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Gut Hormone Concentrations: Satiety-, Nutrient- or Subject-Related?

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Abstract

Against the background of the current human epidemic of overweight and obesity, it is important to study factors that influence appetite and energy intake. The search for physiological biomarkers of appetite is very active. However, changes in "orexigenic" and "anorexigenic" hormone and glucose concentrations or in energy intake do not seem to relate strongly to appetite profiles. An overview of current findings will be presented, as well as the use of an appropriate statistical approach that takes into account the change over time of appetite profiles versus hormone and glucose concentrations by focusing on within-subject relations of these observations.

Glossary of Abbreviations

AUC: Area Under the Curve
BMR: Basal Metabolic Rate
CCK: Cholecystokinin
DER: Daily Energy Requirements
GLP-1: Glucagon-Like Peptide 1
PYY: Peptide Tyrosine-Tyrosine
VAS: Visual Analogue Scales
WHO: World Health Organization

Introduction

The prevalence of overweight and obesity has increased worldwide to epidemic proportions, and severe obesity is a risk factor for many diseases, including type 2 diabetes, cardiovascular diseases and hypertension.[1] The World Health Organization (WHO) has estimated that by 2015, approximately 2.3 billion adults worldwide will be overweight and more than 700 million will be obese. Overweight and obesity results from a positive energy balance, with energy intake exceeding energy expenditure over a prolonged period.[1] It has been shown that physical activity energy expenditure in Europe and North America did not decrease between the 1980s and 2005, a period during which obesity rates increased.[2]

This suggests that the recent rise in obesity may not result from a lowered physical activity but, rather, from an increased energy intake. The regulation of energy intake and appetite is a complex process involving, besides genetic, environmental and behavioral factors, physiological factors such as the dynamics of gastrointestinal hormones and the possibly related feelings of hunger and satiety.[1,3,4] Prevention of overweight and obesity requires sustained or increased satiety in order to prevent energy intake exceeding energy expenditure. Quantification of appetite is necessary in order to characterize effects of interventions. Since overweight and obesity appear to a large extent to be an environmental effect, and since in overweight families the pets usually are overweight as well, the presented physiology is not only relevant for humans but also for pets.[5,6]

Measurement of Appetite

Appetite can be measured by means of questionnaires, such as subjective ratings on 100 unit (mm) visual analogue scales (VAS).[7] The scales can be anchored with "not at all" at one end and "extremely" at the other end and combined with questions on feelings of hunger and satiety, e.g., "How hungry are you right now?" Subjects have to make a single vertical mark at the appropriate point between the two anchors on each scale to indicate their subjective feeling. Measured feelings of appetite expressed as ratings on VAS have shown to be highly reproducible and therefore reliable.[7]

Appetite also can be measured by actual energy intake after an intervention.[8] For calculation of the energy intake, the subject specific energy requirements should be taken into account. The relationship between appetite and food intake can be disrupted by several factors,[4] e.g., eating in the absence of hunger because of availability of palatable food or emotional stress.[9]

Physiological measures, such as changes in gut hormone concentrations, also may be used as indicators of appetite and energy intake and may possibly serve as biomarkers of appetite.[10] The search for physiological biomarkers of appetite currently is very active. A biomarker is, in general, a substance that can provide reliable

early indicators of a biological state.[11] For a biomarker of appetite to be useful, it must meet a number of criteria: the measurement of the biomarker must be feasible, measurable without invasive procedures and reproducible under similar conditions; moreover, the biomarker must clearly relate to appetite physiology and be sensitive to changes in appetite.[11] This also may indicate, in the case of pets, where only physiological measures can be used, to which extent they may predict hunger and fullness.

