

Glucose-dependent insulinotropic polypeptide and insulin-like immunoreactivity in saliva following sham-fed and swallowed meals

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Abstract

Gastrointestinal peptides, including insulin, glucagon and glucose-dependent insulinotropic polypeptide (GIP) have previously been reported in salivary glands. Recent evidence has suggested they might influence postprandial macronutrient metabolism. This study therefore investigated and compared postprandial hormone concentrations in saliva and plasma to determine whether their secretion was influenced by oral food stimuli. In a within-subject randomised cross-over comparison of hormone concentrations in plasma and saliva following a mixed meal, 12 subjects were given two 1708 kJ mixed meals. On one occasion the meal was chewed and swallowed (swallowed meal), on the other it was chewed and expectorated (sham-fed meal). Salivary and plasma levels of immunoreactive insulin, GIP and glucagon-like peptide-1 (GLP-1), total protein, α -amylase, glucose and non-esterified fatty acid were measured before and for 90 min following the meals. Saliva total protein and α -amylase rose following both meals, indicating that the stimulus for salivary protein release is related to the presence of food in the mouth. GLP-1 was not detected in saliva. Fasting

salivary insulin levels were lower in saliva than plasma (28 ± 6 vs 40 ± 25 pmol/l respectively). Both increased following the swallowed meal but the rise in saliva was slower and less marked than in plasma (peak levels 96 ± 18 and 270 ± 66 pmol/l for saliva and plasma respectively, $P < 0.01$). Both were unchanged following the sham-fed meal. GIP was detected in saliva. Fasting GIP levels were significantly higher in saliva than plasma (183 ± 23 compared with 20 ± 7 pmol/l, $P < 0.01$). They decreased in saliva following both swallowed and sham-fed meals to nadirs of 117 ± 17 and 71 ± 12 pmol/l respectively, but rose following the swallowed meal to peak levels of 268 ± 66 pmol/l. These findings are consistent with insulin in saliva being an ultrafiltrate of that circulating in blood, but GIP in saliva being the product of local salivary gland synthesis, whose secretion is influenced, directly or indirectly, by oral stimuli. The function of salivary GIP is unknown, but we speculate that it may play a role in the regulation of gastric acid secretion in the fasting state.

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Introduction

Most mammals possess three pairs of salivary glands, the submandibular, parotid and sublingual glands, the secretory end-pieces of which are drained by a branched duct system to secrete saliva into the mouth. Saliva performs several functions, including lubricating the mouth to facilitate the swallowing of food and prevent the demineralisation of tooth enamel. α -Amylase, secreted into saliva, catalyses the hydrolysis of ingested starch in the buccal cavity. The salivary glands are a rich source of biologically active peptides. A number of growth factors are synthesised within the salivary gland and secreted into saliva, including epidermal growth factor (Boyer *et al.* 1991), insulin-like growth factor-I and tumour necrosis factor- α (Humphreysbeher *et al.* 1994, Kerr *et al.* 1995).

The salivary glands also contain biologically active proteins involved in glucose homeostasis. Human saliva

contains a proline-rich salivary peptide P-C, which potentiates glucose-induced insulin release and inhibits arginine-induced glucagon release in the rat pancreas (Kimura *et al.* 1995, Kimura *et al.* 1998). Insulin-like immunoreactivity has been extensively reported in rodent salivary glands, human parotid (Murakami *et al.* 1982) and submandibular salivary glands (Shubnikova *et al.* 1984), and in human saliva (Fekete *et al.* 1993). Glucagon-like immunoreactivity has been reported in rat salivary glands (Lawrence *et al.* 1977). A native form of peptidylglycine alpha-amidating monooxygenase is secreted into saliva (Kato *et al.* 1992); this is a regulating enzyme in the synthesis of biologically active hormones, such as glucagon-like peptide-1 (GLP-1), that have a C-terminal amide. The gastrointestinal (GI) hormone glucose-dependent insulinotropic polypeptide (GIP) has also been detected in rodent salivary glands and saliva (Tseng *et al.* 1993, 1995). The transcripts have been detected in the

striated ductal cells of the submandibular salivary gland. Pretranslational modulation of the GIP gene by nutrient ingestion has also been demonstrated in salivary glands as well as in the GI tract (Tseng *et al.* 1994, 1995), indicating that the GIP transcript is processed in the salivary gland to produce native GIP, consistent with a physiological role for salivary GIP in the rat.

