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Association of Faecal Elastase 1 with Non-Fasting Triglycerides in Type 2 diabetes

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Abstract

Aims Intestinal absorption of esterified fatty acids depends on exocrine pancreatic function and influences plasma triglycerides levels. The aim was to investigate the association of reduced exocrine pancreatic function (low fecal elastase-1; FE1) with plasma triglycerides in type 2 diabetes and controls without diabetes.

Methods FE1 (μg/g stool) and non-fasting plasma triglyceride measurements were undertaken in 544 type 2 diabetes patients (age: 63 ± 8 years) randomly selected from diabetes registers in Cambridgeshire (UK), and 544 matched controls (age, sex, practice) without diabetes. Linear regression models were fitted using FE1 as dependent and log-triglycerides as independent variable adjusting for sex, age, body mass index, alcohol consumption, serum lipase, HbA1c, and smoking.

Results FE1 concentrations were lower (mean ± sd: 337 ± 204 vs. 437 ± 216 μg/g, $p<0.05$) and plasma triglycerides were higher (geometric mean */: standard deviation factor: 2.2*/:1.9 vs. 1.6*/:1.8 mmol/l, $p<0.05$) in type 2 diabetes compared to controls, respectively. Within the category of type 2 diabetes and controls separately, a 10% increase in plasma triglycerides was associated with 4.5 μg/g higher FE1 concentrations ($p<0.01$) after adjusting for confounders. In contrast, in diabetes patients and controls with pathological FE1 (<100 μg/g), low FE1 levels were associated with high plasma triglycerides (significant only in controls).

Conclusions Non-fasting triglycerides were positively related to FE1 in both type 2 diabetes and controls suggesting that impairment of exocrine pancreas function is influencing plasma triglycerides. Marked loss of exocrine pancreatic function had the opposite effect, resulting in higher levels of plasma triglycerides.

Key words: pancreatic exocrine dysfunction, faecal elastase 1, triglycerides

Introduction

Morphological alterations of the exocrine pancreas are observed in patients with diabetes and a considerable number show mild to moderate impairment of bicarbonate and enzyme secretion (1). The pathophysiological mechanisms leading to impairment of exocrine pancreatic function in diabetes mellitus have not been elucidated, but may include imbalance of islet hormones, pancreatic fibrosis due to angiopathy, autoimmune mechanisms, autonomic neuropathy and altered release of gastrointestinal regulatory mediators (2).

Pancreatic elastase 1 is an enzyme that is highly stable during passage through the gastrointestinal tract (3). The concentration of elastase 1 can be measured in faeces using an enzyme-linked immunosorbent assay (4). Faecal elastase-1 (FE1) levels have been demonstrated to correlate with more sensitive tests of pancreatic secretion, such as the secretin-caerulein test and resulting duodenal lipase activity (4). Low FE1 levels have also been shown to correlate with morphologic characteristics of chronic pancreatitis as detected with endoscopic retrograde pancreatography (5) and magnetic resonance cholangiopancreatography (6).

In patients with type 2 diabetes, the prevalence of pancreatic exocrine insufficiency (PEI) estimated by low FE1 measurements ranged between 12 and 73% in different studies (2). However, most of these studies only included small numbers of highly selected patients and have limited statistical power. In the largest population-based study so far, we randomly selected 544 type 2 diabetic patients (age: 63 years) from local diabetes registers in Cambridgeshire (UK), and 544 individually matched controls without diabetes (7). Low levels of FE1 (<100 µg/g) were found in 11.9% of cases and 3.7% of controls (age-sex-adjusted odds ratio (OR); 95% CI: 3.6; 2.2-6.2) (7).

The most accepted definition of PEI is a reduction of exocrine pancreatic function to a level that results in malabsorption, demonstrated as a decreased coefficient of fat absorption (8,9). However, exocrine dysfunction in patients with diabetes defined by low FE1 is usually mild to moderate and does not lead to steatorrhoea in the majority of cases. Thus, the clinical relevance of low fecal elastase in type 2 diabetes is questionable.

Although decreased lipase activity in the intestine is a hallmark for PEI, little is known about the impact of PEI on plasma triglycerides in general. Intestinal absorption of esterified fatty acids depends on exocrine pancreatic function and influences plasma triglycerides levels. In a recent study, chronic pancreatitis patients with PEI according to the 13C mixed triglycerides breath-test were unexpectedly observed to have higher triglyceride concentrations than to those without PEI (10). Studies investigating the metabolic importance of mild and more severe impaired pancreatic exocrine function on triglyceride concentrations are lacking.

To the best of our knowledge, the association of FE1 concentrations with plasma triglycerides in type 2 diabetes has not been investigated. Therefore, our study aimed (i) to investigate the association between FE1 concentrations and non-fasting plasma triglycerides in type 2 diabetes and in controls without diabetes adjusting for potential

confounders including age, sex, body mass index (BMI), HbA1c, smoking and alcohol intake and (ii) to evaluate the relationship of mild and more severe PEI (FE1 <100 μg/g) on plasma triglycerides.

Methods

Study population

We analysed data from a case-control study in Cambridgeshire (UK) (7). Five-hundred-and-forty-four randomly selected type 2 diabetic patients from general practitioner registers in South Cambridgeshire (UK) were individually matched with 544 controls (diabetes excluded by HbA1c measurement, cut-off 7.0%). The matching variables were sex, age and practice. The presence of type 2 diabetes was primarily based on clinical criteria, e.g. onset of diabetes after the age of 30 years, and no insulin therapy during the first year after diagnosis. Because of the high prevalence of previously undiagnosed diabetes in patients of this age (mean age: 63 years), diabetes was not only excluded in controls by medical record search but also by normal glycated hemoglobin measurement using a cut-point of <7% which was accepted at the time of the study.

