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ARTICLE

Effects of 12 weeks' treatment with a proton pump inhibitor on insulin secretion, glucose metabolism and markers of cardiovascular risk in patients with type 2 diabetes: a randomised double-blind prospective placebo-controlled study

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Abstract
Aims/hypothesis Recent studies suggest that proton pump inhibitor treatment may increase insulin secretion and improve glucose metabolism in type 2 diabetes. In a randomised double-blind prospective placebo-controlled 2 × 2 factorial study, we examined the effect of esomeprazole on insulin secretion, HbA_{1c} and cardiovascular risk factors in type 2 diabetes.
Methods Forty-one patients with type 2 diabetes using dietary control or oral glucose-lowering treatment were randomised to receive add-on esomeprazole 40 mg (n=20) or placebo (n=21) for 12 weeks. Randomisation was carried out prior to inclusion on the basis of a computer-generated random-number list. The allocation sequence was concealed in sealed envelopes from the researcher enrolling and assessing participants. The study was undertaken at Steno Diabetes Center, Gentofte, Denmark. The primary outcome was change in AUC for insulin levels during a meal test. Secondary outcomes were the levels of HbA_{1c} and biochemical markers of cardiovascular risk, including lipids, coagulation factors, inflammation markers, markers of endothelial function and 24 h ambulatory BP measurements.
Results Forty-one participants were analysed. In the esomeprazole-treated group the AUC for insulin did not change (before vs after treatment: 28,049±17,659 vs 27,270±32,004 pmol/l × min (p=0.838). In the placebo group AUC for insulin decreased from 27,392±14,348 pmol/l × min to 22,938±11,936 pmol/l × min (p=0.002). Esomeprazole treatment (n=20) caused a ninefold increase in the AUC for gastrin. HbA_{1c} increased from 7.0±0.6% (53±5 mmol/mol) to 7.3±0.8% (56±6 mmol/mol) in the esomeprazole-treated group and from 7.0±0.6% (53±5 mmol/mol) to 7.4±0.8% (57±6 mmol/mol) in the placebo group (n=21) (p for difference in change >0.05). Except for BP, there were no differences between the groups in the markers of cardiovascular risk (p>0.05). Monitoring of 24 h ambulatory BP showed a significant decrease in daytime systolic BP, daytime diastolic BP and 24 h diastolic BP in the placebo group (p<0.05). No change in BP was seen in the patients treated with esomeprazole.
Conclusions/interpretation Treatment with esomeprazole over 12 weeks did not improve insulin secretion, glycaemic control or cardiovascular disease biomarkers in patients with type 2 diabetes.

Trial registration ClinicalTrials.gov NCT00699426
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Keywords Cardiovascular risk · Diabetes mellitus · Drug therapy · Glucose metabolism · Hypoglycaemic agent · Insulin secretion · Proton pump inhibitors

Abbreviations

Ag	Antigen
CVD	Cardiovascular disease
hs-CRP	High-sensitivity C-reactive protein
PAI-1	Plasminogen activator inhibitor-1
PPI	Proton pump inhibitor
t-PA	Tissue-type plasminogen activator
vWF	von Willebrand factor

Introduction

Gastrin is a hormone that regulates gastric acid secretion and growth of the gastric mucosal cells. Gastrin is released from antro-duodenal G cells in response to a meal and is itself regulated by the acidity of gastric juice; a low pH inhibits gastrin release [1, 2]. Studies in the regenerating pancreas of duct-ligated rats revealed that gastrin stimulates beta cell neogenesis and expansion of beta cell mass from transdifferentiated, but not normal, exocrine pancreas tissue [3, 4]. Similarly, studies on rodents (*Psammomys obesus*) have shown that gastrin treatment stimulates insulin secretion, thereby reducing the blood glucose levels and increasing beta cell mass [5]. Moreover, studies in man have shown that moderate hypergastrinaemia (exogenous as well as endogenous gastrin source) also increases insulin secretion [6–8].

A recent retrospective electronic medical record examination indicated that patients with type 2 diabetes taking a proton pump inhibitor (PPI) (omeprazole, esomeprazole, pantoprazole, rabeprazole or lansoprazole) had reduced HbA_{1c} concentrations compared with a group of patients not taking a PPI [9]. This finding was supported by a retrospective data analysis from our group that showed improved glycaemic control in patients with type 2 diabetes treated with the PPI esomeprazole for approximately 12 months. We found a 0.7% point lower HbA_{1c} in the esomeprazole-treated group compared with the control group. Patients with an HbA_{1c} above 9% had a 1.2% points lower HbA_{1c} level, suggesting an even stronger effect in patients with poor glycaemic control [10]. Recently, two other studies have confirmed an association between HbA_{1c} and PPI treatment [11, 12].