The physical presence of food in the mouth may stimulate hormone secretion from the salivary glands, and salivary hormone secretion into saliva may be modulated by the physical action of masticating food in the mouth, rather than the presence in, or absorption of, food from the gut. Sham-feeding a high-fat test 'meal' following ingestion of encapsulated oil results in higher circulating triacylglycerol levels compared with sham-feeding a low-fat test 'meal' (Mattes 1996), suggesting that oral exposure to dietary fat can influence postprandial lipid metabolism. Sham-feeding during an intra-gastric glucose infusion has also been found to influence circulating plasma glucose levels (Teff & Engleman 1996), and salivary peptide P-C has been found to modulate both insulin and glucagon release from the isolated pancreas (Kimura *et al.* 1990). These experiments indicate that the presence of food in the mouth has the ability to modulate both carbohydrate and lipid metabolism. These effects, consistent with the known biological actions of hormones such as insulin and GIP, might be mediated via the production of these hormones from the salivary glands.

This study was therefore designed to investigate and compare postprandial hormone concentrations in saliva and plasma following ingestion of a mixed meal, and to determine whether their secretion can be stimulated by the physical presence of food in the mouth, following a sham-fed meal, which was chewed and expectorated.

Materials and Methods

Subjects

Twelve healthy subjects, six men and six women aged 21–35 years with body mass indices ranging from 21 to 28 kg/m² were recruited. Subjects were excluded if they had significant current or previous medical history, were receiving regular medication affecting the GI or nervous systems, consumed >20 units of alcohol/week, or were smokers. Written consent for the study was obtained from every subject after a full explanation of the purpose and nature of all procedures used. The investigation was approved by the University of Surrey Advisory Committee on Ethics, functioning according to the guidelines issued by the Royal College of Physicians of London in August 1996.

Study protocol

The study design was a within-subject randomised cross-over comparison of hormone concentrations in plasma and

saliva following a mixed meal which was either chewed and swallowed, or chewed and expectorated (sham-fed).

The mixed test meal (total energy content 1708 kJ, containing 40% total energy as carbohydrate, 40% as fat and 25% as protein) consisted of cream cheese and crackers, together with a 200 ml glass of sugar-free orange squash. On each test occasion, after an overnight fast, a cannula was inserted into the subject's antecubital vein and two basal blood samples were taken. At the same time, two saliva samples were also taken. Subjects were then given the test meal. On one occasion the meal was chewed and swallowed (the swallowed meal), on the other it was chewed and expectorated (the sham-fed meal). Blood and saliva samples were simultaneously taken at intervals for 90 min following the meals (15, 30, 45, 60 and 90 min). Blood and saliva samples were both centrifuged at 1825 g for 10 min to separate plasma from whole blood and any particulate matter from saliva. They were stored at –20 °C until analysis.

Assay procedures

Plasma glucose and non-esterified fatty acid (NEFA) and salivary total protein and α -amylase were analysed by standard automated enzymatic spectrophotometric methods (reagent kits from Roche Products Ltd, Welwyn Garden City, Herts, UK (glucose and protein), Wako Chemicals, Neuss, Germany (NEFA) and Randox, Country Antrim, Northern Ireland, UK (α -amylase)). The interassay coefficients of variation were less than 5% for these assays. Plasma and salivary insulin, C-peptide, GIP and GLP-1 were measured by in-house RIAs (Morgan *et al.* 1978, Elliott *et al.* 1993, Hampton & Withey 1996). The interassay coefficients of variation were less than 10% for these assays. All samples from a single subject were measured in the same assay.

GIP and insulin-like immunoreactive species in saliva were considered to be similar to their gut and pancreatic counterparts on the grounds that (i) serially diluted saliva samples containing GIP and insulin ran parallel to standard curves constructed from GIP and insulin of GI origin, (ii) recovery of GIP and insulin in saliva samples 'spiked' with the hormones of GI origin was 104 ± 5.0 and $106 \pm 6.4\%$ respectively, and (iii) GIP and insulin-like immunoreactivity in saliva samples co-eluted with their respective GI and pancreatic counterparts on HPLC (4.6 i.d. \times 250 mm 5 μ C18 column; trifluoroacetic acid/acetonitrile solvent gradient) (Messenger 2000).