The study was approved by the Cambridgeshire Local Research Ethics Committee. This analysis of the data was approved by the Ethical Committee of the Medical Faculty of the Heinrich Heine University Düsseldorf, Germany. All participants gave formal written consent after they had been instructed in detail about the study aims and investigations.

Measurements

FE1 measurements (μg/g stool) were performed centrally at ScheBo-Tech Institute in Wettenberg (Germany) blind to case-control assignation. Patients sampled a probe of their morning stool into a labeled stool tube that was collected by the investigators, frozen and dispatched to the laboratory on ice. Pathologically low levels of FE1 were defined as <100 μg/g stool.

Anthropometric measures were performed in light clothing using standardized methods. Detailed medical history was recorded by structured interviews and questionnaires. Current and historical alcohol consumption as well as smoking was assessed. Current alcohol intake was calculated as alcohol units per week. Among diabetic patients, diabetes duration and treatment was recorded.

Whole blood was collected and delivered to the central laboratory at Addenbrooke's Hospital, Cambridge, England, for measurement of HbA1c and plasma was collected for measurement of triglycerides using the RA 1000 analyser (Bayer Diagnostics, Basingstoke, England). Other measurements were performed as previously described (7).

Statistical analyses

Demographic variables of the study population were stratified by diabetes depending on their distributions by frequency tables, means ± standard deviations (SD), geometric means */: standard deviation factors (SDF) or non-parametrically by medians (interquartile ranges). FE1 was analysed as a continuous variable assuming an approximate normal distribution, fitting the data better than a log-normal distribution. An approximate log-normal distribution was assumed for triglycerides. Scatter plots of FE1 versus log-triglycerides were carried out. Linear regression models were fitted using FE1 as dependent variable and log-triglycerides as independent variable. Potential confounders were sex, age (continuous), BMI (continuous), alcohol consumption (heavy drinking: ever/never), current heavy drinking (men: ≥21 g/day; women: ≥14 g/day), lipase (continuous), HbA1c (continuous), smoking and in the diabetes sub-population, insulin treatment (yes/no), and diabetes duration (< 5 years, 5-10 years, > 10 years). In a first step, bivariate linear models were fitted using log-triglyceride and one confounder as independent variables in each model. Furthermore, a final model including all potential confounders was calculated using stepwise variable selection (log-triglycerides forced into the model). Analyses were also performed separately in the subgroup of subjects with pathological FE 1 (<100 µg/g).

Finally, linear regression models with log-triglycerides as dependent and elastase as independent variable were fitted, separately for diabetes patients and non-diabetic controls. Sex, age, BMI, history of and current alcohol consumption, HbA1c, lipase, smoking, insulin therapy and diabetes duration were considered as potential confounders. Interaction variables between the subgroup indicator "elastase < 100 µg/g" and elastase were included in univariate and in final multivariate models. To evaluate multicollinearity in the regression models, Pearson and Spearman correlation matrices of all variables were calculated. In addition, the variance inflation factor (VIF) was used to assess the extent to which the variances of the estimated coefficients were inflated. A variable with VIF >10 is considered as an indication of serious collinearity. Furthermore, a collinearity analysis estimating condition indices and proportions of variances was performed. Finally, reduced models excluding some of the covariates were fitted as a sensitivity analysis. Data from the collinearity analyses are not shown in detail.

All analyses were carried out using SAS 9.4.

Results

The clinical characteristics of type 2 diabetes patients and controls are given in Table 1. After matching, the mean age was 63 years with a higher proportion of males (64%) in both groups. The mean diabetes duration was 7 years and two thirds of the patients were treated with oral antidiabetic drugs (insulin: 8.9%). The mean HbA1c was 7.9% in type 2 diabetes and 5.6% in controls ($p<0.05$).

FE1 concentrations were significantly lower and plasma non-fasting triglycerides higher in patients with type 2

diabetes than in controls, respectively (both p<0.05) (Table 1). Whereas prevalence of patients with history of heavy alcohol consumption was higher in patients with type 2 diabetes, current alcohol intake was lower than in controls (p<0.05). There was no significant difference in smoking between the two groups. The prevalence of current gastrointestinal symptoms (diarrhea, constipation), the history of pancreatitis (ever) and serum lipase concentrations were also similar.

A visual analysis of the scatterplot of FE1 and non-fasting triglycerides suggested a weak positive correlation in patients with type 2 diabetes (Figure 1). After fitting the regression line, higher triglyceride levels were associated with increased FE1 concentrations. As shown in Table 2, the positive relationship of triglycerides with FE1 concentrations persisted after adjusting for potential confounders in the multivariate linear regression model (Table 2). A 10% change in triglycerides was related to +4.5 µg/g (95%CI: 1.8-7.1 µg/g) FE1 concentrations. Age, male sex, and HbA1c (non-significant: p=0.060) were inversely related to the parameter of pancreatic exocrine function. Finally, BMI and serum lipase showed a positive relationship with FE1 (Table 2). There was no significant association with smoking. Triglyceride levels and the confounders explained about 10% of the variance in FE1.