Physiological Measures Related to Appetite

The gastrointestinal tract is the source of a large number of signals and mechanisms that play a role in satiety and food intake. This renders the gastrointestinal tract an obvious physiological target of strategies aimed at weight management and weight loss.[12] Exposing the stomach and the small intestine to nutrients leads to release of gut peptides and neurotransmitters and activation of vagal afferents through mechanical and chemical stimuli.[13] These signals can act locally and convey information about energy needs to the hypothalamus for processing, and consequently induce a reduction in hunger levels and food intake.[12-14]

Relevant physiological measures related to appetite, i.e., hunger and satiety, may be the anorexigenic peptides cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1) and peptide tyrosine-tyrosine (PYY), the orexigenic gut peptide ghrelin, and glucose and insulin.[10,15] CCK is produced by endocrine cells of the intestinal mucosa, located in the duodenum and the proximal jejunum, in response to the intraluminal presence of digestion products of fat and protein.[10] CCK appears to reduce appetite, as it has been shown that exogenous administration of CCK suppresses food intake.[16,17] CCK levels rise rapidly, reaching a peak within 15 minutes after a meal.[10]

GLP-1 and PYY are released from the endocrine L cells of the ileum and the colon and appear to reduce appetite.[10,18,19] Intravenous infusion of GLP-1 and PYY demonstrates decreases in energy intake.[18,19] GLP-1 and PYY plasma concentrations are low in the fasting state and rise during a meal.[20] GLP-1 is released rapidly in response to exposure of the gut wall to especially carbohydrates and fat and reaches a peak about 15 to 30 minutes after digestion.[10] GLP-1 also increases glucose-dependent insulin-secretion, reduces glucagon secretion and increases pancreatic β cell growth.[21] PYY is released within 15 minutes of food intake, in proportion to the amount of ingested calories, with fat being the most potent macronutrient, followed by carbohydrates, followed by proteins, and reaches a peak at 1 to 2 hours.[10,22]

In the ileum, exposure of the gut mucosa to fat, carbohydrates and protein activates the ileal brake.[23,24] The ileal brake is a negative feedback mechanism that potently inhibits gastric emptying and small intestinal transit and results in a reduction in gastric acid secretion, pancreatic enzyme secretion and bile acid secretion.[23] Furthermore, it may stimulate central satiety centers in the brain.[24] Consequently, activation of the ileal brake significantly increases satiety and reduces food intake. GLP-1 and PYY are thought to be mediatory peptides for ileal brake activation. Furthermore, it has been demonstrated that different neural mechanisms are involved in mediating the different effects of ileal brake activation.[23]

Ghrelin is a peptide secreted primarily by the stomach and appears to increase appetite, as it has been shown that intravenous infusion of ghrelin in humans increases food intake.[25,26] Ghrelin plasma concentrations peak before a meal and rapidly drop postprandially in response to nutrient ingestion.[10] Ghrelin responses are dependent on caloric intake and circulating nutritional signals, with fat causing less suppression than carbohydrates or protein.[27,28]

Glucose has a central role in the regulation of energy metabolism and is the only energy source for the central nervous system.[8,29] Circulating glucose concentrations, from the time glucose is ingested to its absorption in the gut, and its increase or decrease in blood concentration are tightly monitored.[29] Glucose is hypothesized to play a role in meal initiation, as feeding is usually preceded by a decrease in blood glucose concentrations.[8,29-31] Glucose triggers insulin secretion by the β cells of the pancreatic islets.[29] Insulin stimulates the uptake of glucose by peripheral tissues and suppresses hepatic glucose production.[8] Similar to blood glucose, insulin has been hypothesized to be involved in appetite regulation.[32,33]

Relationship VAS Appetite Ratings Vs. Physiological Measures

A possible association between VAS appetite ratings and physiological measures remains a subject of debate. Several studies showed no relationship between appetite ratings and endogenous CCK, GLP-1, PYY and ghrelin concentrations,[34-36] while others found significant correlations ($p<0.05$, $R^2<0.3$) at a few time points or for the area under the curve (AUC).[18,37-40] The latter papers suggest a relationship between appetite ratings and gastrointestinal hormone concentrations, although correlation coefficients are mostly too low to presume that the gastrointestinal hormones serve as a reliable biomarker. Regarding glucose and insulin concentrations, it has been shown that insulin concentrations were inversely correlated with feelings of hunger ($p<0.02$, $R^2<0.1$), while glucose concentrations were not correlated with feelings of hunger or satiety.[15] Literature has indicated that it is not clear yet whether blood glucose and insulin

concentrations can act as biomarkers of appetite as the relation is confounded by many metabolic processes.[8,15]

In the studies that find significant correlations between VAS appetite ratings and gut hormone and glucose concentrations,[15,18,34-39] correlation analyses were based upon the calculated AUC or the measured values per time point, not taking into account the factor time. In one of our studies (submitted for publication), the dynamics of VAS hunger and fullness ratings were compared with the dynamics of GLP-1, PYY, ghrelin, glucose and insulin concentrations, using a statistical approach that includes the factor time by concentrating on the within-subject relations of these observations. We hypothesized that including the factor time might strengthen the possible relationship between VAS appetite scores and hormone and glucose concentrations. Moreover, we investigated whether the changes in VAS scores are synchronized with, or lag behind or in front of, the changes in hormone and glucose concentrations.