Statistical analyses

All hormone and metabolic data were compared by repeated-measures ANOVA with time and treatment as within-subject factors, followed by a post-hoc Duncan's

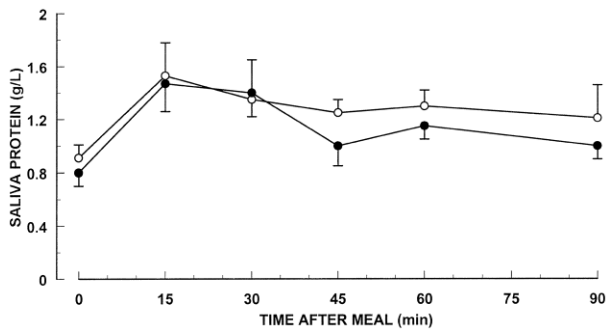


Figure 1 Salivary total protein levels in healthy subjects following a 1707 kJ test meal which was either swallowed (○) or sham-fed (●). Means \pm S.E.M., $n=12$.

multiple range test of critical ranges, where appropriate, using the Statistica statistical package (Statsoft, Tulsa, OK, USA).

Results

Postprandial metabolite responses

Postprandial plasma levels of both glucose and NEFA were significantly different following the two test meals ($P<0.01$). Both were unchanged following the sham-fed meal. Mean glucose levels rose from basal levels of 4.87 ± 0.1 mmol/l to a maximum of 5.26 ± 0.2 mmol/l at 30 min and NEFA levels fell from basal levels of 0.47 ± 0.04 mmol/l to plateau at 0.18 ± 0.02 mmol/l, 60 min following the swallowed meal.

Salivary total protein and α -amylase concentrations following the two test meals are shown in Figs 1 and 2. Salivary total protein rose following both sham-fed and swallowed meals, to reach peak levels at 30 min. There were no significant differences between the sham-fed and swallowed meals ($P=0.38$). α -Amylase activity also rose following both meals to reach peak levels at 15 min following the swallowed meal and 60 min following

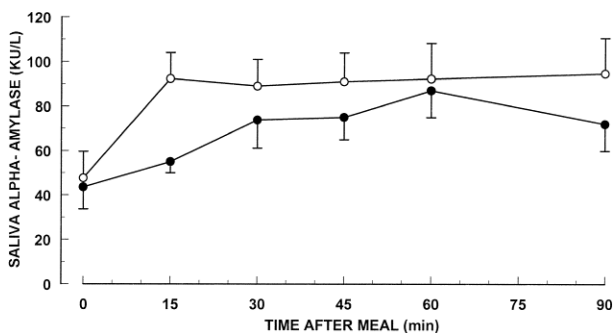


Figure 2 Salivary α -amylase levels in healthy subjects following a 1707 kJ test meal which was either swallowed (○) or sham-fed (●). Means \pm S.E.M., $n=12$.

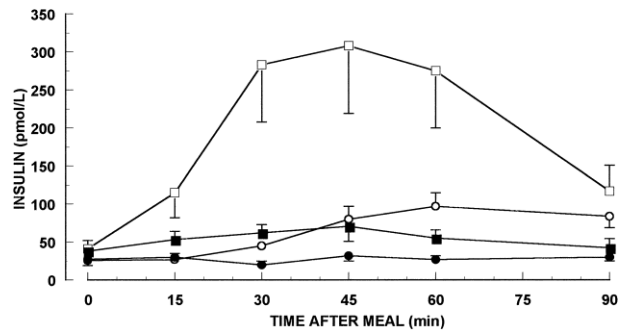


Figure 3 Salivary and plasma insulin levels in healthy subjects following a 1707 kJ test meal which was either swallowed or sham-fed. Means \pm S.E.M., $n=12$. ○=saliva, swallowed meal; ●=saliva, sham-fed meal; □=plasma, swallowed meal; ■=plasma, sham-fed meal.

the sham-fed meal. The total response was significantly higher following the swallowed than the sham-fed meal ($P<0.05$).

