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Research letter

Carbohydrate metabolism improvement after *Helicobacter pylori* eradication

1. Introduction

Several studies have found controversial relationships between *Helicobacter pylori* (*H.pylori*) infection and components of the metabolic syndrome (MetS) and hormones involved in energy homoeostasis, such as ghrelin and glucagon-like peptide-1 (GLP-1). *H. pylori* colonization and eradication have been linked to gastric ghrelin production. Nweneka et al. [1] concluded that plasma ghrelin levels were significantly lower in *H. pylori*-infected subjects *vs* uninfected subjects, with no significant effect of circulating ghrelin on *H. pylori* eradication. Discrepancies between studies may be due to differences in the populations studied, disease complications (with or without gastric atrophy), type of *H. pylori* strain, type of immunoassay and duration of the study period, and also because ghrelin is not only produced in the stomach. Other authors have described relationships between *H. pylori* infection, ghrelin and weight [2].

The aim of the present study was to assess, for the first time to our knowledge, changes in incretins and carbohydrate metabolism with an oral glucose tolerance test (OGTT) before and after antibiotic eradication treatment of patients colonized by *H. pylori*.

2. Material and methods

The present prospective case study was conducted in 32 non-diabetic patients infected by *H. pylori*. The sample size was calculated based on the average levels of ghrelin before and after eradication therapy in *H. pylori*-positive patients [3]. A minimum sample size of 15 subjects was the result of a *t* test for paired samples of the FIRST programme [α error: 0.05, mean difference: 10.63, standard deviation (SD): 10.23, 95% statistical power].

Our 32 patients, who were all positive for *H. pylori* antigen in faeces as determined by immunochromatography (Laboratorios LETI, Barcelona, Spain), were recruited through the Virgen de la Victoria Clinical University Hospital Department of Microbiology. The study was approved by the ethics committee of the Hospital and conducted in accordance with the Declaration of Helsinki. Patients were informed of the characteristics and objectives of the study before signing two copies of fully informed consent forms, thereby agreeing to participate in the study. Changes in incretins and carbohydrate metabolism were assessed by OGTT before and after successful antibiotic eradication treatment of *H. pylori* colonization. As other authors have described relationships between *H. pylori* infection, ghrelin and weight, the results were also evaluated for obese ($n = 7$) and non-obese ($n = 19$) patient subgroups.

Physical examination included body mass index (BMI), waist circumference (WC) and blood pressure (BP). A venous blood sample was taken following a minimum fasting period of 8 h. These samples were maintained at 4 °C, centrifuged, had the plasma distributed in aliquots and stored at –80 °C until needed for the laboratory analyses. Plasma glucose, insulin, glucagon-like peptide (GLP)-1 and ghrelin were measured after a 75-g OGTT, which was performed before and 2 months after antibiotic eradication treatment (omeprazole 20 mg, clarithromycin 500 mg and amoxicillin 1000 mg, every 12 h for 10 days). *H. pylori* antigen detection in faeces by immunochromatography (Laboratorios LETI) was used to confirm its eradication.

2.1. Laboratory analyses

Plasma glucose (mmol/L) was analyzed using the Modular DPD biochemical system (Roche Diagnostics, Risch-Rotkreuz, Switzerland). Glycosylated haemoglobin (HbA_{1c}, in % and mmol/mol) was measured using an immunoturbidimetric method for completely haemolyzed anticoagulated blood (COBAS INTEGRA 700 autoanalyzer, Roche Diagnostics). Plasma insulin (pmol/L) was assessed by electrochemiluminescence (E170 module, Roche Diagnostics) and C-peptide (μg/L) also by electrochemiluminescence (LIAISON, DiaSorin SpA, Saluggia, Italy). The homoeostasis model assessment (HOMA) index score was calculated by the formula: [fasting insulin (mU/mL) × fasting glucose (mmol/L)] ÷ 22.5. The patients' lipid profiles, including total cholesterol (Total-Chol, mmol/L),

Abbreviations: BP, blood pressure; BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; GLP-1, glucagon-like peptide-1; *H. pylori*, *Helicobacter pylori*; HbA_{1c}, glycosylated haemoglobin; HC, hip circumference; HDL-Chol, high-density lipoprotein cholesterol; HOMA, homoeostasis model assessment; LDL-C, hollow-density lipoprotein cholesterol; MetS, metabolic syndrome; OGTT, oral glucose tolerance test; OR, odds ratio; SE, standard error; TG, triglycerides; Total-Chol, total cholesterol; WC, waist circumference.

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Table 1
Clinical and analytical variables in patients before and after successful *Helicobacter pylori* eradication.

