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# Pancreatic and gut hormones as predictors of new-onset prediabetes after non-necrotising acute pancreatitis: a prospective longitudinal cohort study

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## Abstract

*Objective:* Early identification of individuals at high risk for metabolic derangements after an attack of acute pancreatitis (AP) is critical with a view to tertiary preventing of this disease. The aim was to investigate whether fasting pancreatic and gut hormones at baseline were predictive of future risk of new-onset prediabetes after acute pancreatitis (NOPAP) in individuals with non-necrotising AP.

*Methods:* This was a prospective longitudinal cohort study that included 69 consecutive non-diabetic participants with AP, of whom 55% ($n = 38$) had normoglycaemia both at baseline and during follow-up, 25% ($n = 17$) had prediabetes both at baseline and during follow-up, and 20% ($n = 14$) were normoglycaemic at baseline but developed NOPAP during follow-up. The associations between the study groups and circulating fasting levels of pancreatic and gut hormones (insulin, glucagon, C-peptide, amylin, glucose-dependent insulinotropic peptide, glucagon-like peptide-1, pancreatic polypeptide, and peptide YY) were studied using multinomial regression in both unadjusted and adjusted analyses.

*Results:* Elevated plasma insulin and glucagon at baseline were significantly associated with NOPAP (adjusted odds ratio 1.99, 95% CI 1.01 to 3.92 and adjusted odds ratio 3.44, 95% CI 1.06 to 11.19, respectively). The same hormones had no significant association with antecedent prediabetes in AP. The other studied hormones were not significantly associated with the study groups.

*Conclusions:* Normoglycaemic AP individuals with elevated fasting levels of insulin and glucagon at baseline constitute a high-risk group for future NOPAP.

## Introduction

Post-pancreatitis diabetes mellitus, the largest contributor to diabetes of the exocrine pancreas (1), is the second most common type of adult-onset diabetes (2). Post-pancreatitis diabetes mellitus is associated with a higher risk of all-cause mortality and hospitalisation (for gastrointestinal and infectious diseases-related complications) than type 2 diabetes (3), putting a considerable burden on healthcare resources. Post-pancreatitis diabetes is a frequent sequela

of acute pancreatitis (AP). A projection study estimated the annual incidence for post-pancreatitis diabetes mellitus in AP individuals to increase from 5.2 per 100,000 persons in 2020 to 13.6 per 100,000 persons by 2050 (4). Further, aggregated evidence from several studies suggests that individuals with AP are at a two-times increased risk of new-onset diabetes than the general population, independent of the severity of AP (5, 6, 7). Given that the cumulative incidence of new-

onset diabetes increases with time (8), there is a need for early identification of high-risk individuals to introduce targeted strategies for preventing and managing this sequela of AP.

Prediabetes is one of the most prominent risk factors for new-onset diabetes mellitus (9). It is estimated that up to 75% of individuals with prediabetes may progress to diabetes mellitus (10, 11). Data from several diabetes prevention trials suggest the benefits of screening for prediabetes for timely and effective prevention of diabetes mellitus (12, 13, 14, 15). Ideally, a blanket mass surveillance of all AP patients at regular intervals would ensure early detection of individuals at a substantial risk of developing glucose abnormalities. However, AP is the most common disease of the exocrine pancreas, with the annual incidence of 34 cases per 100,000 persons worldwide (16). Therefore, an indiscriminate surveillance is unlikely to be practical and cost-effective. This brings to the fore the importance of identifying predictors that enable accurate identification of high-risk individuals shortly after AP diagnosis with the ultimate goal of targeted surveillance. Our longitudinal prospective cohort as part of the LACERTA project includes prospectively diagnosed individuals with AP who were followed up after hospital discharge at regular intervals for up to 2 years (8). This prospective longitudinal cohort provides a unique framework for the investigation of blood biomarkers at baseline that could distinguish individuals who subsequently develop NOPAP from those who remain normoglycaemic after AP. The DORADO project (a cross-sectional study of individuals with a history of AP), which preceded and did not overlap with the LACERTA project, sieved more than 50 blood biomarkers and identified a number of pancreatic and gut hormones that play a role in derangements of glucose homeostasis after an attack of AP (17). Because derangements of glucose homeostasis after necrotising pancreatitis are typically a function of the extent of pancreatic necrosis (and, hence, relatively straightforward to predict), the current challenge is to predict derangements of glucose homeostasis after non-necrotising AP.

The aim was to investigate whether fasting levels of pancreatic and gut hormones measured at baseline can predict future risk of NOPAP in individuals with non-necrotising AP.

## Methods

### Study design

This prospective longitudinal cohort study was part of the LACERTA project. The study conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by the Health and Disability Ethics Committee (approval number 13/STH/182). The study included adults with non-necrotising AP admitted to Auckland City Hospital (New Zealand) who were followed up over a period of 24 months after hospital discharge. Blood samples collected at baseline (mean 0.9 months, from the date of hospitalisation for AP) and during follow-up were used in the present study.

### Study cohort

Individuals who had a primary diagnosis of non-necrotising AP (determined based on the absence of pancreatic necrosis on CT during hospitalisation), were at least 18 years of age, and provided informed consent were included in the study.

Individuals who had diabetes mellitus (defined as glycated haemoglobin (HbA1c) ≥ 48 mmol/mol (6.5%)) (18) and/or taking antidiabetic medications), chronic pancreatitis, intra-operative diagnosis of pancreatitis, post-endoscopic retrograde cholangiopancreatography pancreatitis, malignancy, cognitive disability, or were pregnant at the time of hospitalisation for AP or during follow-up were excluded from the study.

### Study groups

Individuals were categorised into three study groups based on their fasting plasma glucose (FPG) and HbA1c measurements at both baseline and during follow-up.