Examination of the scatterplot and regression line in controls without diabetes also showed a weak positive correlation between non-fasting triglycerides and FE1 (Figure 2). After adjusting for potential confounders in linear regression analysis, a 10% change in triglycerides was related to +4.5 µg/g (95%CI: 1.4-7.7 µg/g) FE1 in the control group, which was very similar to diabetes patients. Age, current heavy alcohol drinking and HbA1c showed an inverse association with FE1 in non-diabetic subjects, whereas a positive relationship was found with serum lipase concentrations (Table 3). The association with smoking was not significant. Triglyceride levels and confounders explained about 9% of the variance in FE1. Similar results were observed after excluding 13 patients with HbA1c values between 6.5-6.9% (data not shown).

Visual inspection of the scatterplots indicated that the relationship between FE1 and triglycerides values was inverse in people with pathological FE1 (<100 µg/g) values. Therefore, an explorative regression analyses were carried out for the subgroup with pathological FE1 concentrations (<100 µg/g), consistent with a high probability of PEI. The prevalence of diarrhea or constipation was not significantly different comparing subjects with fecal elastase <100 and ≥100 µg/g in diabetes patients (diarrhea: 0.0 vs. 3.1%, p=0.24; constipation: 15.6 vs. 12.2%, p=0.42) or non-diabetic (diarrhea: 0.0 vs. 2.1%, p=1.00; constipation: 5.0 vs. 13.2%, p=0.495) patients.

In contrast to the whole sample, in patients with type 2 diabetes with low FE1 levels (n=65) the plot indicates an inverse association of the pancreatic enzyme and triglycerides (Figure 3A). Similar result were also found in controls without diabetes (n=20) (Figure 3B). In both patients and controls regression parameters for log-triglycerides (dependent variable FE1) in the univariate linear regression models were negative (ß-coefficient [95%

confidence limits] for diabetes: -7.98 [-18.50 to 2.55], p=0.135; patients without diabetes: -28.68 [-50.18 to -7.18], p=0.012). Multiple adjustment using the same confounders as in the whole sample (diabetes: sex, age, BMI, lipase, HbA1c, smoking; controls: age, alcohol, lipase, HbA1c, smoking) did not change these results.

Finally, linear regression models with log-triglycerides as dependent and FE1 as independent variable were fitted (type 2 diabetes and controls). Overall, in type 2 diabetes patients there was a significant positive association between FE1 and (log-)triglycerides, which persisted after adjusting for the confounders (age, sex, BMI, HbA1c, diabetes duration, smoking). An increase of 100 µg/g FE1 was estimated to be related to an increase of 4.6% of triglycerides (95%CI: 2.0% to 7.3%). In the diabetes subgroup with FE1 concentrations <100 µg/g a non-significant negative association between the marker of PEI and triglycerides was found, and the interaction between the subgroup and FE1 was also non-significant (p=0.092) in the confounder adjusted interaction model including the whole diabetic population.

In controls, there was also a significant positive association between FE1 and (log-)triglycerides after adjusting for the confounders (age, sex, BMI, HbA1c, current alcohol consumption and smoking). An increase of 100 µg/g FE1 was associated with a 3% increase of triglycerides (95%CI: 0.8% to 5.3%). In contrast, in the subgroup (FE1 <100 µg/g) a significant negative association was estimated, and the interaction between the subgroup and FE1 was significant (p=0.036) in the confounder adjusted interaction model including the whole non diabetic population.

There were some moderate significant correlations between the covariables of the model in both diabetes and non-diabetic subjects (data not shown). Furthermore, some low to moderate collinearity was observed in both models, but sensitivity analyses showed that the main result of association between triglycerides and elastase was not affected (data not shown).

Discussion

The novel finding of the present study is that non-fasting plasma triglyceride concentrations are positively correlated with FE1 concentrations, a measure of pancreatic function, in patients with type 2 diabetes and in age-, sex- and practice-matched people without diabetes. Although lower FE1 values were found in type 2 diabetes patients than controls, the positive correlation between the marker of pancreatic function and non-fasting triglycerides was found in both groups. In contrast to this finding, a post-hoc analysis of subjects with pathological values of FE1 (<100 µg/g stool) demonstrated an inverse correlation between non-fasting plasma triglycerides and FE1. Thus, the study indicates that variation within the normal range of the exocrine pancreas function influence plasma triglyceride levels. In subjects with PEI, further loss of exocrine pancreatic function appears to have an opposite and pathophysiologically different effect, resulting in higher levels of plasma triglycerides.

Studies on the clinical consequences of moderately decreased FE1 concentrations are rare. Some studies have indicated that only a very pronounced reduction of FE1 is clinically relevant. One study in a cohort of patients with chronic pancreatitis found that steatorrhea was present only in cases with FE1 below 15 µg/g (11). Other studies have indicated that less pronounced reductions of FE1 might be relevant. For example, a high prevalence (59%) of pathological faecal fat excretion (>7g/day) was found in a study on patients with diabetes and FE1 <100 µg/g stool (12). The mean FE1 concentration in this selected study sample was 56 µg/g (12). However, no significant correlation between FE1 and fat excretion was observed (12).

