92). While it has been demonstrated that the non-acylated ghrelin peptide acts as anorexigenic peptide (93).

Originally thought of as gastric hormone acting directly on the hypothalamus, ghrelin can stimulate appetite via the vagus nerve (90). However, ghrelin knockout mice exhibit normal body weight and food intake, raising questions as to the importance of ghrelin as an orexigenic factor and the potential value of ghrelin antagonists as anti-obesity agents (94). The postprandial decrease in ghrelin levels is less important in obese compared with lean individuals (95–96). Interestingly, the drop in ghrelin level after a standardized meal is normalized in obese individuals who lost weight upon dieting (97,98).

Multiple studies have shown that intravenous infusion of ghrelin in humans increases food intake (99,100), thus supporting its relevance in food intake behaviour. Moreover, similar effects have been found by subcutaneous infusion of growth hormone-releasing peptide-2, an agonist of ghrelin (101). In addition, several studies reported an increase in the palatability of the food after receiving ghrelin infusion (98,99). Recently, it has been shown that administering ghrelin to subjects increases the neural response to food pictures, especially in areas that are involved in the reward and hedonic values of visual cues (102).

Ghrelin concentrations appear to be positively associated with appetite scores and inversely associated with intermeal interval (97,103,104). Such an association suggests that the suppression of ghrelin concentrations may postpone initiation of the next meal. However, there is also strong evidence that ghrelin levels rise in anticipation of a meal, and is conditioned by the habitual meal pattern. Cross-sectionally meal patterns are closely related to ghrelin patterns (105). On the contrary, Callahan found that within individuals time-blinded spontaneous meal requests were proportional to caloric content of the previous meal and not to the ghrelin response (106). When the caloric content of meals is varied but the volume, macronutrient distribution and all other features are kept constant, the depth and duration of prandial ghrelin suppression are dose-dependently related to the number of ingested calories (106). In other words, large meals suppress both ghrelin and hunger more thoroughly than do small meals. Furthermore, the magnitude of the subsequent preprandial recovery of ghrelin levels has been reported to correlate with the number of calories consumed in the following meal (95,106). Together, these observations present a compelling picture of ghrelin as a meal initiator (see Table 3 for illustration). The data, however, are circumstantial, and definitive loss-of-function experiments with ghrelin-blocking agents or genetic ablations are required to prove or disprove this hypothesis.

Modulation of ghrelin by food/nutrients. Circulating ghrelin levels are high during fasting, rapidly fall after a meal (103,107) and are thought to be regulated by both calorie intake and circulating nutritional signals. Surprisingly, prandial ghrelin suppression does not require luminal nutrient exposure in the stomach or duodenum, the principal sites of ghrelin production (95). Instead, signals mediating this response originate further downstream in the intestine and from post-absorptive events. Contributors include changes in plasma insulin, intestinal osmolarity and enteric neural signalling, whereas gastric distension, the vagus nerve and GLP-1 are not required (95).

Ghrelin responses are dependent on energy dose and on the type and composition of the macronutrients (108). The mechanisms by which nutrients suppress ghrelin levels are beginning to be elucidated. It seems that ingested lipids suppress ghrelin levels less effectively than carbohydrates or proteins (at equal energy loads) (109). The relatively weak suppression of this orexigenic hormone by enteral lipids (110) could represent one of many mechanisms promoting high-fat (HF) diet-induced weight gain.

Although the kinetics of the ghrelin response to ingested proteins differs from that following carbohydrate consumption, the overall magnitude of suppression rendered

by isocaloric intake of these two macronutrient types is relatively similar. Hence, ghrelin levels were found to be equivalent among people in energy balance consuming isocaloric high- vs. normal-protein diets, in which the carbohydrate content was varied and fat content held constant (111).

Recent results demonstrate that following a high carbohydrate breakfast given to healthy young women, plasma acylated ghrelin decreases to reach the lowest value at 30 min, whereas the drop of total ghrelin is maximal after 90 min (112). This suggests that the kinetics of release of ghrelin depends on the form of ghrelin measured in the plasma (113).