On this background, we performed a randomised placebo-controlled study to prospectively examine the effect of esomeprazole treatment on insulin secretion, HbA_{1c} and risk factors for cardiovascular disease (CVD) in patients with type 2 diabetes. Our central hypothesis was that esomeprazole increases the secretion of gastrin, which then stimulates insulin secretion and reduces the concentration of HbA_{1c}. Furthermore, the potential beneficial effects of esomeprazole may include a range of non-glycaemic risk factors for CVD.

Methods

We conducted a randomised double-blind prospective placebo-controlled 2×2 factorial design study in 41 patients with type 2 diabetes. Twenty patients were randomised to receive esomeprazole and 21 to placebo. In a separate randomisation, the patients received 300 ml Cardi04 yogurt or 300 ml placebo yogurt. The primary aim of the Cardi04 yogurt part of the factorial study design, which was independent of the scope for the esomeprazole treatment, was to investigate the extent to which yogurt intervention might influence intestinal flora and, as a result, reduce BP and/or associated dysmetabolic traits, including insulin resistance, glycaemic control and plasma lipid levels. The combined design was chosen strictly for feasibility reasons, and not because of any anticipated interaction. In Fig. 1, a flow diagram illustrates the inclusion process. The study started in June 2008 and the physical examinations were finished in June 2009.

Esomeprazole was given at a dosage of 40 mg/day for 12 weeks. Both the esomeprazole capsule and the yogurt drink were taken in the morning before breakfast. The study medication was added to the patients' ongoing medication. The type and dose of prior antihypertensive or diabetic treatment remained unchanged throughout the study (Table 1). Drug compliance was assessed by tablet counts. Randomisation was carried out prior to inclusion on the basis of a computer-generated random-number list and concealed in envelopes from the researcher enrolling and assessing

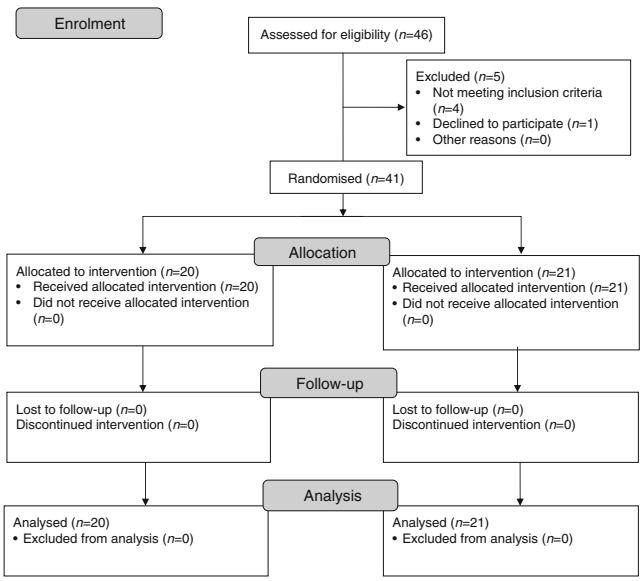

Fig. 1 Patient flow diagram

Table 1 Patient characteristics at baseline

	Esomeprazole			Placebo		
	Yogurt (n=10)	Placebo (n=10)	p value	Yogurt (n=13)	Placebo (n=8)	p value
Age (years)	60.5 (8.4)	61.4 (5.0)	0.776	56.9 (7.0)	59.5 (5.7)	0.390
BMI (kg/m^2)	29.4 (3.9)	27.1 (0.7)	0.178	29.0 (3.8)	28.5 (3.2)	0.774
HbA_{1c} (%)	6.7 (0.3)	7.2 (0.7)	0.209	6.9 (0.5)	7.4 (0.6)	0.048*
HbA_{1c} (mmol/mol)	50 (2)	55 (5)	0.209	52 (4)	57 (5)	0.048*
SBP (mmHg)	129.7 (11.8)	139.1 (14.9)	0.142	136.1 (17.1)	132.3 (9.3)	0.569
DBP (mmHg)	76.0 (6.7)	75.4 (3.7)	0.830	75.2 (9.3)	77.5 (10.7)	0.602
Diabetes duration (years)	7.5 (5.4)	8.4 (3.9)	0.673	5.6 (3.1)	10.5 (6.8)	0.035
Macrovascular complications (n)	1	0		1	2	
Microvascular complications (n)	3	3		8	5	
Hypertension (n)	7	9		13	4	
Diet only (n)	2	0		2	0	
Oral glucose-lowering medication (n)	8	10		11	8	
SU only	0	1		0	1	
Metformin only	4	1		4	1	
SU + metformin	4	8		7	6	
BP medication (n)	6	9		12	4	
One BP medication	2	2		5	1	
Two BP medications	3	3		5	0	
Three BP medications	1	3		2	1	
Four BP medications	0	1		0	2	

Data are means (SD)

* p<0.05 vs placebo

SU, sulfonylurea

participants. The code was not broken until the study was completed.