There are several different mechanisms that may have contributed to the observed positive correlation between non-fasting triglyceride concentrations and FE1 concentrations in the total cohort. It could be speculated that an increased pancreatic lipase activity associated with higher FE1 levels (4), could contribute to a larger uptake of fatty acids and therefore increased production of chylomicrons in the intestine and very-low density lipoproteins in the liver (15,16). Also an association between impaired exocrine function and increased breakdown of very-low density lipoproteins (VLDL) is possible; if less chylomicrons are produced, enhanced hydrolysis of VLDL triglycerides is expected to occur in the post-prandial state contributing to reduced non-fasting triglyceride levels. Differences in dietary habits are another possible explanation as consumption of a high-fat diet is associated with high plasma triglycerides and may also increase pancreatic enzyme output. A study on the influence of diet composition on exocrine pancreatic output has demonstrated that ingestion of a high fat diet (40% of caloric content) resulted in a four times higher pancreatic enzyme output compared to a low fat diet (10% of total caloric content) (17). We did not have access to information on dietary habits in the current study and dietary fat intake may have confounded the observed association between FE1 and non-fasting triglycerides. New studies would be of value to understand how reduced exocrine function of the pancreas influences food intake and food preferences to fully understand the complex relationships between FE1 levels and plasma triglycerides. More research on gastrointestinal symptoms in type 2 diabetes patients related to low FE1 levels is also warranted. Additional factors that may have an impact on fecal elastase levels that we were not able to adjust for include undiagnosed celiac disease and some medications known to influence pancreatic secretion (i.e. cloroquine, octreotide). Increased plasma levels of triglycerides are associated with obesity, physical inactivity, smoking, excessive alcohol consumption, consuming a diet rich in carbohydrates and fat, some diseases (type 2 diabetes and insulin resistance, chronic renal failure, nephritic syndrome), use of certain medications (e.g. corticoid steroids, β-blockers, estrogens), genetic factors, low HDL, and high non-HDL cholesterol (13,14). We have been able to adjust for some but not all of these potential confounders in the present study, which needs to be further investigated.

The present study also identified factors other than triglycerides that are related to FE1 concentrations. In type 2 diabetes and controls, higher age was inversely related to FE1. This finding confirms a previous cross-sectional study in subjects without gastrointestinal disease or diabetes (18). FE1 concentrations correlated negatively with age (Pearson r=-0.3531, $P<0.001$) and were significantly lower among people over 70 years old compared to younger controls (20-28 years old) (18). Thus, the mean age of study populations need to be taken into account when comparing results of various studies on FE1 concentrations. In type 2 diabetes, male sex was related to lower FE1 concentrations, which was also found in a previous study in type 1 diabetes (19). Men with type 1 diabetes were about five times more likely to have FE1 values <100 µg/g compared to female patients (19). Furthermore, BMI was positively related to FE1 in type 2 diabetes. Also in patients with type 2 diabetes, PEI (defined by low FE1 and faecal chymotrypsin) was associated with lower BMI, without evidence of weight loss (20). In contrast with this finding, another cross-sectional study found predominance of FE1 levels <200 µg/g in obese diabetes patients (21). The authors raised the hypothesis that obesity and type 2 diabetes may have synergistic effects on the development of pancreatic exocrine insufficiency (21). Therefore, longitudinal studies are required to investigate the association of FE1 with body weight and weight change in patients with and without type 2 diabetes. Current heavy alcohol drinking was related to lower FE1 in people without diabetes. Interestingly, after excluding people with an alcohol consumption over 20 g per day, the prevalence of PEI based on low FE1 levels was considerably lower in a recent study compared to other investigations (22). Thus, the role of alcohol consumptions also needs to be considered when comparing studies on PEI in type 2 diabetes and controls. Serum lipase was positively related to FE1 levels both in diabetes and controls, indicating that the leakage of lipase is correlated to the function of exocrine pancreas. Finally, an inverse relationship was found between HbA1c and FE1 in both groups, which is in line with a previous study (23).

In the sub-analysis of subjects with a pathological FE1 (<100 µg/g), there was an inverse association of triglycerides and FE1 concentrations that was statistically significant in controls with and without adjustment for potential confounders, but not significant in diabetes patients. Post-hoc analyses with log-triglycerides as dependent and FE1 as independent variable, separately for diabetes patients and non-diabetic controls, confirmed these relationships. There was a significant positive association between FE1 and triglycerides in type 2 diabetes patients and in the subgroup with low FE1 a negative association was found, although this was not statistically significant. Similar relationships were observed in controls. In the subgroup with FE1 <100 µg/g, a significant negative association was found in controls, and the interaction term indicated a significant effect modification of mild or severe PEI on the association between FE1 and triglycerides.

Hence, in the participants with a high probability of clinically significant PEI, a further decrease of residual pancreatic exocrine function is associated with increased plasma levels of triglycerides. Similar observations have been done in previous studies. In a recent study, blood nutritional markers were investigated in 114 people with chronic pancreatitis, 38 with and 76 without PEI, according to the ^{13}C-mixed triglycerides breath tests (10). Chronic pancreatitis patients with PEI had higher average fasting triglyceride levels compared to those without PEI (mean (SD): 232 (445) mg/dl vs. 125 (88) mg/dl; p=0.05) (10). Another study comparing 20 patients with moderate to advanced chronic pancreatitis with 20 healthy controls reported differences in apolipoproteins and total cholesterol, and a non-significant trend towards higher plasma levels of triglycerides in patients with chronic pancreatitis (24).

As a potential clinical consequence of our findings, a direct association between cardiovascular disease and chronic pancreatitis (severe exocrine pancreatic insufficiency) has been proposed (25) and more pronounced exocrine insufficiency leading to higher plasma triglycerides may be a contributing factor. This speaks in favor of early diagnosis and treatment of PEI in type 2 diabetes patients that already have an increased risk for cardiovascular events. Future research should also investigate the importance of enzyme supplementation in the regulation of plasma triglycerides in patients with very low FE1 and hypertriglyceridemia.