	Pretreatment visit ($n = 26$)	Post-treatment visit ($n = 26$)	P
Body mass index (kg/m^2)	27.1 ± 0.8	27.1 ± 0.9	0.861
Waist circumference (cm)	91.4 ± 2.1	90.7 ± 2.3	0.520
SBP (mmHg)	124.2 ± 3.0	121.8 ± 4.1	0.457
DBP (mmHg)	77.9 ± 1.6	79.9 ± 2.1	0.283
Triglycerides (mmol/L)	1.12 ± 0.09	1.06 ± 0.08	0.485
TotaL-Chol (mmol/L)	5.16 ± 0.20	5.01 ± 0.17	0.601
HDL-Chol (mmol/L)	1.22 ± 0.06	1.25 ± 0.07	0.084
LDL-Chol (mmol/L)	3.20 ± 0.18	3.09 ± 0.17	0.606
C-reactive protein (mg/L)	3.9 ± 0.4	3.7 ± 0.5	0.943
C-peptide (mcg/L)	2.4 ± 0.1	2.4 ± 0.15	0.819
HOMA index	15.2 ± 3.7	15.1 ± 3.7	0.776
HbAjc (%)	5.5 ± 0.1	5.4 ± 0.1	0.035*
$HbAi_c$ (mmol/mol)	37 ± 0.6	36 ± 0.6	
Fasting glucose (mmol/L)	5.2 ± 0.4	5.2 ± 0.5	0.444
30 post-OGTT glucose (mmol/L)	8.5 ± 2	8.2 ± 1.7	0.373
60 post-OGTT glucose (mmol/L)	8.3 ± 3	7.8 ± 3	0.062
120 post-OGTT glucose (mmol/L)	6.41 ± 0.4	5.91 ± 1.8	0.022*
Fasting insulin (pmol/L)	62.4 ± 48.1	60.3 ± 48.1	0.569
30 post-OGTT insulin (pmol/L)	331.5 ± 188.7	424.0 ± 255.4	0.115
60 post-OGTT insulin (pmol/L)	496.5 ± 346.6	479.3 ± 447.0	0.996
120 post-OGTT insulin (pmol/L)	436.2 ± 290.6	354.5 ± 306.4	0.267
Fasting GLP-1 (ng/mL)	4.9 ± 4.4	4.6 ± 4.1	0.752
30 post-OGTT GLP-1 (ng/mL)	5.6 ± 5.1	5.1 ± 4.3	0.440
60 post-OGTT GLP-1 (ng/mL)	5.2 ± 4.8	4.9 ± 4.5	0.619
120 post-OGTT GLP-1 (ng/mL)	5.0 ± 4.5	4.6 ± 4.3	0.559
Fasting ghrelin (pg/mL)	15.7 ± 15.1	11.6 ± 10.8	0.224
30 post-OGTT ghrelin (pg/mL)	6.5 ± 5.9	6.3 ± 6.8	0.795
60 post-OGTT ghrelin (pg/mL)	6.5 ± 5.6	6.5 ± 5.4	0.826
120 post-OGTT ghrelin (pg/mL)	13.7 ± 9.7	9.3 ± 9.8	0.073

Data are expressed as means ± standard error (SE) and as *n* (%).
* $P < 0.05$ (by Wilcoxon's test); SBP/DBP: systolic/diastolic blood pressure; Total-Chol: total cholesterol; HDL-Chol: high-density lipoprotein cholesterol; LDL-Chol: low-density lipoprotein cholesterol; HOMA: homoeostasis model assessment; HbA_{1c}: glycosylated haemoglobin; 30/60/120: min after OGTT; OGTT: oral glucose tolerance test.

triglycerides (TG, mmol/L), low-density lipoprotein cholesterol (LDL-Chol, mmol/L) and high-density lipoprotein cholesterol (HDL-Chol, mmol/L), were quantified by enzymatic colorimetry using the Modular DPD biochemical autoanalyzer (Roche Diagnostics). Plasma GLP-1 and ghrelin levels were measured manually using commercial kits (human GLP-1 EIA Kit, GENTAUR Belgium, Kampenhout, Belgium, and Human Ghrelin Fluorescent EIA Kit, Phoenix Pharmaceuticals, Burlingame, CA, USA, respectively) and expressed in ng/mL and pg/mL, respectively.

2.2. Statistical analyses

Descriptive statistics included means ± standard error (SE), ranges for quantitative variables and percentages for qualitative variables. The magnitude of association was calculated using odds ratios (ORs) and Cornfield's approximation with a 95% confidence interval (95% CI). Information from each visit was compared by Student's *t* test. Differences in the frequency distribution of qualitative variables between groups were assessed by Fisher's test. Non-parametric variables were evaluated by Mann–Whitney and Wilcoxon tests. Correlation between variables was evaluated using Pearson's correlation. A level of $P < 0.05$ was considered statistically significant.

3. Results

Thirty-two patients were studied. Their average age was 49 ± 2.03 years; 75% were women and 46.9% had a personal history of gastrointestinal disease (15.6% gastroesophageal reflux, 12.5% peptic ulcer, 3.1% irritable bowel). Of these patients, 81.3% ($n = 26$) achieved eradication of *H. pylori*. Clinical and analytical variables are summarized in Table 1. Significant correlations were found between HbA_{1c} and levels of GLP-1 post-treatment at all time points after OGTT (Fig. 1). Levels of ghrelin correlated, with close to statistical significance, with differences at 120-min post-OGTT in glucose at baseline ($r = –0.366$, $P = 0.060$) and in HbA_{1c} ($r = –0.353$, $P = 0.071$) after antibiotic treatment. There was also a significant positive correlation between fasting ghrelin before antibiotic treatment and HbA_{1c} ($r = 0.414$, $P = 0.032$).

The prevalence of obesity (defined as BMI ≥ 30 kg/m^2) at baseline was 21.9%. No significant differences were found in obese patients either before or after treatment, whereas non-obese patients showed significant decreases in levels of 120-min post-OGTT glucose (6.24 ± 0.44 mmol/L *vs* 5.53 ± 0.43 mmol/L, $P = 0.032$) and HbA_{1c} (5.5 ± 0.1% *vs* 5.3 ± 0.07%, $P = 0.038$) after successful *H. pylori* eradication. On comparing the results before and after eradication