Patients with type 2 diabetes, diabetes duration of more than 1 year, HbA_{1c} level between 6.0 and 10.0% (42 and 46 mmol/mol) and age between 40 and 70 years who were using oral glucose-lowering medication only (metformin, sulfonylurea or diet) were invited by letter to participate in the study, provided that they were not currently taking a PPI. Patients were recruited through the electronic medical record database of Steno Diabetes Center, Denmark. Both men and women were invited to take part in the study, but only men responded to the invitation. A total of 300 invitation letters were sent out. Exclusion criteria were serious organic or metabolic diseases including malignant disease, liver or severe kidney disease (serum creatinine level above the normal range or macroalbuminuria) or severe heart failure (New York Heart Association class 3 or above), fasting C-peptide concentrations <0.3 pmol/l, alcohol or drug addiction, potential medication interaction with the study medication, severe neuropathy (symptoms + vibration perception threshold >50 mV measured using a biotensiometer), anaemia, treatment with insulin, neutropenia, treatment with warfarin or other coumarin derivates or with medication for gastric diseases.

A total of 46 persons responded to the invitation letter, and we enrolled all 46 respondents in the study. One dropped out for personal reasons, one dropped out because of a cancer diagnosis and three persons were excluded after screening: one because of alcohol abuse, one with anaemia and one with elevated liver enzymes. Thus, 41 patients participated in and completed the study (Fig. 1). The patients were not allowed to participate in other clinical intervention studies during participation in the current trial. All patients were instructed to maintain their usual lifestyle with regard to food and exercise habits.

The patients had to be at Steno Diabetes Center on four occasions during the 3 month study period: (1) the inclusion day; (2) the baseline test and randomisation day; (3) after 6 weeks for clinical assessment and compliance check; and (4) after 12 weeks for the final examination and outcome measures.

Ethics

The study protocol was approved by the Central Ethics Committee, the Danish Medicine Agency and the Danish

Data Protection Agency and was conducted in accordance with the guidelines of the Declaration of Helsinki. Written and oral informed consent was obtained from all patients enrolled in this study. The study was registered at ClinicalTrials.gov (registration no. NCT00699426).

Study procedures

Meal test After an overnight fast the examination began between 07:00 and 08:00 hours. Blood samples were collected from an intravenous cannula inserted into an antecubital vein. A meal test was performed to assess insulin secretion. The 2,200 kJ meal consisted of 15% protein, 55% carbohydrate and 30% fat. The ingredients of the meal test were 50 g rye bread, a 50 g wholemeal bun, 10 g butter, a choice of either 40 g of 20% cheese or 40 g of salty meat, 125 g apple, 20 g marmalade and 30 g milk (0.5% fat). The meal had to be consumed within 10 min. Blood samples were drawn 10 min before and 0, 15, 30, 60, 90, 120 and 180 min after meal intake.

On the day before the last meal test the patients were taking the study medication twice a day and then with the meal on the day of the meal test. This was done to maximise the potential benefit of the study medication.

Blood samples Blood samples were taken for measurement of the plasma concentrations of gastrin, insulin, proinsulin, C-peptide, glucose, lipids, high-sensitivity C-reactive protein (hs-CRP), plasminogen activator inhibitor-1 (PAI-1) antigen (Ag), tissue-type plasminogen activator (t-PA) Ag, TNF-α and von Willebrand factor (vWF) Ag. For further details, please refer to the electronic supplementary material (ESM) Methods.

BP measurements BP was measured using an electronic BP device (UA-779; A&D Instruments, Abingdon, UK) after at least 10 min of rest in the supine position on the test day.

Ambulatory BP (ABP) over 24 h was measured by the Takeda TM2421 oscillometric device (A&D Medical, Tokyo, Japan). The device was set to obtain BP readings at 15 min intervals during daytime from 07:00 hours to 23:00 hours and at 30 min intervals during the night from 23:00 hours to 07:00 hours. The patients were instructed to continue with their usual daily activities but to remain still at measurement times. The recordings were analysed to obtain 24 h average systolic BP (SBP), diastolic BP (DBP) and heart rates as well as average daytime and night-time SBP, DBP and heart rates. Values were averaged for each hour before calculating day, night and 24 h BP values. The meal test, including the 24 h ABP measurement, was repeated after 12 weeks of intervention.

