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# Relationship between circulating levels of pancreatic proteolytic enzymes and pancreatic hormones

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ABSTRACT

*Background:* While the close morphological relationship between the exocrine and endocrine pancreas is well established, their functional interaction remains poorly understood. The aim of this study was to investigate the associations between circulating levels of pancreatic proteolytic enzymes and insulin, as well as other pancreatic hormones.

*Methods:* Fasting venous blood samples were collected and analyzed for trypsin, chymotrypsin, insulin, glucagon, somatostatin, and pancreatic polypeptide. Linear regression analysis was used in unadjusted and two adjusted (accounting for prediabetes/diabetes, body mass index, smoking, and other covariates) statistical models.

*Results:* A total of 93 individuals with a history of acute pancreatitis were included in this cross-sectional study. Chymotrypsin was significantly associated with insulin in the two adjusted models ($p = 0.005$; $p = 0.003$) and just missed statistical significance in the unadjusted model ($p = 0.066$). Chymotrypsin was significantly associated with glucagon in both unadjusted ($p = 0.025$) and adjusted models ($p = 0.014$; $p = 0.015$); as well as with somatostatin - in both unadjusted ($p = 0.001$) and adjusted models ($p = 0.001$; $p = 0.002$). Trypsin was not significantly associated with insulin in any of the models but was significantly associated with glucagon in both unadjusted ($p < 0.001$) and adjusted models ($p < 0.001$), and pancreatic polypeptide in both unadjusted ($p < 0.001$) and adjusted ($p < 0.001$) models.

*Conclusion:* The state of hyperinsulinemia is characterized by a dysfunction of the exocrine pancreas. In particular, chymotrypsin is increased in the state of hyperinsulinemia and trypsin is significantly associated with glucagon and pancreatic polypeptide.



## Introduction

The pancreas is an intricate organ with a dual functionality of both endocrine and exocrine tissues. These parts of the pancreas are linked closely both anatomically and physiologically [1,2], and play an important role in digestion and metabolism. Morphological studies show that the endocrine islet cells are scattered amongst the exocrine tissue [3]. Blood supplied to the islets drains into the surrounding acinar tissue to form islet-acinar portal venous system [4]. As blood leaving the islets flows into the acinar capillaries, the acinar cells are exposed to high concentrations of the islet hormones (such as insulin, glucagon, somatostatin, and pancreatic polypeptide (PP) [5–8]) that regulate pancreatic exocrine function, in particular, the synthesis and secretion of pancreatic enzymes [9]. This has led to the notion of 'insulo-acinar axis', explaining the regulatory system based on the interaction between the endocrine and exocrine pancreas [10].

The endocrine islets are made up of five types of cells, with the insulin-producing β cells comprising about 60% of the total cellular population [11–13]. Insulin is known to have a trophic effect on the exocrine pancreas, with high local concentrations of insulin resulting in larger peri-insular acini containing more zymogen granules than the tele-insular acini [14–18]. Other islet hormones are believed to have an inhibitory effect on the function of the exocrine pancreas [19–21]. Relationship between the endocrine

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and exocrine pancreas is not possible to investigate directly in humans ante-mortem but it could be investigated by studying proxies for the endocrine function (circulating levels of pancreatic hormones - insulin, glucagon, somatostatin, and pancreatic polypeptide) and proxies for the exocrine function (circulating levels of pancreatic proteolytic enzymes (PPE), such as trypsin and chymotrypsin that are, unlike amylase or lipase, unique to the pancreas). To date, studies investigating the association between PPE and pancreatic hormones have been mainly conducted in hypoinsulinemic states [22–27]. To the best of our knowledge, no clinical study has investigated these associations in a hyperinsulinemic state. Hyperinsulinemia has long been recognized as a key pathogenic mechanism associated with obesity [28,29]. More recent data suggest that hyperinsulinemia may also play a causative role in tumorigenesis in general and obesity-associated pancreatic cancer in particular [30,31]. Findings from the DORADO study [32–34] show that insulin levels are also frequently elevated in patients after acute pancreatitis; hence DORADO provides a valuable framework for investigating the association between hyperinsulinemia and circulating levels of PPE.