The study design comprised consumption of a four-course lunch spread over two hours (staggered) and consumption of the same four-course lunch in half an hour (nonstaggered). This design gave us the ability to measure and compare postprandial appetite and hormone and glucose dynamics throughout different meal patterns and thereby different timings of nutrient delivery to the gut. Subjects (n=38, age=24±6y, BMI=25.1±3.1kg/m^2) came to the university twice for consumption of a staggered or nonstaggered four-course lunch (randomized cross-over design). The amount subjects consumed of the four-course lunch corresponded to 40% of their daily energy requirements (DER). For each subject, the DER was calculated by multiplying the basal metabolic rate (BMR) by the appropriate physical activity factor (1.5 to 1.8).41 The BMR (kcal/day) was calculated according to the equation of Harris–Benedict.[42]

During the two test days, subjects were seated separately, blood samples were drawn, and VAS on appetite were completed. To assess the strength of the within-subject relation between changes in VAS scores for hunger and fullness and changes in hormone and glucose concentrations, we calculated, separately for each subject, regression slopes and R^2 values for the regression of VAS scores on hormone and glucose concentrations, for the corresponding measuring moments (fullness vs. GLP-1, fullness vs. PYY, hunger vs. ghrelin, fullness vs. glucose, fullness vs. insulin). To investigate whether the changes in VAS scores were synchronized with, or lagged behind or in front of, the changes in hormone and glucose concentrations, the analysis was repeated with the VAS score versus the hormone and glucose concentration of the previous and of the following measuring moments. Student's one-sample t-tests were used to determine whether the means of the regression slopes were different from zero.

Analyses of regression slopes and R^2 values showed that VAS appetite scores and hormone and glucose concentrations changed synchronously and that the mean explained variation was, depending on the actual physiological measure, ~70% for fullness vs. insulin, ~55% for fullness vs. GLP-1 and PYY, ~50% for hunger vs. ghrelin, and ~30% for fullness vs. glucose. The analysis of the hormone and glucose dynamics, in relation to feelings of appetite, may be useful to determine differences between experimental conditions and differently characterized groups.

Furthermore, analyses showed strong correlations between the cumulative energy intake over the four courses and the changes in hormone concentrations (R^2=0.6-0.8). These relatively high correlations indicate that the hormone releases are directly nutrient related. The relationship between gut hormone and glucose levels and energy intake in pets still needs to be established. Overall, it appeared that GLP-1, PYY, glucose and insulin concentrations changed synchronously with VAS fullness scores. In contrast, changes in ghrelin concentrations lagged behind (10 to 30 minutes) changes in hunger scores and insulin concentrations (R^2=0.6-0.7), suggesting a role for insulin as negative regulator of ghrelin. Prandial ghrelin suppression does not require luminal nutrient exposure in the stomach or duodenum, the principle sites of ghrelin production.[43,44] Instead, signals mediating this response originate farther downstream in the intestine and from postabsorptive events.[45] Insulin may be required for postprandial ghrelin suppression.[46-48] There were no main differences in slopes and R^2 values between the meal patterns. Thus, meal pattern had no major impact on the relationship between VAS scores and hormone and glucose concentrations. It is of great interest to execute these analyses in different species of pets since they differ largely in their meal patterns.[49]

Conclusion

Relevant correlations of changes in appetite profiles and "orexigenic" and "anorexigenic" hormone and glucose concentrations are present in humans, as being, depending on the physiological measure used, ~70 % for fullness vs. insulin, ~55% for fullness vs. GLP-1 and PYY, ~50% for hunger vs. ghrelin, and ~30% for fullness vs. glucose. The relationships between these gut hormone and glucose concentrations and energy intake in pets still need to be established.

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