The patients were clinically assessed (weight, waist and hip circumference, clinic BP, glucose, HbA_{1c}) at baseline, after 6 weeks of treatment and at the end of the study.

Biochemical analyses Plasma gastrin concentrations were measured using a radioimmunoassay with a high-affinity antibody with precisely defined epitope specificity that bound all bioactive gastrins with equimolar potency [13, 14]. Plasma glucose concentrations were determined by the hexokinase glucose 6-phosphate dehydrogenase enzymatic assay method using an automated analyser (model no. 912; Roche Hitachi, Basel, Switzerland). Plasma insulin concentrations were measured using solid-phase two-site fluoroimmunometric assays (AutoDELFIA Insulin kit, Wallac, Turku, Finland). HbA_{1c} measurements were obtained using ion-exchange HPLC (HLC-723G7; Tosoh, Tokyo, Japan) for routine analysis. Plasma proinsulin and C-peptide concentrations were measured using the AutoDELFIA C-peptide kit (Wallac). The assay was a solid-phase two-site fluoroimmunometric test based on the direct sandwich technique, in which two monoclonal antibodies are directed against separate antigenic determinants on the C-peptide molecule.

Statistical analysis

Continuous variables were compared using unpaired and paired Student's *t* tests, as appropriate. The effects of esomeprazole treatment were tested for potential interactions with the yogurt treatment using linear regression analysis. All analyses were performed with and without adjustment for the yogurt intervention. Sample size calculation (assuming a 25% difference in the insulin secretion during meal tests between the groups, with a CV 0.286 and an SD for AUC insulin=20 pmol/l×time) showed that a total sample size of 34 was necessary to reach statistical significance with a statistical power of 80%. The primary outcome was a priori decided to be a change in the AUC of plasma insulin levels during meal tests. From a previous study of the effect of repaglinide vs metformin treatment in patients with type 2 diabetes, we knew that an increase in insulin secretion of approximately 25% would be sufficient to cause a clinically meaningful reduction in the blood glucose AUC during a meal test [15].

Furthermore, in a previous study in an animal model of type 2 diabetes (*Psammomys obesus*), the animals treated with lansoprazole, compared with vehicle, displayed a 2.3-fold increase in the intensity of insulin staining in the beta cells ($p=0.0002$) and a 50% higher beta cell mass ($p=0.04$) [5]. Thus, we believe that our power estimates are

Table 2 Clinical characteristics before and after esomeprazole or placebo treatment

	Esomeprazole (n=20)		Placebo (n=21)		$p_{unadjusted}$[a]	$p_{adjusted}$[b]
	Before	After	Before	After		
Age (years)	61±6.8	–	57.9±6.5	–	–	–
Height (cm)	177±7.1	–	176.9±6.8	–	–	–
Weight (kg)	88.9±16.8	89.4±17.2	90.4±13.9	90±13.1	0.129	0.126
BMI (kg/m^2)	28.2±3.8	28.4±3.9	28.8±3.5	28.7±3.3	0.185	0.187
Waist circumference (cm)	100.6±12.6	100.9±13.7	101.1±9.2	101.6±8.7	0.902	0.928
Hip circumference (cm)	103.1±9.7	103±10	103.5±6.6	103±7	0.258	0.188
HbA_{1c} (%)	7.0±0.6	7.3±0.8*	7.0±0.6	7.4±0.8*	0.297	0.276
HbA_{1c} (mmol/mol)	53±5	56±6	53±5	57±6	0.297	0.276
HOMA-IR	2.5 (1.4, 4.3)	2.4 (1.8, 4.3)	3.4 (1.7, 4.7)	3.3 (1.9, 5.5)	0.528	0.164
Plasma glucose (mmol/l)	7.7 (7.1, 9.7)	8.3 (7.5, 10.3)*	8.8 (7.8, 10)	8.6 (7.8, 11.5)	0.863	0.872
C-peptide (pmol/l)	867 (660, 1288)	810 (701, 1385)	942 (556, 1169)	932 (589, 1140)	0.714	0.530
Insulin (pmol/l)	49.5 (30, 73)	42.5 (35.8, 80.5)	43.5 (30, 81)	46 (32.5, 75)	0.523	0.197
Proinsulin (pmol/l)	29.5 (14.5, 36)	30.3 (20.8, 46.5)	25 (19, 39)	25 (19.5, 44)*	0.351	0.082
Gastrin (pmol/l)	7.3 (5.5, 8.5)	34.3 (21.3, 71)*	8.5 (6.5, 15.5)	11 (7, 15)	<0.0001	<0.0001
Total cholesterol (mmol/l)	3.9±0.95	4.3±1.1*	3.5±0.6	3.7±0.9*	0.358	0.488
HDL-cholesterol (mmol/l)	1.1±0.3	1.1±0.3	1.2±0.3	1.2±0.3	0.993	0.737
LDL-cholesterol (mmol/l)	2.0±0.8	2.2±0.8*	1.5±0.6	1.8±0.7	0.438	0.547
Triacylglycerols (mmol/l)	1.4 (1.2, 1.8)	1.9 (1.0, 2.4)	1.4 (0.9, 1.9)	1.3 (1.1, 2.5)	0.648	0.706
hs-CRP (mg/l)	1.3 (0.6, 2.7)	1.6 (0.6, 2.6)	0.9 (0.6, 2.1)	0.9 (0.5, 2.0)	0.908	0.560
PAI-1 Ag (ng/ml)	32±14	34.1±18.3	35.8±15.7	35.0±14.5	0.503	0.444
TNF-α (pg/ml)	1.1 (0.9, 1.6)	1.3 (1.0, 1.5)	1.1 (0.8, 1.2)	1.2 (0.9, 1.3)	0.910	0.551
t-PA Ag (ng/ml)	13.0±4.0	13.6±4.5	13.1±4.1	13.9±3.4	0.770	0.647
vWF Ag (%)	128.5±38.3	142.8±42.8*	122.2±29.4	128.3±37.2	0.183	0.117