Interestingly, consumption of beverages sweetened with fructose suppresses ghrelin less well than does ingestion of isocaloric glucose-sweetened beverages, probably because of different capacities of these monosaccharides to increase insulin levels (114). Beverages sweetened with either sucrose or high fructose corn syrup were not examined.

As shown in Table 3, there are several characteristics that allow ghrelin to be considered as an interesting biomarker directly related to appetite.

Focus on cholecystokinin

Cholecystokinin and appetite regulation. Cholecystokinin is an amino acid peptide that is produced by endocrine cells of the intestinal mucosa located in the duodenum and the proximal jejunum. CCK exists in multiple molecular forms ranging from 8 to 58 amino acids. It has been shown in *in vitro* studies that the affinity of the different isoforms to CCK receptors is similar (115). However, CCK-8 and CCK-33 are mostly studied with regard to appetite (116). Second to the endocrine cells, a small proportion of CCK is produced by neurons in the gastrointestinal tract and nervous system (117).

Cholecystokinin causes gallbladder contraction (118) and stimulates the release of digestive enzymes by the pancreas, such as amylase, peptidase and lipase. Next to facilitating digestion, CCK is a negative feedback signal to the stomach; it slows gastric emptying (119) and therefore increases stomach distension. CCK binds to the CCK-A receptor that has been identified on the gallbladder, pancreas and stomach.

Multiple studies have shown that exogenous administration of CCK suppresses food intake (e.g. (120,121)). The mean estimated effect of the 16 studies performed until 2004 was 22.5% (116). Ballinger *et al.* (122) used a dosage of CCK-8 that produced similar plasma concentrations to that of a meal (7.28 ± 2.43 pmol L^{-1}). Although they studied only six subjects, a statistically significant decrease in food intake of 20% ($P = 0.03$) was found. Lieverse *et al.* (123) infused CCK-33 in a relatively low dosage (plasma levels increased to 10–14 pmol L^{-1}). They found a borderline significant decrease in food intake of 20% ($n = 10$).

Several studies found a suppressing effect of CCK on food intake or subjective hunger ratings (124–126). Moreover, blocking the CCK-A receptor, with MK-329 or Loxiglumide, showed to diminish the satiating effect of a test meal (123,127–130). This clearly demonstrates that CCK has a causal role in appetite regulation.

Effect of food/nutrients on cholecystokinin. It has been shown in multiple studies that CCK is secreted shortly after the start of a meal (e.g. (118,126,131)). Especially, fats and proteins (compared with equal calories of carbohydrate) have shown to stimulate CCK production (115). Studies with inflatable gastric balloons have shown that stomach distension alone stimulates CCK release. However, studies in which nutrients are infused directly in the duodenum also show an increase in CCK concentration in plasma (17,18,132).

Recently, several studies have noticed that, in equivalent conditions, women might be more sensitive with regard to CCK than men (126,133,134). However, this was found in secondary analyses and needs to be investigated in more detail.

Here, also the illustration by Diepvens is of relevance (135). With respect to effects of different proteins and hydrolysates, Diepvens *et al.* investigated the effects of whey protein (WP), pea protein hydrolysate (PPH), a combination of WP + PPH and control (milk protein [MP] which consists of 80% casein and 20% WP) on appetite ratings, postprandial changes in hunger/satiety hormones and energy intake, and observed that both CCK and GLP-1 were increased by MP ($P < 0.05$), while no effect on energy intake was seen. They conclude that different exogenous biopeptides produced differences in release of endogenous peptides – including CCK- that had inconsistent relationships with satiety.

Focus on glucagon-like peptide-1

Glucagon-like peptide-1 and appetite regulation. GLP-1 is released by the endocrine cells of the ileum and colon. The GLP's are synthesized from proglucagon by the tissue specific prohormone convertase (136). Shortly after secretion $GLP\text{-}1^{7-36}$ is degraded by dipeptidyl peptidase 4 (DPP-IV) into the biologically non-active $GLP\text{-}1^{9-36}$. GLP is an incretin hormone, which means that it stimulates glucose dependent insulin secretion by the pancreas and inhibits glycogen release from the liver (see e.g. (137,138)). Intravenous infusion of $GLP\text{-}1^{7-36}$ has shown to decrease gastric emptying (e.g. (138,139)), which might enhance satiation.

