PostScript

LETTERS

Circulating ghrelin levels in patients with inflammatory bowel disease

Ghrelin, the gut-brain peptide recently identified as the natural endogenous ligand for growth hormone secretagogue receptors, exerts various endocrine and non-endocrine effects, including control of food intake and energy homeostasis.¹ It could also play a role in modulating immune responses and inflammatory processes.¹ Indeed, ghrelin exerts potent anti-inflammatory effects in vitro and in vivo,¹⁻⁵ and high circulating ghrelin levels have been found in rats with septic shock, cysteamine induced duodenal ulcers, and adjuvant induced arthritis.⁴⁻⁶ Also, we have recently demonstrated that in patients with newly diagnosed coeliac disease, circulating ghrelin levels are abnormally high, correlate positively with intestinal mucosal lesion severity, and normalise in successfully gluten free diet treated patients.⁷ However, normal or even decreased ghrelin levels have been found in other inflammatory diseases, including rheumatoid arthritis and *Helicobacter pylori* associated gastritis.^{8,9}

No data on ghrelin pattern are available in inflammatory bowel disease (IBD). Therefore, we evaluated fasting serum total ghrelin levels and the inflammatory markers, tumour necrosis factor α (TNF- α) and C reactive protein (CRP), in 96 consecutive IBD patients and 40 healthy controls (table 1).

The IBD group included 53 ulcerative colitis (UC) patients (27 with active disease, 26 in remission) and 43 Crohn's disease (CD) patients (15 with active disease, 28 in remission). Serum ghrelin was measured by radioimmunoassay, as previously described,⁷ and TNF- α by a highly sensitive immunoassay (Quantikine HS; R&D Systems, USA). Results were evaluated by one way analysis of variance, followed by the Student-Newman-Keuls multiple comparison test, univariate linear regression, and multiple regression analysis. Results are summarised in table 1.

Table 1 Clinical and biochemical data in patients with active and inactive inflammatory bowel disease (IBD) and in healthy controls

	IBD patients		Healthy controls
	Active	Remission	
No of cases	42	54	40
Sex (M/F)	28/14	30/24	20/20
Age (y)	49 (16)	48 (16)	42 (14)
Body mass index (kg/m ²)	22.1 (3.4)	24.1 (3.1)	22.1 (2.5)
C reactive protein (mg/dl)	3.5 (6.3)***	0.6 (0.4)	0.4 (0.1)
Tumour necrosis factor α (pg/ml)	1.83 (1.13)***	0.94 (0.29)	0.86 (0.39)
Ghrelin (pmol/l)	323.6 (119.2)***	217.4 (64.9)	203.0 (81.1)

Data are given as n or mean (SD).

***p<0.001 versus IBD in remission and healthy controls (one way analysis of variance followed by the Student-Newman-Keuls multiple comparison test for unequal replicates).

There were no age or sex related differences for each variable in any group. Patients with active IBD had a normal body mass index and high serum CRP and TNF- α levels (p<0.001 v controls and inactive IBD). TNF- α pattern was similar in UC and CD patients (fig 1A).

Ghrelin values were abnormally high in active IBD patients (p<0.001 v controls and inactive IBD), with no significant differences between UC and CD patients (fig 1B), and normal in IBD patients in remission. There was a significant negative correlation between ghrelin and body mass index in normal subjects (r = -0.428, p<0.006) and IBD patients in remission (r = -0.464, p = 0.0004) whereas no correlation was found in active IBD (r = -0.161, NS). However, in active IBD patients, ghrelin correlated significantly with log transformed CRP (r = 0.425, p = 0.0051) and TNF- α levels (r = 0.712, p<0.0001) (fig 1C). Multiple regression analysis identified TNF- α as the only variable significantly associated with ghrelin in active IBD (p<0.0001), and body mass index in both the inactive IBD group (p = 0.002) and healthy subjects (p = 0.02).

This study demonstrates for the first time that high ghrelin levels, correlating positively with TNF- α , are a feature of active IBD and normalise in patients in remission. TNF- α is a proinflammatory cytokine with anorectic and catabolic properties.^{2,10} A positive correlation

between ghrelin and TNF- α levels has previously been found in cachectic states.^{1,10} However, our active IBD patients had normal body mass index values, and their TNF- α pattern would be consistent with its role as mediator of intestinal inflammation.

In agreement with previously reported data,²⁻⁷ our findings in IBD could suggest a possible role for endogenous ghrelin in the control of the inflammatory process. Moreover, in IBD, some inflammatory mediators and/or products could play a role in stimulating ghrelin release from gastrointestinal or extraintestinal tissues, such as immune cells, which are activated in the disease.^{1,2}

In conclusion, endogenous ghrelin secretion increases in active IBD although the real pathophysiological significance of its upregulation and the mechanisms involved remain unclear.

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Figure 1 (A, B) Serum ghrelin and tumour necrosis factor α (TNF- α) levels in 27 patients with active ulcerative colitis (a-UC), 26 with ulcerative colitis in remission (r-UC), 15 with active Crohn's disease (a-CD), 28 with Crohn's disease in remission (r-CD), and 40 healthy controls. Data are mean (SD). (C) Correlation between serum ghrelin and TNF- α levels in patients with active inflammatory bowel disease.

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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.