Data are means ± SD or medians (interquartile range)

* p<0.05 vs before value in the same group (unadjusted)

[a] p for difference in change between esomeprazole- and placebo-treated groups (unadjusted)

[b] p for difference in change between esomeprazole- and placebo-treated groups (adjusted for yogurt treatment, age and baseline value)

meaningful and justified from a clinical perspective, both for the expected impact of PPI on insulin secretion and for a clinically meaningful reduction of plasma glucose levels.

A p value <0.05 was considered to be statistically significant. Statistical analysis was performed using the Statistical Analysis System (SAS 9.2; SAS Institute, Cary, NC, USA).

Results

Treatment with esomeprazole was generally well tolerated. Only three patients reported minor adverse events; two of these reported flatulence and one patient reported diarrhoea. Two patients reported flatulence and one patient reported intermittent diarrhoea in the placebo group also.

Table 1 shows the patient characteristics at baseline for the subgroups.

The esomeprazole and placebo groups were comparable with regards to age, BMI, HbA_{1c}, BP and diabetes duration. Thirteen patients in the placebo group presented with microvascular complications compared with six in the esomeprazole group. The two groups had almost the same distribution of glucose-lowering treatment and BP medication.

In the subgroups receiving esomeprazole treatment, those receiving placebo–yogurt tended to have a higher SBP, longer diabetes duration and a higher HbA_{1c}. This is also reflected in more microvascular complications and more use of glucose- and BP-lowering medications. In the subgroups of placebo–esomeprazole, those receiving placebo–yogurt had a higher HbA_{1c} and longer diabetes duration. The yogurt subgroup had higher SBP, higher intakes of BP- and glucose-lowering medication and more microvascular complications.

There were significant interactions between esomeprazole and yogurt treatment on the following variables: Δweight (p=0.029), ΔBMI (p=0.038), Δheart rate (p=0.006), ΔvWF (p=0.043), ΔHbA_{1c} (p=0.036), Δdaytime

SBP (p=0.014), Δnight-time SBP (p=0.019), and Δ24 h SBP (p=0.006). Adjustment for yogurt treatment, however, did not change any of the results (Table 2).

Within the esomeprazole-treated group, those receiving yogurt experienced a weight gain of 2.0 kg (95% CI 0.32, 3.77; p=0.027) and BMI increased by 0.6 kg/m^2 (95% CI 0.0, 1.2, p=0.046) compared with those receiving placebo–yogurt in whom there was no effect on body weight (p=0.330) or BMI (p=0.288).

