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Postprandial response of salivary ghrelin and leptin to carbohydrate uptake

Circulating ghrelin rapidly responds to food intake, being decreased by carbohydrates and fat¹ and increased by proteins¹ or amino acids.² Leptin also affects energy expenditure. While ghrelin stimulates food intake,³ leptin induces satiety.⁴ It has therefore been postulated as an antagonist of ghrelin.⁴

The salivary glands are additional sources of both peptides.^{5–6} Determination of salivary values has been suggested as a non-invasive alternative to serum.⁷ We investigated the postprandial response of salivary ghrelin and leptin to determine whether this

Figure 1 Changes in serum and salivary ghrelin in healthy male adults (oral glucose tolerance test) (A) and in the saliva of children suspected of lactose/fructose malabsorption (B) before (0 min) and after (120 min) carbohydrate uptake (1 mg/kg body mass). The decline was present in all of the pre-post samples in both environments (***) $p < 0.001$. No difference was found between either carbohydrate.

non-invasive application is useful in clinical practice.

Serum and saliva were obtained from healthy males ($n = 11$; 18–40 years; body mass index (BMI) 19–27 kg/m²), before and two hours after intake of 1 g/kg glucose (fig 1). Patients ($n = 18$, 9.9 (4.4) years, BMI 13–26.5 kg/m²), underwent oral fructose ($n = 9$) or lactose tolerance tests ($n = 9$) because of suspected carbohydrate malabsorption. The sugar load was adjusted to 1 g/kg body mass. For ethical reasons only saliva was collected. Participants and parents gave informed consent.

Ghrelin was measured by RIA (Mediagnost) with an LLOQ of 17 ng/l. Intra- and interassay coefficients of variation were 8.5% and 14%. Leptin was analysed by an inhouse RIA.⁸

Agreement between values obtained from matched serum and saliva was assessed by plotting the method differences against the method means.⁹ Differences between saliva and serum and between the two time points were calculated by the Wilcoxon signed rank test.

- (A) In the glucose group, a significant correlation between BMI and preprandial salivary and serum levels was found for ghrelin ($r^2 = 0.46$; $p < 0.001$) and leptin ($r^2 = 0.49$; $p < 0.001$). No comparable results were obtained for patients.
- (B) The mean absolute difference (%) between basal ghrelin in serum and saliva was -18.03 (30.51) (95% confidence interval -87.05 to 50.99). Values after glucose uptake yielded a mean absolute difference of -68.97 (15.48) (95% CI -104 to -33.95). Combination of pre- and postprandial serum and saliva yielded absolute differences of -43.5 (78.9) (95% CI -80.43 to -6.56).

- (C) In the glucose group, initial ghrelin values ranged between 1.07 and 3.79 ng/ml (2.05 (1.03) ng/ml) in serum and 0.42 and 3.13 ng/ml (1.40 (0.90) ng/ml) in saliva. After glucose uptake these values decreased significantly ($p < 0.0001$) in all participants by 25.9 (22.4)% in serum and 39.5 (28.1)% in saliva, yielding final concentrations of 0.62–3.13 ng/ml (1.47 (0.72) ng/ml) in serum and 0.33–2.11 ng/ml (0.76 (0.59) ng/ml) in saliva.
- (D) The same was found in patients after fructose or lactose uptake. Initial salivary ghrelin values ranged from 0.62 to 7.90 ng/ml (2.11 (1.85) ng/ml). After sugar intake, ghrelin decreased significantly ($p < 0.0001$) by 45.2 (24.7)%, reaching final concentrations of 0.22–3.29 ng/ml (1.06 (0.86) ng/ml).

Several studies demonstrated a strong correlation between BMI and serum levels of both peptides, being positive for leptin and negative for ghrelin. In our study we found only a weak correlation between basal salivary values and BMI but this may have been due to the homogeneity of the participants' BMI values.

We found that leptin was not affected by carbohydrate uptake whereas ghrelin was decreased significantly in both environments two hours after sugar load. This was not surprising as leptin is a long term regulator of energy expenditure whereas ghrelin rapidly stimulates food intake in response to hunger.