It is reasonable to assume that the mechanisms behind the inverse association between FE1 and triglycerides in subjects with impaired exocrine pancreatic function are different from the mechanisms explaining the positive association between the same variables in subjects without evidence for PEI. It could be speculated that reduced intestinal fatty acid uptake and increased dietary intake of simple carbohydrates as a consequence of fat intolerance result in insulin resistance and increased de novo lipogenesis in the intestine and the liver, which in turn enhance triglyceride production in both tissues (15,16,26). The association between PEI and triglycerides may also be related to glucagon-like peptide 2 (GLP-2) (27,28). Interestingly, PEI increases the secretion of GLP-2, which is most likely due to delivery of a larger nutrient load to the distal part of the small intestine, where GLP-2 secreting L-cells are abundant (28). In healthy volunteers, an increase in postprandial triglycerides and free fatty acid concentrations was observed during GLP-2 administration (27). Moreover, experiments in mice and hamsters also showed that GLP-2 enhances absorption of fatty acids as well as increases production and secretion of chylomicrons (29,30). Thus, further studies are necessary to investigate the role of GLP-2 in PEI and lipid metabolism.

There are a number of drawbacks of the present study that need to be mentioned. The use of a direct pancreatic function test like the secretin test would have given more reliable estimates of exocrine pancreatic function (31). However, direct pancreatic function tests are invasive and not suitable to use as screening tests. Fecal elastase was

selected as method for estimation of exocrine pancreatic function in this study based on the previously established correlation between fecal elastase and the secretin test (32, 33). In-depth analysis of clinically overt signs of PEI in patients with low fecal elastase levels was not possible in this study since limited information was available. Furthermore, beyond HbA1c, we had no other measures of glucose metabolism and insulin sensitivity in patients with type 2 diabetes and the control group. Next, plasma triglycerides were measured in a non-fasting state. However, it has been shown that at most plasma triglycerides changed minimally in response to normal food intake in individuals in the general population (34). New studies would be of value to understand how fasting and post-prandial triglyceride levels are influenced by exocrine dysfunction of the pancreas. Furthermore, assessment of important confounders (e.g., alcohol intake) was undertaken using self-report questionnaires, which introduces the possibility of measurement error in these confounding factors and, therefore, residual confounding when they are adjusted for in the analysis. We also had no information on dietary habits that potentially can influence both FE1 levels and plasma triglycerides. Finally, controls were not randomly selected from the practices but were individually matched to patients with type 2 diabetes. However, because we matched only by age, sex, and general practice and participants were randomly selected from the general practice register, the group of controls is broadly representative of people in Cambridgeshire of a similar age to the people with diabetes. The analyses for FE1 < 100µg/g were planned post hoc and are based on small subsamples with low power. The results on the subgroups should therefore be interpreted in an explorative manner. The proportion of the variance in the FE1 values explained by all the independent variables in the regression equation together was only 9-10%, which is often found in epidemiological studies. The other covariables in the linear model were primarily considered as confounders, because it was not the goal to fit a model describing the full functionality of elastase depending on different risk factors.

In conclusion, in this cross-sectional study we have observed that high FE1 levels are associated with high non-fasting triglycerides in the total cohort of participants with and without diabetes. The opposite was observed in the subgroup of people with a suspicion of clinically relevant PEI as indicated by FE1 <100 µg/g, where low FE1 was associated with high non-fasting plasma triglycerides. Our findings suggest that exocrine pancreatic function measured as FE1 may have an impact on triglyceride turn-over and that this association is different in participants with and without PEI. However, the importance of exocrine pancreatic function for plasma triglyceride regulation needs to be confirmed in longitudinal investigations with more complete analysis of plasma lipid profile and full characterization of dietary habits.

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authors declare that there is no duality of interests associated with this manuscript.

Contribution statement

All authors made substantial contributions to the conception and design, analysis and interpretation of data, as well as to the drafting and revising of the manuscript. In detail, BH undertook the primary data analysis, and WR drafted the article. All authors approved the final version of the manuscript.