Changes in glycaemic control

The clinical characteristics of the study population before and after treatment are reported in Table 2. As shown in Table 2, there was a significant increase in the HbA_{1c} concentration after esomeprazole treatment of 0.3% points (p=0.002) and after placebo treatment of 0.4% points (p=0.031), with no significant difference in change between the two groups (p=0.276). After 6 weeks, HbA_{1c} was 7.06±0.7 (p=0.275) in the esomeprazole-treated group and 7.07±0.8 (p=0.529) in the placebo group, with no significant difference in change between the two groups. However, there was an interaction between esomeprazole treatment and yogurt treatment on 12 week change in HbA_{1c}. In those receiving placebo–yogurt the HbA_{1c} increased by 0.5% (95% CI 0.15, 0.80; p=0.010) in the esomeprazole group compared with the controls. Plasma glucose increased by 0.6 mmol/l in the esomeprazole group (p=0.054), but not in the placebo group. There was no significant change in the AUCs for glucose (Fig. 2a) or proinsulin (Fig. 2b) after treatment with esomeprazole or placebo. The AUC for C-peptide decreased significantly after both esomeprazole treatment and placebo (Fig. 2c). The AUC for insulin decreased significantly only in the placebo group (Fig. 2d). In contrast, the AUC for gastrin increased more than ninefold in the group receiving esomeprazole treatment (p<0.001) compared with the placebo group, for whom the gastrin concentration did not change over time (p>0.453) (Fig. 2e). There was no significant difference in HOMA-insulin resistance (HOMA-IR) between the two treatment groups (Table 2).

Changes in lipid profiles

The plasma cholesterol concentration increased after esomeprazole treatment (p=0.001) and after placebo (p=0.041), with no difference in change between the groups (Table 2). LDL-cholesterol only increased significantly in the esomeprazole-treated group. There was no significant difference in HDL-cholesterol or triacylglycerols between the two treatment groups (Table 2).

Changes in inflammation and antithrombosis

After esomeprazole treatment, vWF had increased by 11% (p=0.009), but because of a slight (non-significant) increase in vWF in the placebo group during the intervention, the

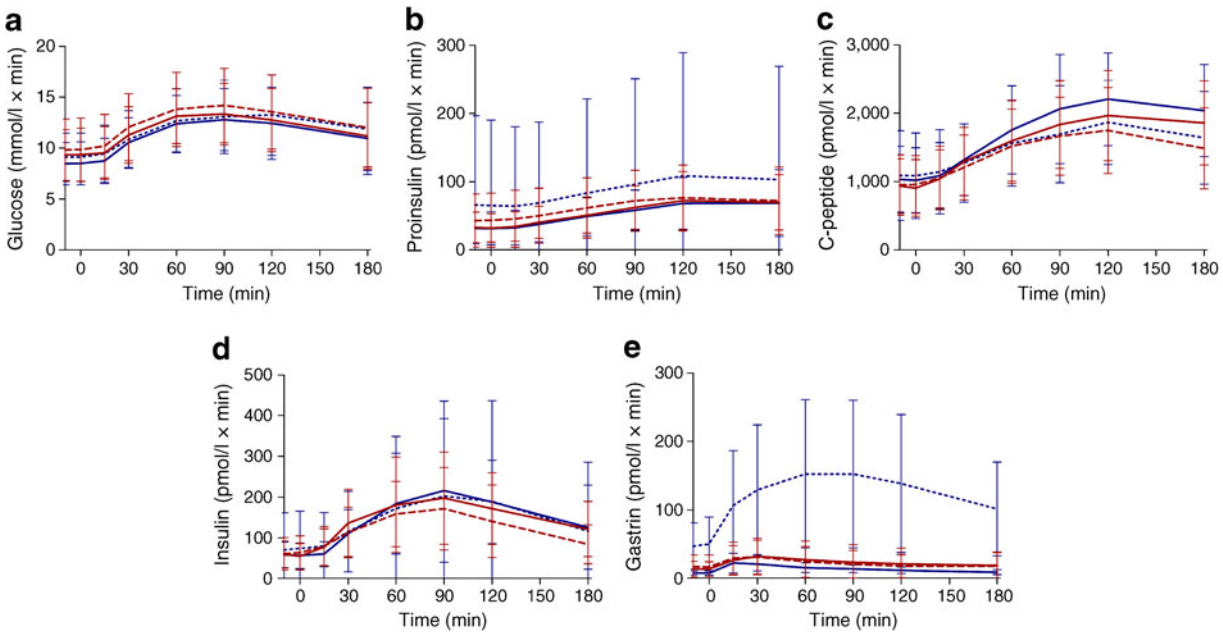

Fig. 2 Plasma glucose (**a**), plasma proinsulin (**b**), plasma C-peptide (**c**), plasma insulin (**d**) and plasma gastrin (**e**) over 0–180 min during the meal test before esomeprazole treatment (n=20) (solid dark blue), after esomeprazole treatment (n=20) (dotted dark blue), before placebo treatment (n=21) (solid dark red) and after placebo treatment (dashed dark red). Data are means ± SD; p<0.05 for difference within groups in C-peptide AUC before and after treatment (**c**), for difference in insulin AUC before and after placebo treatment (**d**) and for gastrin AUC before and after esomeprazole treatment (**e**)