In conclusion, we agree with Aydin and colleagues⁷ that salivary ghrelin may offer a non-invasive alternative to qualitative analysis, especially for dose-response or kinetic studies. Yet there was no difference between healthy volunteers and patients with innocuous gastrointestinal disturbances. In ongoing studies we will investigate whether salivary ghrelin and leptin are potential diagnostic markers of gastrointestinal diseases.

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Bravo wireless versus catheter pH monitoring systems

Pandolfino *et al* (*Gut* 2005;**54**:1687–92) and Bruley des Varannes *et al* (*Gut* 2005;**54**:1682–6) presented data from simultaneous pH monitoring by wireless Bravo and catheter mounted antimony electrode systems. Both studies demonstrated that the wireless system recorded significantly fewer reflux events; the effect on overall acid exposure was more limited. Further analysis revealed that events “missed” by the Bravo system were shorter and less acidic than those detected by both systems. The authors suggested that the different recording characteristics of the two systems explain the apparent “higher sensitivity” of the catheter pH system, including the lesser sampling rate of the Bravo system, and the systematic inaccuracy in catheter electrode calibration (not assessed by Bruley des Varannes *et al*).

Beyond these technical concerns, the significance of short “reflux events” is questionable because short drops to pH <4 can be caused by factors unrelated to gastro-oesophageal reflux: firstly, ingestion of mildly acidic fluids (despite instruction); secondly, as suggested by Pandolfino *et al*, movement of the catheter relative to the mucosa (that is, “drying” or “loss of contact” of the catheter electrode—the internal reference of the Bravo reduces this source of error); and thirdly, movement of the catheter relative to the gastro-oesophageal junction (GOJ) during swallowing (fig 1). The last point requires

Figure 1 Concurrent high resolution manometry (HRM) and pH recording during a transient lower oesophageal sphincter relaxation (TLOSr) in a normal volunteer. The positions of the Bravo capsule, catheter electrode, and LOS are indicated on the schematic diagram and the HRM plot. TLOSr was associated with brief oesophageal contraction with ~5 cm shortening. The position of the Bravo capsule remained constant relative to the LOS and the Bravo pH recording remained at ~pH 6; however the catheter electrode approached the LOS and recorded a short pH drop to <4 as it entered the “acid pocket”. Relaxation of the oesophagus restored the position of the LOS and the catheter pH recording normalised without swallowing activity. This “reflux event” was an artefact, related to oesophageal shortening rather than gastro-oesophageal reflux. UOS, upper oesophageal sphincter.

explanation: on swallowing, the oesophagus shortens by several centimetres due to longitudinal muscle contraction,^{1,2} an event that stabilises the oesophageal wall and increases the effectiveness of peristaltic contraction and bolus transport.³ Even greater shortening can occur during oesophageal spasm⁴ and transient lower oesophageal sphincter relaxation (with or without acid reflux). As the oesophagus shortens, the catheter electrode moves distally towards the GOJ and may pass into the proximal stomach, a region in which highly acidic conditions may be present.^{5,6} Thus the catheter electrode may dip into the “acid pocket” at the GOJ before relaxation of the oesophagus returns the catheter into its original position. This cannot occur with the Bravo because it is fixed to the oesophageal wall. As a result, a short drop in pH is recorded by the catheter electrode but not the Bravo system.

As stated by the authors, pH studies alone cannot explain the discrepancies between the catheter and Bravo systems. Studies that combine pH monitoring (chemical reflux and clearance) and multichannel intraluminal impedance (volume reflux and clearance) with manometry are required to define

the physiology of these events and help determine their relevance (if any) in eliciting symptoms. Studies using polymodal measurements have shown that oesophageal volume clearance is considerably faster than chemical clearance.^{7,8} Chemical clearance usually progresses by stepwise increases in pH as swallowing activity brings bicarbonate containing saliva into contact with acid reflux. However, short pH drops (often <20 seconds) can occur with peristaltic contractions and resolve without further swallowing activity. Short pH drops are also seen during transient lower oesophageal sphincter relaxation (fig 1), again resolving without swallowing activity. These observations strongly suggest that many short “reflux events” recorded by catheter systems may be artefacts, related to oesophageal shortening rather than gastro-oesophageal reflux.

The Bravo system is a well tolerated alternative to catheter based pH measurement, experience with the technique is increasing, and normal values are being established. If the accuracy (specificity) of catheter based detection of reflux events is shown to be limited, the Bravo system may establish itself as the new standard for pH