Moreover, GLP-1 is thought to directly affect the brain. GLP-1, produced peripherally, may enter the CNS via the subfornical organ and area postrema, which lack a blood-

brain- barrier (136). Numerous GLP-1 receptors have been located in the hypothalamus (140). Recently, a brain mapping study has shown associations between postprandial plasma GLP-1 concentrations and an increased activation in the hypothalamus (141). More important, GLP-1 is thought to play an important role in the so called 'ileal brake' mechanism that causes a moderate and stable (digestible) flow of nutrients from the stomach into the small intestine; thereby being part of a feed back loop to enhance efficient nutrient uptake (136).

Several studies have consistently found that intravenous infusions of GLP-$1^{7–36}$ reduces food intake and appetite (for a meta-analyses see (139)). And although infusion of GLP-$1^{7–36}$ could not show the same appetite suppressing effects as GLP-$1^{9–36}$, this meta-analysis showed that the total circulating concentration of GLP, including both GLP-$1^{9–36}$ and GLP-$1^{7–36}$, showed a correlation with appetite parameters (prospective consumption $r = –0.43$; hunger $r = –0.26$ and fullness $r = 0.38$).

Effects of food /nutrients on glucagon-like peptide-1. It has been reported that GLP-1 shows a biphasic response after meal ingestion (136). As the first peak of GLP-1 secretion is observed before the nutrients enter the distal ileum and colon, it is thought that the first peak is not due to nutrients present in the large intestine. About 15–30 min after digestion the first peak, which may be involved in meal termination, is observed (see e.g. (108,131,141,142)). The second peak is probably triggered by nutrients in the large intestinal lumen and therefore likely to be dependent on the production of metabolites by the microbiota, most importantly short chain fatty acids: acetate; propionate and butyrate. This peak occurs after several hours and is more difficult to observe, hence good timing of the blood sampling is essential. Especially carbohydrates and protein (relative to equal calories from fat) have an effect on the first peak of GLP-1 secretion (141,143).

It has been hypothesized that GLP-1 secretion is under neural regulation (144). A second hypothesis is that GLP-1 secretion is under hormonal control, i.e. by other gut hormones such as CCK (144) or leptin (145). Recently, Brennan and colleagues observed that infusion of physiological dosages of CCK-8 increased GLP-1 plasma levels (55). However, these effects were not observed during CCK-33 infusion in physiological doses (146). Moreover, interaction effects of combined CCK and GLP-1 infusions on gastric motility, appetite or food intake were not observed in these studies (55,146).

Studies confirming effects of GLP-1 agonists and DPP-IV inhibitors might enhance the evidence for a role of this mechanism in appetite regulation.

In some studies in humans, an increase in postprandial response of GLP-1 was observed after ingestion of different types of fibre or indigestible carbohydrates (62,147,148), and in some cases, but certainly not in all, this is related to a significant increase in satiety (149). The criteria relating to the efficacy of dietary fibres (i.e. fermentable vs. non-fermentable) on gut peptide release and satiety remains debatable (150). The 'kinetics' of fermentation – assessed by the hydrogen breath test – would be taken into account when assessing the influence of fermented nutrients on circulating gut peptides (62,148). But the link with food intake behaviour in humans has been rarely explored until now.

Interestingly, Smeets *et al.* (151) recently reported an example of GLP-1 being mainly nutrient-related, i.e. showing the highest release after a high carbohydrate lunch compared with a iso-energetic high-protein (HP) lunch. However, the HP lunch was more satiating, illustrating that GLP-1 release would only be secondarily and not always quantitatively synchronized with satiety.

Focus on peptide tyrosin-tyrosin

Peptide tyrosin-tyrosin and appetite regulation. PYY, or peptide tyrosine-tyrosine, is synthesized and released from the endocrine cells of the ileum and the colon. PYY is released postprandially as $PYY^{1–36}$ and cleaved by DPP-IV into $PYY^{3–36}$. The $PYY^{3–36}$ amide belongs to same peptide family as neuropeptide Y (NPY) and pancreatic polypeptide (PP). All three members of the family influence food intake, NPY acts as orexigenic peptide, PP from the pancreas has a satiety effect (152). PYY is secreted predominantly from entero-endocrine cells of the ileum and colon. The L-cells of the intestine release PYY in proportion to the amount of calories ingested at a meal. Circulating PYY exists in two major forms: $PYY^{1–36}$ and $PYY^{3–36}$, due to the cleavage by DPP-IV.