Table 3 ABP monitoring variables before and after treatment

	Esomeprazole (n=20)		Placebo (n=21)		$p_{unadjusted}$[a]	$p_{adjusted}$[b]
	Before	After	Before	After		
Daytime SBP (mmHg)	142±18	149±15	145±16	138±16*	0.015	0.010
Daytime DBP (mmHg)	70±6	72±6	74±7	71±9 *	0.009	0.018
Daytime heart rate (bpm)	77±8	77±10	77±10	77±10	0.521	0.373
Night-time SBP (mmHg)	123±19	124±20	126±24	124±15	0.714	0.737
Night-time DBP (mmHg)	62±7	61±7	63±8	63±7	0.528	0.315
Night-time heart rate (bpm)	69±11	71±9	71±12	70±8	0.316	0.351
24 h SBP (mmHg)	136±18	140±15	139±18	132±14	0.123	0.142
24 h DBP (mmHg)	67±5	68±6	70±6	67±6*	0.159	0.331
24 h heart rate (bpm)	74±9	75±9	75±10	74±8	0.285	0.279

Data are means ± SD

*p<0.05 vs before value in the same group (unadjusted)

[a]p value for difference in change between esomeprazole- and placebo-treated groups (unadjusted)

[b]p value for difference in change between esomeprazole- and placebo-treated groups (adjusted for yogurt treatment, age and baseline value)

difference in change between the two groups was not significant (p=0.117) (Table 2). However, there was interaction between esomeprazole and yogurt on the change in vWF. In those receiving placebo–yogurt, vWF increased by 20.5% (95% CI 5.9, 35.1; p=0.012) in the esomeprazole group compared with controls (Table 2). In those receiving yogurt there was no effect of esomeprazole on vWF (p=0.800) (Table 2).

Changes in 24 h ambulatory BP

In general, esomeprazole treatment did not affect BP (Table 3). However, in the placebo group there was a significant decrease in daytime SBP, daytime DBP and 24 h DBP and a non-significant decrease in 24 h SBP (Table 3). These findings resulted in significantly different changes in daytime SBP and DBP in the two groups (p for difference in Δ<0.05).

However, there was interaction between esomeprazole and yogurt on change in daytime SBP, night-time SBP and 24 h SBP. In those receiving yogurt, the daytime SBP and 24 h SBP increased by 17.4 mmHg (95% CI 6.1, 28.7; p=0.0006) and 13.5 mmHg (95% CI 3.3, 23.7; p=0.0016), respectively. In those receiving placebo–yogurt there was no change in the esomeprazole group compared with controls.

There was no significant change in daytime heart rate, night-time heart rate or 24 h heart rate (Table 2). However, there was interaction between esomeprazole and yogurt on the change in heart rate. In those receiving placebo–yogurt the heart rate decreased by 4.7 bpm (95% CI −8.8, −0.6; p=0.034) in the esomeprazole group compared with the controls. In those receiving yogurt there was no effect of esomeprazole on HbA_{1c} (p=0.061).

Discussion

In this placebo-controlled intervention study, we showed that there was no overall effect of esomeprazole treatment on insulin secretion, HbA_{1c} and biochemical CVD markers. However, patients treated with esomeprazole did not experience the same beneficial decrease in BP as those in the placebo group. Previous studies have indicated that treatment with a PPI enhances gastrin secretion, which increases insulin secretion and thereby causes a reduction in HbA_{1c} [5]. Our results, however, showed that HbA_{1c} increased in both the esomeprazole-treated group and in the placebo-treated group (Table 2).

As expected, the gastrin concentrations increased significantly in the esomeprazole-treated group; however, gastrin did not increase the secretion of insulin. This is in contrast to previous animal and retrospective studies and to human studies using exogenous gastrin in modest doses [6] or endogenous hypergastrinaemia correlating with insulin secretion [7, 8]. In one animal study [5], rodents were fed a high-energy diet until they reached a morning blood glucose level >10 mmol/l. They subsequently received the PPI lansoprazole in doses up to 15 mg/kg for 17 days [5]. The study showed that treatment with lansoprazole resulted in up to a ninefold dose-dependent increase in endogenous gastrin concentration, a significant reduction in morning blood glucose and a 2.3-fold increase in the intensity of insulin staining in beta cells. The different outcome of the animal study and the present intervention study in humans may have several explanations. First, the lansoprazole doses used in animals are relatively high compared with doses used in humans. In comparison, the maximal human dose is typically 80 mg for an average person of 70 kg. Extending the doses from those used in the animal study, a person of 70 kg should receive up to 1,050 mg lansoprazole per day. Second, the blood glucose concentrations of the rodents were considerably higher compared with the patients investigated in this study. The rodents all had blood glucose levels >10 mmol/l whereas our patient population had a fairly mild blood glucose elevation with an HbA_{1c} of 7% (53 mmol/mol). However, even with our dose of esomeprazole of

40 mg, the increment in gastrin secretion was comparable with the increment observed in the animal study. This may indicate that even a higher dose of esomeprazole would probably not have influenced the insulin secretion.