Postprandial hormone responses

Plasma and salivary insulin levels following the two test meals are shown in Fig. 3. Plasma levels were significantly different following the two meals ($P<0.01$), remaining unchanged following the sham-fed meal but rising significantly 15 min following the swallowed meal, to reach a peak at 45 min. Salivary insulin levels were also significantly different following the two meals ($P<0.01$), remaining unchanged following the sham-fed meal but rising more slowly than plasma levels, to reach a peak 60 min following the swallowed meal. Fasting salivary insulin concentrations were similar to those in plasma; however, the insulin response to a swallowed meal was significantly lower in saliva than in plasma ($P<0.01$).

Plasma and salivary GIP levels following the two test meals are shown in Fig. 4. Plasma levels were significantly

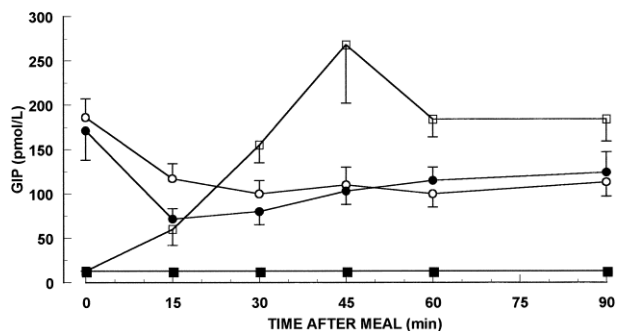


Figure 4 Salivary and plasma GIP levels in healthy subjects following a 1707 kJ test meal which was either swallowed or sham-fed. Means \pm S.E.M., $n=12$. ○=saliva, swallowed meal; ●=saliva, sham-fed meal; □=plasma, swallowed meal; ■=plasma, sham-fed meal.

different following the two meals ($P < 0.01$), remaining unchanged following the sham-fed meal but rising significantly following the swallowed meal, to reach a peak at 45 min. Fasting salivary GIP levels were significantly higher than the following plasma levels. They decreased immediately following both sham-fed and swallowed meals and remained significantly lower than fasting values for the rest of the study ($P < 0.025$).

Plasma GLP-1 levels following the two test meals followed a similar pattern to plasma GIP. Basal circulating GLP-1 levels were 8.6 ± 2.2 pmol/l, rising to mean peak values of 29 ± 4.2 pmol/l 30 min following the swallowed meal. Plasma levels were unaffected by the sham-fed meal. No GLP-1-like immunoreactivity could be detected in fasting saliva samples, or following either meal.

Discussion

Plasma levels of hormones and metabolites were measured in order to enable a direct comparison between circulating and salivary hormones concentrations, to elucidate the origin of hormones found in saliva, and to verify that none of the food in the sham-fed meal was inadvertently swallowed. Plasma insulin and glucose levels rose and plasma NEFA fell in a manner typical of data that we have previously obtained following ingestion of a mixed meal and its absorption from the gut (Elliott *et al.* 1993, Lund *et al.* 2001). The lack of any change in plasma hormone or metabolite levels following the sham-fed meal is consistent with none of the meal contents being swallowed.

In contrast to plasma hormone and metabolite profiles, which were unchanged following the sham-fed meal, but which were altered in response to the swallowed meal, salivary total protein increased following both sham-fed and swallowed meals and the responses in saliva were similar for both meals. This would indicate that the signal for salivary protein release comes from the buccal cavity. The concentration of total protein in saliva has not previously been reported in response to food, but the stimulation of the salivary flow by the presence of food in the mouth has been widely investigated (Turner & Camden 1990, Turner 1993). Saliva contains many biologically active compounds, including the enzyme α -amylase, which is secreted from serous cells within the parotid gland, a gland which contributes approximately 20% of the total unstimulated saliva volume. Although α -amylase activity rose following both meals, indicating that a signal for its release originates in the buccal cavity, the response following the sham-fed meal was significantly blunted and delayed, suggesting that a gastric, or post-absorptive stimulus occurs for secretion of α -amylase into saliva, in addition to the oral one.