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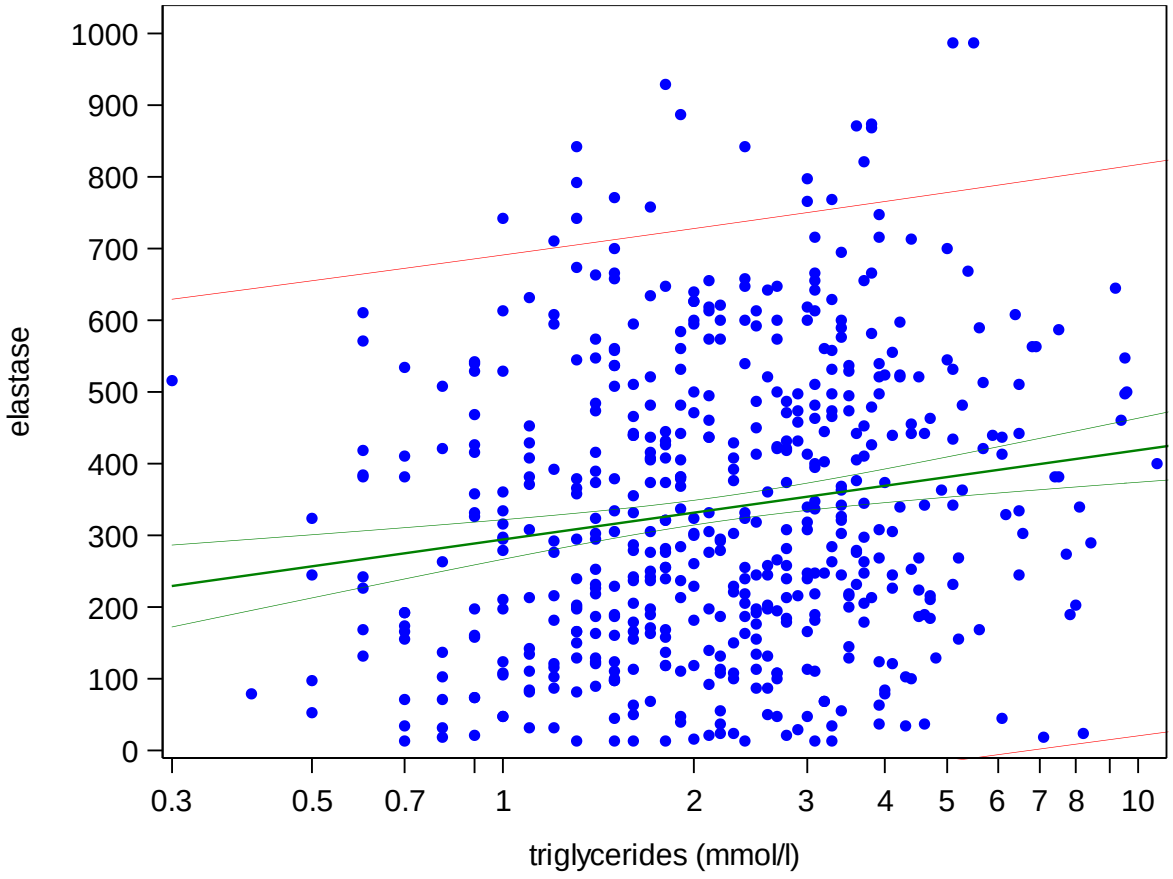
Table 1 Characteristics of 544 patients with type 2 diabetes and 544 age-, sex-, and practice-matched non-diabetic controls (Cambridgeshire, UK)

Variables	Type 2 diabetes	Controls
Age (years)	63 ± 8	63 ± 8
Males (%)	64	64
Body mass index (kg/m^2)	29.7 ± 5.2*	27.4 ± 4.2
HbA1c (%)	7.9 ± 1.4*	5.6 ± 0.4
Diabetes duration (years)	7.3 ± 7.0	-
Oral antidiabetic agents (%)	65.6	-
Insulin treatment (%)	8.9	-
Faecal elastase 1 (µg/g stool)	337 ± 204*	437 ± 216
Triglycerides (non-fasting) mmol/l	2.2 [1.9]*	1.6 [1.8]
Alcohol intake:		
Heavy drinking (ever, %)	16*	9
Alcohol units/week	1.5 (0.0-9.0)*	4.9 (0.8-12.4)
Smoking:		
Current (%)	14	13
Ex-smoker (%)	60	58
Current gastrointestinal symptoms (%):		
Diarrhea	2.8	2.0
Constipation	12.6	12.9
Pancreatitis (ever, %)	0.9	0.6
Lipase (U/L)	247 ± 79	247 ± 61

N <10 missings in each variable, except for alcohol intake (24 missing)
Data are mean ± SD, median (interquartile range), proportions or geometric mean */: [standard deviation factor] (triglycerides)
* p<0.05 (diabetes vs controls): paired t-tests, McNemar or Bowkers tests

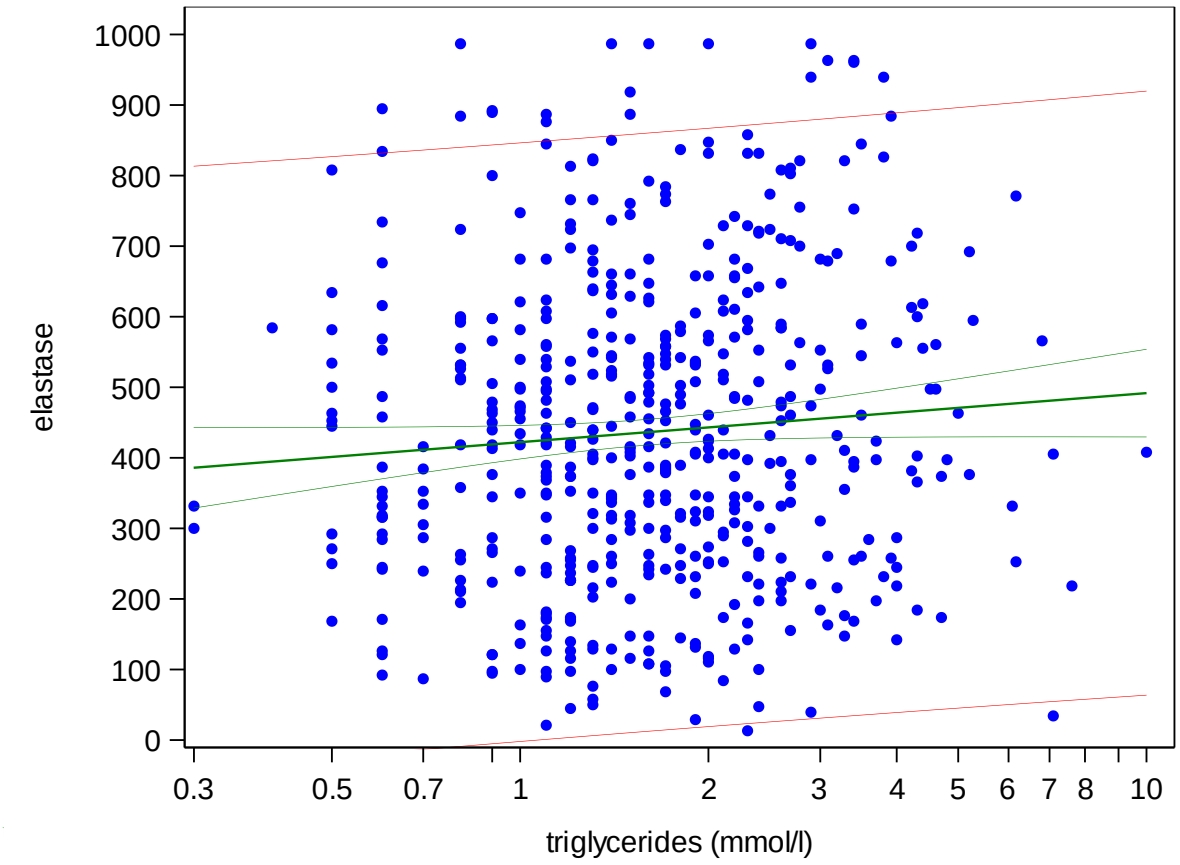
Figure 1 Scatterplot and regression line of faecal elastase 1 (µg/g stool) and non-fasting triglycerides (mmol/l) in type 2 diabetes patients