Receptors that mediate the effect of PYY belong to the NPY receptor family and include Y1, Y2, Y3, Y4 and Y5. $PYY^{3–36}$ has a high affinity with the Y2 receptor, which is present in the CNS and hypothalamus (2).

Intravenous infusions of $PYY^{3–36}$ demonstrate dose dependent decreases in energy intake (153). Several data have been obtained through intravenous infusions with $PYY^{3–36}$ at pharmacological doses, showing the kinetics of effects on food intake behaviour (137,152,154). Additive effects are obtained through the combination of $PYY^{3–36}$ and GLP-$1^{7–39}$ (155). However, one must be careful with conclusions as the effects of high dosages could also be due to adverse effects, such as nausea and vomiting as observed in the study of Sloth (156).

Effects of food/nutrients on peptide tyrosin-tyrosin. PYY levels increase approximately 1–2 h after ingestion of a meal. In addition, the size of a meal is important in terms of PYY response (153,157,158). To determine if the macronutrient composition influences postprandial serum PYY

levels Essah *et al.* compared 1 week on a weight-maintenance, low carbohydrate, high-fat (LCHF) diet with a low-fat, high-carbohydrate (LFHC) diet in obese subjects. They found that the LCHF diet stimulates PYY secretion more than a LFHC diet in obese individuals (159).

Comparing HF, high-carbohydrate and HP diets in obese women, Helou *et al.* have shown that the HF meal induced a significantly higher increase in postprandial $PYY^{(3–36)}$ levels, at 15 and 30 min compared with the HP meal, whereas the postprandial increase following the HP meal became significantly higher than that following the HF meal at 120 min. Postprandial increase in $PYY^{(3–36)}$ was highest in the first hour following the HF meal, while following the HP meal the increase was delayed by 1 h. They conclude that increasing both protein and fat content of a meal may induce an immediate and prolonged increase in $PYY^{(3–36)}$, resulting in increased satiety and its maintenance for a longer period of time (160). Finally, with respect to PYY release, Diepvens *et al.* found only little effects with respect to different proteins and hydrolysates (135).

Technological aspects related to peptides measurement

The amount of gut peptides in biological fluids may be measured by several techniques including sophisticated techniques (high-performance liquid chromatography coupled to mass spectrometry), or more affordable techniques, based on immunoreactivity of the different forms of the peptide (enzyme immunoassay (EIA), radio immunoassay (RIA)). For most peptides, many commercially available kits will take into account the total amount of peptides, and not the specific forms that coexist. Even if circulating total GLP-1 concentrations have been shown to correlate with appetite by some authors (139), this remains debatable.

A key point is to choose antibodies, which allow quantification of the biologically active form of the peptide (acylated form of ghrelin, $GLP\text{-}1^{7–36}$ amide and $PYY^{3–36}$ amide), which is relevant when a link with hunger or satiety must be established. For CCK, several isoforms coexist (such as CCK-8, CCK-33 and CCK-58 (161,162)), that do not have similar biological activities. Even if selective antibodies are available, the price and the sensitivity of the method remain a problem in most cases.

The problem of sensitivity is crucial for most peptides and sometimes requires concentration and/or extraction of the biological sample. This is particularly true when the measurement of the peptides is performed in the fasting state (except for ghrelin, which is released upon fasting).

For some peptides, cross-reactivity with other peptides exists: gastrin, e.g. has an identical carboxyl terminal pentapeptide sequence as CCK. Moreover, the plasma concentrations of gastrin are 20–100 fold to that of CCK. These problems can be tackled by concentrating plasma and using very specific and sensitive antibodies, such as an antibody for the CCK-specific tyrosyl sulfate in position 7 (163).