Until recently, the inability to accurately measure circulating levels of PPE, particularly trypsin, posed a major problem. This was largely because of the use of radio-immunoassays to measure trypsin in blood. However, trypsin in blood is found either as proenzyme trypsinogen [35] or as a complex with the protease inhibitors α1 anti-trypsin and α2 macroglobulin [36], making it difficult to measure the exact concentration of trypsin. While radioimmunoassays could measure concentrations of both trypsinogen and trypsin-protease complex in blood [37–40], they could not differentiate between the two [41]. Further, radio-immunoassays-obtained trypsin values are not reproducible [35,37,39]. Development of the new, highly sensitive and specific enzyme linked immunosorbent assays (ELISA) resulted in more accurate measurements of PPE. Enzyme linked immunosorbent assays are quick, inexpensive, do not require handling of radioactive substances, and provide reproducible results [42–44].

The primary aim of this study was to investigate the associations between circulating levels of trypsin and chymotrypsin and insulinemia. The secondary aim was to investigate the associations between trypsin and chymotrypsin and other pancreatic hormones, as well as their contribution to insulinemia.

## Methods

### *Study protocol*

The study design was a cross-sectional study. The study protocol was described in detail elsewhere [33,34]. In brief, individuals with a primary prospectively established diagnosis of acute pancreatitis as per international guidelines [45] were followed up and invited to participate in the study. The study was approved by the Health and Disability Ethics Committee (13/STH/182).

### *Sample collection and storage*

A certified phlebotomist collected fasting venous blood from all patients. The blood samples were then centrifuged for 7.5 min at 4000 g at 4 °Celsius. The serum separated and stored in Eppendorf tubes at −80 °C until use.

### *Laboratory assays*

Blood tests for insulin, glycated haemoglobin (HbA1c), and fasting blood glucose (FBG) were conducted at LabPlus, an International Accreditation New Zealand (IANZ) accredited medical laboratory, at Auckland City Hospital. Insulin was measured using a chemiluminescence sandwich immunoassay (Roche products and Roche Diagnostics NZ) while HbA1c was measured using boronate affinity chromatography assay (Trinity Biotech). Fasting blood glucose was measured using enzymatic colourimetric assay (F.Hoffmann-La Roche).

Serum from all samples was analyzed for trypsin using the Novateinbio standard sandwich ELISA assay. The standard detection range of the assay was between 0.03 ng/ml −2 ng/ml, with a sensitivity of 0.01 ng/ml, and an intra-assay and inter-assay variation of <10%. Chymotrypsin in serum was analyzed using the Cusabio quantitative sandwich ELISA assay. The detection range of the assay was between 0.16 ng/ml −10 ng/ml, with a sensitivity of 0.04 ng/ml. The intra- and inter-assay variation for the assay was <8% and <10%, respectively. Somatostatin was measured using the Merck-Millipore ELISA assay. The results were recorded with the help of a Rayto Microplate Reader (V 2100C, Santa Fe) with an absorbance range of 405–630 nm). All assays were analyzed according to the user's manuals.

Glucagon and PP were analyzed using MILLIPEX MAP Human metabolic hormone magnetic bead panel based on Luminex xMAP (Luminex) technology in accordance with the user's manuals. The results were measured based on fluorescent reporter signals recorded by the Luminex xPONENT software (MILLIPLEX analyst 5.1).

### *Definitions*

Dysglycemia: was defined as prediabetes (FBG between 5.6 and 6.7 mmol/l and/or HbA1c between 39 and 48 mmol/mol) or diabetes (FBG > 6.7 mmol/l and/or HbA1c > 48 mmol/mol) as per the American Diabetes Association guidelines [46].

Hyperinsulinemia: was defined based on fasting serum insulin levels as the >75th percentile group, in line with previous studies in the field of Diabetology [47–50].

Body Mass Index (BMI) ($kg/m^2$): was measured using a digital scale and stadiometer. Study participants were requested to remove their shoes and any head attire for height measurement (cm). For their weight measurement (kg) participants were asked to empty their pockets and remove their shoes, belt, watch, and jacket.

Physical activity: was recorded as a binary variable, based on whether or not patients exercised for at least 2.5 h per week or 30 min per day [51].

Smoking: was recorded as a binary variable, based on whether or not patients smoked any cigarettes or tobacco-related products.

Chronic alcohol consumption: was deemed to be present if individuals had alcohol etiology of pancreatitis.

Severity of pancreatitis: was defined as per the 2012 determinant-based classification [52].

Recurrence of pancreatitis: was deemed to be present if individuals had one or more episodes from first hospital admission with acute pancreatitis to the time of their participation in the study.

Duration: was defined as the time (months) from individuals' first hospital admission due to acute pancreatitis to their participation in the study.

### *Statistical analyses*

All statistical analyses were conducted using SPSS for Windows (version 23.0). For all analyses, $p$-value $\leq 0.05$ was accepted as statistically significant.

Data on characteristics of all study participants were presented as either a mean and standard deviation (SD), median and

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