thin green lines: 95% confidence limits for the expected value (mean) of the dependent variable
thin red lines: 95% confidence limits for an individual prediction

Data are presented as triglycerides on a log scale (area restricted to 0.3-10 mmol/l)

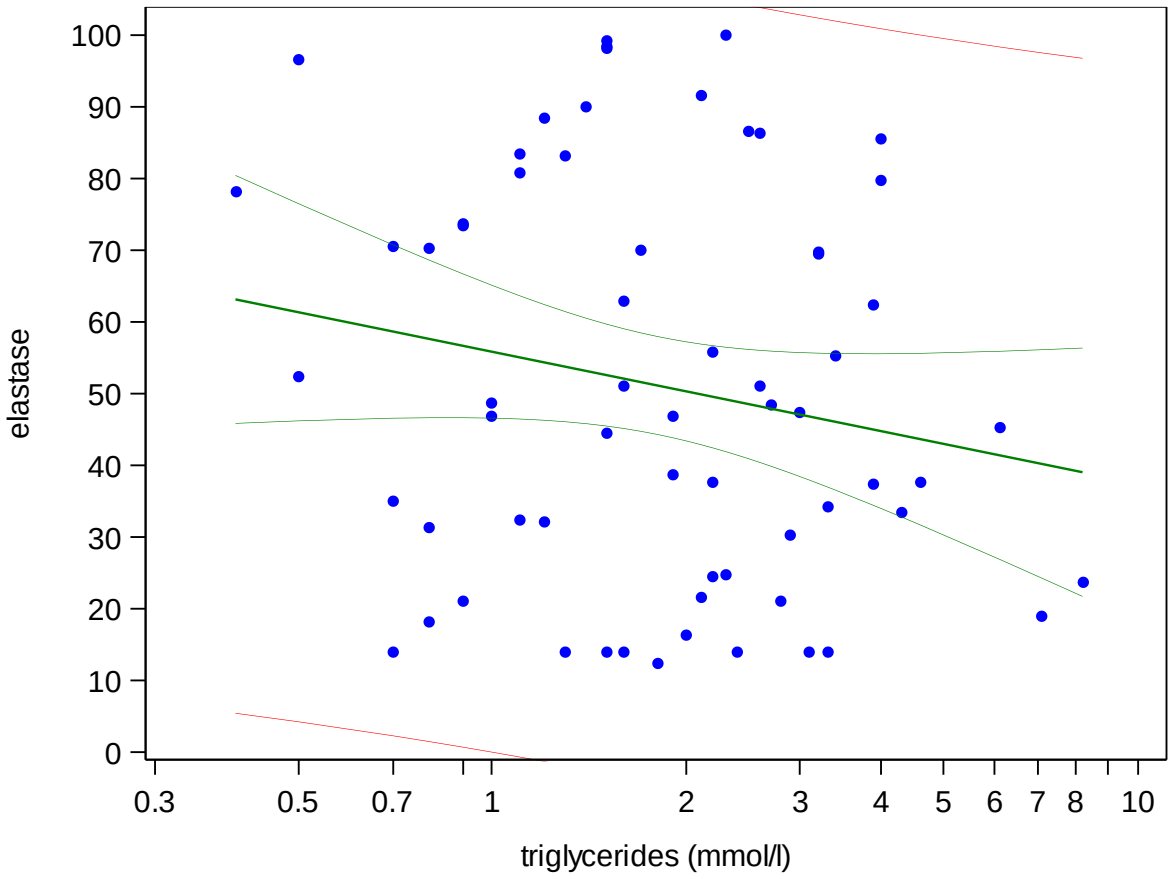
Figure 2 Scatterplot and regression line of faecal elastase 1 (µg/g stool) and non-fasting triglycerides (mmol/l) in non-diabetic controls



thin green lines: 95% confidence limits for the expected value (mean) of the dependent variable
thin red lines: 95% confidence limits for an individual prediction

Data are presented as triglycerides on a log scale (area restricted to 0.3-10 mmol/l)