The degradation of the peptide upon sampling and storage is also crucial and requires precautions to avoid misinterpretation. It might be that immediate acidification of plasma is needed to prevent degradation of the longer isoforms of CCK (e.g. (162)).

Glucagon-like $peptide\text{-}1^{7–36}$ is quickly hydrolysed into $GLP\text{-}1^{9–36}$ by DPP-IV; therefore, DPP-IV inhibitors should be added immediately after blood collection for the measurement of the biologically active $GLP\text{-}1^{7–36}$ amide. On the other hand, the active form of PYY is the $PYY^{3–36}$ amide (obtained upon cleavage by DPP-IV): the presence of DPP-IV inhibitor in the blood sample could avoid the abnormal release of this form upon storage and measurement, thus avoiding false interpretation of PYY increase.

Therefore, treating plasma samples carefully is crucial when measuring the active form of peptides, to avoid false negative, or positive, results depending on the peptide.

Future developments

Some ideas for future developments relating physiological functions and biomarkers to satiation, satiety and/or appetite are proposed below. They take into account some physiological targets poorly explored until now, but also present the need for new technological and scientific development in the field.

Glycaemia: a future for this 'old' biomarker?

Following the proposal of the 'glucostat' theory in the control of food intake, the role of glycaemia in meal initiation has been extensively investigated. The measurement of blood glucose is an easy and well-validated technique. Absolute concentrations of glucose do not seem to be very important in the regulation of food intake; but transient and dynamic declines in blood glucose concentration seem to be strongly related to meal initiation (164).

In addition, intraduodenal glucose influences appetite possibly through glucoreceptors or osmoreceptors in the intestine, which may induce satiety through direct vagal stimulation of the release of insulin and/or GLP-1 (165). The relevance of the hypo-or hyperglycaemic effect of food or ingredient – in the control of appetite or satiety – remains difficult to establish. While authors stated that 'dynamic' falls in blood glucose influenced meal initiation in both animals and humans, the satiating qualities of low glycaemic index foods, e.g. may be more closely related to compositional aspects that promote slower rates of digestion and absorption in the gut, rather than postprandial glycaemia *per se*. Habitual meal frequency is based upon a cluster of related factors including macronutrient composition of the food, sweetness perception, hunger suppres-

sion, but also on blood glucose declines and average blood glucose levels (166). Changes in glycaemia thus appear as only one of the criteria involved in food intake regulation.

Energy metabolism, an additional target to take into account?

One theory proposed is that there is a link between energy expenditure and satiety, thus suggesting that nutrient metabolism could be considered as criteria when assessing the interaction with appetite. The increased energy expenditure at rest (dietary-induced thermogenesis) may translate into satiety feelings (167). The relationship between satiety and diet-induced thermogenesis has been shown under conditions of elevated diet-induced thermogenesis, measured over 24 h (167). An increase in fatty acid oxidation in the liver has been proposed as a satiety signal (168). For example, a number of studies have shown medium chain fatty acids to be more satiating than long chain fatty acids in both animal and human subjects, and specific types of lipids (such as diacyl glycerol) may also influence both fatty acid oxidation and appetite (169). Therefore, regardless of their influence on energy expenditure, fatty acids that are more rapidly and extensively oxidized may play a role in satiety. This area of research is broad, but will be particularly interesting as it opens the door to a common mechanism/target for short-term (effect on appetite) and long-term regulation of energy homeostasis by nutrients or ingredients.

Peripheral control of appetite: not only the gut

In view of studies (mostly performed in animals) insulin has been proposed as the first peptide secreted by the pancreas that could induce satiety at the central level (1). This was followed by the discovery of the role of other pancreatic peptides that could play a relevant part in food intake, namely PP and amylin (170). Only few data report the endogenous modulation of those pancreatic peptides upon feeding, and the relevance with the physiology of satiety (171). However, their role should be considered further in the near future.

Interestingly, other peripheral peptides, which are not secreted by organs directly related with digestion or fat storage, may also influence food intake, e.g. some cytokines (interleukin [IL]-1 or IL6). The data remains scarce but the link between low-grade inflammation and food intake behaviour remains an interesting area to study.