Previously, we and others have shown that type 2 diabetic patients taking a PPI had lower levels of HbA_{1c} compared with patients not taking a PPI [9–12]. In particular, patients taking metformin or sulfonylurea or patients with poor glycaemic control had the largest difference in HbA_{1c} compared with patients using insulin or thiazolidinedione treatment [9, 10].

Using state-of-the-art methods we could not confirm these findings. One possible confounder in the retrospective analyses could be that patients with dyspepsia changed or modified their diet while using esomeprazole: the relief of gastrointestinal symptoms could have made them eat differently. Moreover, the effect of esomeprazole on the secretion of other gastrointestinal hormones is unknown. Several of these hormones (i.e. other than gastrin) may influence islet cell secretion of insulin, glucagon and somatostatin.

Our patients had well-regulated blood glucose concentrations and relatively well-preserved beta cell function. There were no differences in baseline HbA_{1c} between the esomeprazole group and the placebo group. It cannot, however, be excluded that a greater effect would have been seen in a group with worse blood glucose regulation or with a poorer beta cell function. We chose a study period of 12 weeks; a longer study period or a higher dose of esomeprazole would probably not make any difference as we had a significant increase in gastrin within our study period, but it did not, however, cause an increase in insulin secretion. With respect to duration of the PPI intervention, it would, of course, have been even more convincing if the study duration had been longer than 12 weeks. However, from previous experience with virtually all known glucose-lowering agents, we consider that 12 weeks is a reasonable time period to study changes in, for example, HbA_{1c} level, plasma lipids and BP. Also, even though only patients with relatively mild diabetes were examined in this study, we sincerely consider that at least some minor effect of the esomeprazole treatment would have occurred if there was any biological or clinical relevance. As for the previous report of a disproportionately larger effect of PPI treatment on HbA_{1c} levels in patients with the highest HbA_{1c} levels, it must be kept in mind that these data, because of the retrospective design, were only hypothesis generating, and that the higher effect in those with the highest plasma glucose levels could represent the statistical phenomenon of regression towards the mean. Most study participants were elderly men, but these were the individuals who volunteered for the study, and we are unaware of any data indicating sex-specific effects of PPI treatment on metabolic outcome variables. In the placebo group there was a significant decrease in daytime SBP, daytime DBP and 24 h DBP. In the esomeprazole-treated group, the daytime SBP and 24 h DBP tended to increase. The mechanism behind this increase is unclear as treatment was unchanged; further studies are warranted to clarify this possible effect of esomeprazole on BP.

Recent nationwide cohort studies based on linked administrative registry data, however, have shown that PPI therapy is associated with an increased risk of cardiovascular events (cardiovascular death or rehospitalisation for myocardial infarction or stroke) both in patients treated with a PPI alone and in aspirin-treated patients with a first-time myocardial infarction [16, 17]. The authors explain this increased cardiovascular risk associated with PPI use as caused by unmeasured confounders such as comorbid conditions (smoking, lipid levels, body mass index and left ventricle ejection fraction). Our BP finding, however, raises the possibility that increased mortality and morbidity may also possibly be caused by an increase in BP.

Lundell et al and Galmiche et al reported increased cardiovascular events (heart attacks, heart failure and heart-related sudden deaths) after conducting two long-term studies in patients with severe gastroesophageal reflux disease treated with omeprazole and esomeprazole [18, 19]. The US Food and Drug Administration (FDA) therefore performed a safety review of omeprazole (Prilosec) and esomeprazole (Nexium). The conclusion from this safety review was that long-term use of omeprazole and esomeprazole was not likely to be associated with an increased risk of heart problems [20].

In conclusion, daily intake of 40 mg esomeprazole did not result in increased insulin secretion or reduction of HbA_{1c} or any markers of cardiovascular risk in patients with type 2 diabetes. This is in contrast to earlier animal and register studies. Hence, esomeprazole does not appear to have the beneficial effects on insulin secretion, glucose metabolism and markers of cardiovascular risk in type 2 diabetic patients that are seen with other well-established medications used for the effective treatment of type 2 diabetes and the prevention of complications.

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Contribution statement AV, JSP, AEK, PR and KDH designed the study. The data were analysed and interpreted by KF, CB, KDH, SSL, JFR and AV. All authors contributed to critical revision of the article. All authors gave their final approval of the current version to be published.

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