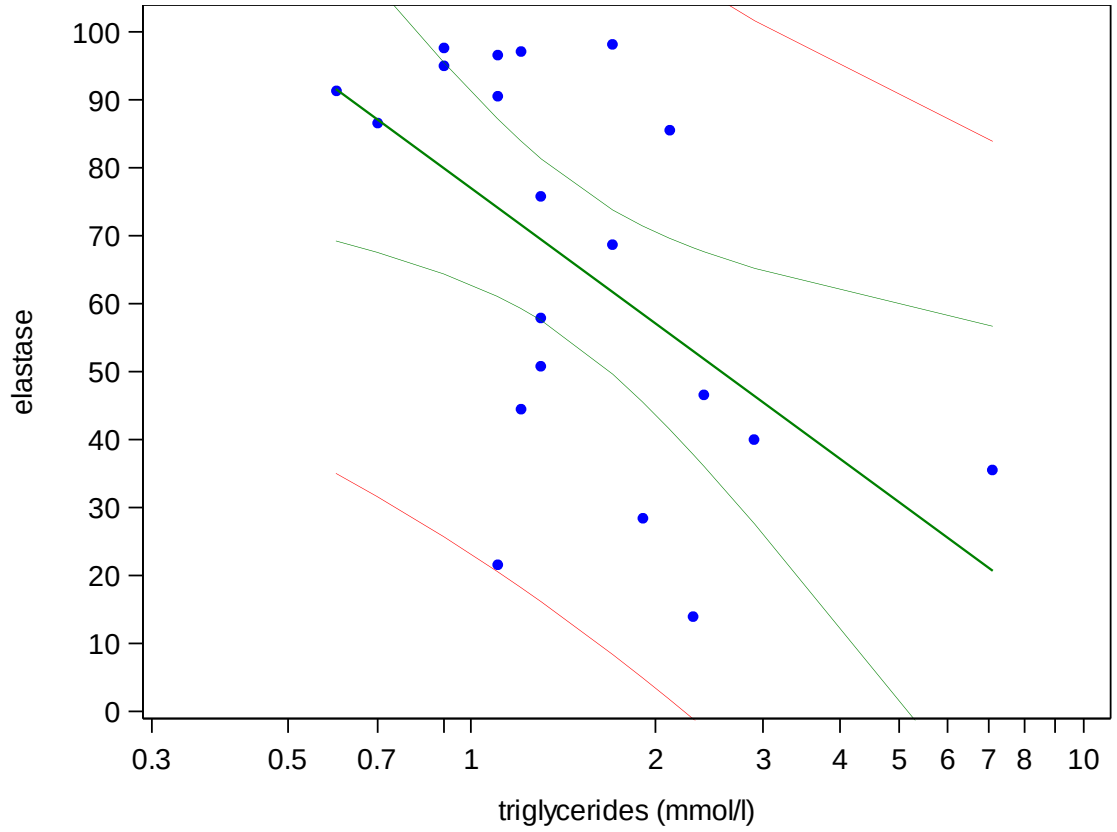
Figure 3A Scatterplot and regression line of faecal elastase 1 (µg/g stool) and non-fasting triglycerides (mmol/l) in type 2 diabetes patients with elastase 1 <100 µg/g (n=65)



thin green lines: 95% confidence limits for the expected value (mean) of the dependent variable
thin red lines: 95% confidence limits for an individual prediction

Data are presented as triglycerides on a log scale (area restricted to 0.3-10 mmol/l)

Figure 3B Scatterplot and regression line of faecal elastase 1 (µg/g stool) and non-fasting plasma triglycerides (mmol/l) in people without diabetes with elastase 1 <100 µg/g (n=20)



thin green lines: 95% confidence limits for the expected value (mean) of the dependent variable
thin red lines: 95% confidence limits for an individual prediction

Data are presented as triglycerides on a log scale (area restricted to 0.3-10 mmol/l)

Table 2 Association of faecal elastase 1 concentrations (dependent variable) with non-fasting plasma triglycerides in type 2 diabetes: multivariate linear regression analyses (after stepwise selection)

Variables	ß-Coefficient	95%CI (ß)	p-value
Intercept	417.68	195.98, 639.38	-
Triglycerides (mmol/l) (log)	46.88	18.78, 74.98	0.001
Male sex	-69.44	-106.60, -32.29	< 0.001
Age (year)	-2.85	-5.05, -0.66	0.011
Body mass index (kg/m^2)	3.98	0.54, 7.42	0.023
Lipase (U/l)	0.30	0.09, 0.51	0.005
HbA1c (%)	-11.72	-23.94, 0.50	0.060
Current Smoker	-37.90	-93.19, 17.39	0.179
Ex-Smoker	18.87	-21.65, 59.39	0.361

R^2: 0.103

n=535 (9 patients excluded because of missing values in the independent variables)

Table 3 Association of faecal elastase 1 concentrations (dependent variable) with non-fasting plasma triglycerides in non-diabetic controls: multivariate linear regression analyses (after stepwise selection)

Variables	ß-Coefficient	95%CI (ß)	p-value
Intercept	1153.17	875.76, 1430.57	-
Triglycerides (mmol/l) (log)	47.72	14.88, 80.56	0.004
Age (year)	-3.78	-6.16, -1.40	0.002
Heavy alcohol drinking (last month)	-58.31	-112.48, -4.14	0.035
Lipase (U/l)	0.30	0.001, 0.59	0.049
HbA1c (%)	-97.20	-143.65, -50.75	<0.001
Current Smoker	-55.65	-116.23, 4.93	0.072
Ex-Smoker	-23.17	-64.64, 18.30	0.273

R^2: 0.093

n=520 (24 patients excluded because of missing values in the independent variables)