Insulin-like immunoreactivity in saliva increased following the swallowed meal but remained unchanged following the sham-fed meal. Salivary insulin levels in this

study are consistent with those previously reported in the literature following a meal (Fekete *et al.* 1993). The pattern in saliva was very similar to that for plasma insulin. However, insulin levels in saliva rose more slowly and were lower than their corresponding plasma concentrations. The origin of immunoreactive insulin in saliva is of interest. Both immunoreactive insulin and insulin mRNA have been found in the salivary glands of mice (Kerr *et al.* 1995) and rats (Taouis *et al.* 1995), and immunoreactive insulin secretion from mouse salivary glands is sensitive to changes in glucose concentrations (Shubnikova *et al.* 1984). It is therefore possible that the insulin found in saliva could be the product of local synthesis. However, the correspondence between circulating and salivary insulin concentrations together with lower insulin levels in saliva makes it more likely that salivary insulin represents an ultrafiltrate from blood. The observation that salivary insulin has been shown to increase following an i.v. injection of insulin in humans, dissociated from any rise in blood glucose concentrations (Vallejo *et al.* 1984), supports this hypothesis.

No immunoreactive GLP-1 was detected in saliva. However, early reports of glucagon-like activity in salivary glands could be accounted for either by the non-specificity of the antiserum used, or by an artefact of salivary protease activity (Tahara *et al.* 1983).

To the best of our knowledge, this study is the first to report the presence of immunoreactive GIP in human saliva. GIP mRNA has only been detected within the GI tract and the salivary glands (Tseng *et al.* 1993). The GIP found in saliva must therefore originate from one of these sources. Peptide endocrine hormones are able to enter the saliva from the circulation via the ultrafiltration route, through the tight junctions between the salivary acinar cells. However, their concentration in saliva is typically several-fold lower than the corresponding concentrations in blood and saliva levels mimic blood levels (Vining *et al.* 1983). This was what was observed in our study for insulin, leading to our conclusion that insulin in saliva is probably an ultrafiltrate of blood. In contrast, however, immunoreactive GIP in saliva was some 9-fold higher than the corresponding plasma level in the fasting state and the pattern of GIP secretion in saliva following the meals bore no resemblance to the corresponding plasma profiles. It is therefore very unlikely that salivary GIP is an ultrafiltrate of blood (originating from GIP cells in the GI tract), but is instead much more likely to be a product of local synthesis. The demonstration of parallelism, good recovery and co-elution on HPLC with GIP of GI origin indicates that the molecular species in saliva identified by immunoassay is similar to the peptide found in the GI tract. The putative physiological role for GIP in saliva is of interest. A major function of GIP of GI origin is the potentiation of glucose-induced insulin secretion via the entero-insular axis (Morgan 1996), but the fall in salivary GIP following both meals mediates against GIP in saliva playing any part

in stimulating postprandial insulin secretion. However, GIP was initially isolated and characterised on the basis of its gastric acid inhibitory properties in denervated stomach pouches of dogs (Brown *et al.* 1975), a biological activity which gave rise to its original name of 'gastric inhibitory polypeptide'. Interest in this biological activity waned when it was questioned whether circulating GIP levels were sufficiently high to inhibit gastric acid secretion from the intact human stomach, but interest in the enterogastrone activity of GIP has recently been revived (Rossowski *et al.* 2002). It is possible that GIP in saliva is the product of local salivary gland synthesis. Its secretion could be stimulated either directly, by oral stimuli, or regulated by central nervous stimuli, due perhaps to the anticipation of food ingestion. GIP is a low molecular mass peptide of 4000 Da, whose activity is preserved in an acid environment. Sham-feeding has been shown to be a potent stimulator of gastric acid secretion (Feldman & Richardson 1986). The decrease in salivary GIP levels observed in this study following sham-feeding could enable gastric acid secretion to be released from an inhibitory control exerted by high levels of salivary GIP in the fasting state. However, in the absence of any additional evidence to support the descriptive findings of this study, any physiological role for salivary GIP must remain speculative. The biological activities of saliva have received comparatively little attention, and most work has concentrated on its local action within the buccal cavity. This study raises the intriguing possibility that GIP, transported in saliva rather than blood, might, in a manner analogous to its endocrine role, have biological actions at a site remote from its site of synthesis.

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