Finally, recent data obtained mainly in animals also suggests that the dietary modulation of gut microbiota and related metabolic activity can also modify gut short chain fatty acids (SCFA) and peptides production and satiety (172). Although, poorly explored until now, gut bacteria could thus become a new physiological target to take into account in appetite regulation.

Besides the above targets, some stress factors are also associated with appetite deregulation, and the investigation into the mechanism(s) behind these effects is now in progress to elucidate novel neuro- and psychobiological factors controlling food intake and energy expenditure. For example, the paradoxical result of prolonged sleep deprivation is the activation of the hypothalamic-pituitary-adrenal axis activation and loss of body weight despite an apparent increase of food intake (173). Another situation is the context of alcoholism and perturbations of appetite sensation upon alcohol craving and withdrawal (174).

Biomarkers in the control of food intake: not only peptides

The cannabinoid system, including fatty acids derivatives, and protein targets, appear as a complex but integrative system (175). This illustrates the fact that not only circulating peptides, but also endogenous compounds with various chemical structure such as lipids: oleyethanolamide (with anorexigenic properties) or anandamide (that conversely promotes food intake) are putative targets to take into account when assessing the interaction between nutrients/food components and food intake. The difficulty remains linked to the development of sophisticated techniques (High Performance Liquid Chromatography (HPLC)-mass spectrometry (MS), gas chromatography (GC)-MS) required to assess the modulation of endocannabinoid in biological fluids between food intake episodes.

Exploring the brain

It is well known that specific areas of the brain are stimulated by hunger and satiety, and that in turn controls and coordinates food intake behaviour. Estimation of the effect of specific dietary manipulation on specific brain activity related to food intake requires the development of imaging techniques. In that context, ^{15}O- PET has already been used to correlate the satietogenic effect of a liquid formula with changes of neuronal activity in lean and obese individuals. However, to date, human studies on the role of the hypothalamus in the regulation of food intake suffer significant limitations related to the small size, the deep location and the high vascular network surrounding this part of the brain (176). Impressive, technological improvements, such as functional MRI, suggest a promising future for this area in understanding better the experimental models and where and how the brain responds to peripheral signals. Using manganese ion accumulation as a marker of neuronal activity changes in signal intensity in key appetite centres within the hypothalamus following peripheral injection of gut hormones have been demonstrated. Manganese-enhanced MRI offers several advantages over methodologies currently used for the study of gut hormone interactions with

the CNS and has the potential for application in fields beyond appetite regulation (177).

Conclusions

In conclusion biomarker measurement can be useful to provide information on the mechanisms underlying the action of nutrients, ingredients, food and beverages on appetite regulation, as evident in both animal and human studies. There are several examples of the quantitative and qualitative impact of some potentially important gastrointestinal influences on satiety and food intake, independent of (although related to) hormonal and neural measures. Only some of the parameters described in this paper have been tested extensively in relation to ingestion behaviour, and, when tested, they are often studied in isolation. Furthermore, the methodology applied could also result in errors of interpretation. For example, variations within subjects should also be estimated before interpreting the results.

It is advised to study several aspects of the gastric and intestinal phase at the same time, using the technique that most feasibly measures the parameter/nutrient of interest in the context of that particular meal. MRI is a relatively new and promising technique that can measure several of these aspects and therefore could generate new insights into relevance of these parameters for appetite regulation. More validation studies of MRI for these specific aspects are needed.

Biomarkers and key parameters related to appetite-related physiological functions may be indicators to help understand the mechanisms by which nutrients or ingredients may act on food intake. However, measuring the level, extent and changes in biomarkers cannot be quantitatively related to satiety. These biomarkers will not replace the appreciation of the different sensations of

Table 4 Peptides signals involved in the regulation of food intake and stimulation of appetite

	Origin	Target	Circulating	Level of evidence	Reference
a. Peptides involved in the stimulation of appetite					
Neuropeptide Y	CNS	CNS	No	+++	(4,6)
Agouti-related protein	CNS	CNS	No	+++	(4)
Melanin concentrating hormone	CNS	CNS	No	+	(4)
Hypocretins/orexins	CNS	CNS/others	No	++	(4)
Growth hormone-releasing hormone	CNS	CNS	No?	++	(4)
Galanin	CNS	CNS	No	+/–	(4)
Dynorphin	CNS	CNS	?	+/–	(4)
Apelin	Adipose tissue	CNS	Yes	+	(92)
Ghrelin	Stomach	CNS	Yes	+++	(92)
b. Peptides involved in satiety					
Pro-opiomelanocortin	CNS	CNS	No	+++	(4)
Cocain- amphetamin-regulated transcript	CNS	CNS	No	++	(4)
Calcitonin gene-related peptide	CNS	CNS	?	+	(4)
Melanocyte-stimulating hormone	CNS	CNS			(4)
Thyrotropin-releasing hormone	CNS	CNS			(4)
Prolactin-releasing peptide	CNS	CNS	No	+	(4)
Motilin	Stomach	CNS	?	+	(4)
Glucagon-like peptide 1	Gut, CNS	CNS	Yes	+++	(4–6)
Oxyntomodulin	Gut, CNS	CNS	Yes	++	(4)
Galanin-like peptide	CNS	CNS	No	+/–	(4)
Peptide tyrosin-tyrosin	Gut	CNS	Yes	+++	(4,6)
Cholecystokinin	Gut	CNS	Yes	+++	(4,6)
Insulin	Pancreas	CNS	Yes	++	(4)
Obestatin	Fat, stomach	CNS	Yes	++	(4,5)
Nesfatin 1	Stomach, gut	CNS	Yes	+/–	(4)
Amylin	Pancreas	CNS	?	+	(170)
Pancreatic polypeptide	Pancreas	CNS	Yes	++	(4,170)
Interleukin1/6	Pancreas/gut	CNS	Yes	+	(6)
	Immune cells	?	yes	+/–	(4)

Peptides presented in bold are described in detail in the present paper.
Level of evidence is estimated upon the available published data; +++ referring to several relevant human studies; +/– refer to a low number of available studies, sometimes with controversial results depending on the strain and effect on appetite.
See more details in Table 2.
CNS, central nervous system.

hunger, satiety and desire to eat – analysed through validated scales. They can be useful to estimate the kinetics of persistence of satiety in postprandial periods, to assess a putative mechanism, or to help target specific population (obese people who have a low level of satietogenic peptides such as GLP-1). As illustrated in Table 4 for ghrelin, and as explained throughout the text for other targets, there are some persuasive arguments for some peptides to be considered as physiologically relevant. At this stage, possibilities for choosing a specific biomarker related to the expected effect of one nutrient or a particular food or beverage is limited. There are a number of peptide candidates prone to modulate satiety and food intake but only a few studies measure several peptides in parallel. Their relationship with satiety is not always straightforward. Evidence derived from a supposed biomarker for satiety does not guarantee the highest satiety. The classical technique (RIA, EIA) for measuring the peptides are subject to major limitations such as the amount of samples needed for the measurement, and the costs related to this. Moreover, the measurement of many peptides requires special conditions of sampling (addition of protease inhibitors) not necessarily adapted to routine blood sampling.

To conclude, a lot of progress has been made to assess the physiological processes regulating appetite. It would be rather difficult to restrain the complexity of appetite regulation to the assessment of one or two measurable biomarkers. But their measurement becomes essential to understand the mechanism by which food components can drive or restrain appetite and food intake.

Conflict of Interest Statement

No conflict of interest was declared.

Acknowledgements

This work was commissioned by the Appetite Regulation Task Force of the European branch of the ILSI (ILSI Europe). Industry members of this task force are: Ajinomoto Europe; Cadbury; Cargill; Coca-Cola Europe; Colloïdes Naturels International; Cosucra; Danisco; Groupe Danone; Kellogg Europe; Kraft Foods; Lipid Nutrition; National Starch Food Innovation; Nestlé; PepsiCo International; Sensus; Südzucker/BENEO Group; Tate & Lyle; Unilever. For further information about ILSI Europe, please email info@ilsieurope.be or call +32 2771 00 14. The opinions expressed herein are those of the authors and do not necessarily represent the views of ILSI Europe. The work of the expert group and its publication was coordinated by Fiona Samuels, Scientific Project Manager at ILSI Europe.

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