(i) Normoglycaemia after AP (NAP): individuals with HbA1c < 39 mmol/mol at baseline, and FPG < 5.6 mmol/L (100 mg/dL) and HbA1c < 39 mmol/mol (5.7%) during follow-up were deemed to have NAP.

(ii) Antecedent prediabetes before AP (APAP): individuals with HbA1c between 39 and 47 mmol/mol at baseline, and FPG between 5.6 and 6.9 mmol/L (100–125 mg/dL) and/or HbA1c between 39 and 47 mmol/mol (5.7–6.4%) during follow-up were deemed to have APAP.

(iii) New-onset prediabetes after AP (NOPAP): individuals with HbA1c < 39 mmol/mol at baseline, and FPG between 5.6 and 6.9 mmol/L (100–125 mg/dL) and/or HbA1c between 39 and 47 mmol/mol (5.7–6.4%) during follow-up were deemed to have NOPAP.

The used thresholds were in line with the DEP criteria (19). Given that FPG > 5.6 mmol/L (100 mg/dL) at baseline might be due to stress hyperglycaemia (20), FPG at baseline was not taken into consideration in grouping the study participants.

## Laboratory measurements

Fasting venous blood samples were collected after an overnight fast (≥ 8 h) both at baseline and during follow-up. Glycated haemoglobin and FPG were measured on whole never-frozen blood immediately after blood collection. Glycated haemoglobin was measured using boronate affinity chromatography assay (Trinity Biotech, County Wicklow, Ireland) and FPG was measured using enzymatic colourimetric assay (F. Hoffmann-La Roche, Basel, Switzerland). The other collected tubes of blood were centrifuged at 4000 $g$ for 5.5 min at 4°C, plasma separated, aliquoted, and stored at –80°C for future analyses. Amylin, C-peptide, glucagon, glucose-dependent insulinotropic peptide (GIP), glucagon-like peptide-1 (GLP-1), insulin, pancreatic polypeptide, and peptide YY were measured in plasma samples using the MILLIPLEX® MAP human metabolic hormone magnetic bead panel. Protease (Merck KGaA) and DPP-IV inhibitors (Merck KGaA) were added to the samples. All assays were conducted as per the user's manual. Results were quantified using the Belysa™ immunoassay curve fitting software (Merck KGaA).

## Definitions of covariates

Sex was a binary variable and categorised into 'men' and 'women'. Age at baseline was categorised (based on interquartile range (IQR)) into 'young adults' (≤ 25th percentile: ≤ 36 years), 'middle-aged adults' (25th–75th percentile: 37–64 years), and 'older adults' (≥ 75th percentile: ≥ 65 years). BMI (kg/m$^2$) was determined using a stadiometer. BMI was categorised into normal ≤ 25 kg/m$^2$ and overweight/obese > 25 kg/m$^2$. Smoking was a binary variable. The smoking status was categorised as 'yes' for individuals who were current smokers at the time of baseline blood collection and as 'no' for other individuals. Aetiology was categorised into biliary, alcohol-related, and other. Recurrence was a binary variable and was deemed to be present if individuals had one or more episodes of AP (at least 30 days apart) prior to their participation in the present study. Cholecystectomy was a binary variable and was deemed to be present if participants underwent cholecystectomy within 3 months of baseline blood collection.

## Statistical analyses

All statistical analyses were done using SPSS 27.0. For all analyses, $P$ values < 0.05 were deemed statistically significant. Baseline characteristics of individuals in the study groups were presented as frequency or median (IQR). Differences in baseline characteristics between individuals in the three groups were compared using Fisher's exact tests or one-way ANOVA. The assumption of normality for ANOVA was not met and, therefore, the pancreatic and gut hormones variables were log-transformed. Other statistical analyses were conducted in the following steps.

First, multinomial logistic regression analyses were used to investigate the associations between the APAP and NOPAP groups and pancreatic and gut hormones in both unadjusted (i.e. model 1) and adjusted (i.e. model 2) models. For all the analyses, the NAP group was used as the reference. The analyses were adjusted for age, sex, BMI, and aetiology. Data were presented as odds ratio (OR) with corresponding 95% CI and $P$ values.

Second, receiver-operating characteristic (ROC) curves were generated from univariate analyses comparing NOPAP with NAP. The area under the curve (AUC) was calculated to quantify the discriminatory power of pancreatic and gut hormones in predicting NOPAP (21). Cut-off thresholds, predictive values, and likelihood ratios were derived for each hormone (22, 23).

Third, backward regression analyses were used to investigate the effects of several patients- and pancreatitis-related characteristics (age, sex, BMI, smoking, aetiology, recurrence, and cholecystectomy) on the studied hormones, separately in the three groups. Each hormone was analysed individually as a continuous dependent variable and age, sex, BMI, smoking, aetiology, recurrence, and cholecystectomy as independent variables. Data for statistically significant covariates were presented as B coefficients with corresponding 95% CI, $P$ values, and adjusted $R^2$ values.

## Results

### Characteristics of participants

The study included a total of 69 individuals, of whom 55% ($n$ = 38) had NAP, 25% ($n$ = 17) had APAP, and 20% ($n$ = 14) developed NOPAP during follow-up. The mean (s.e.m.) time to diagnosis of NOPAP was 6 (2) months from baseline blood collection. Other baseline characteristics of study participants are presented in Table 1.

### Pancreatic and gut hormones in the study groups

The median (IQR) for insulin was 91.08 pmol/L (45.40, 170.71) in the NAP group, 127.74 pmol/L (65.22, 391.42)

in the APAP group, and 202.60 pmol/L (85.54, 344.63) in the NOPAP group ($P=0.099$). Insulin was not significantly associated with NOPAP in the unadjusted model but was significantly associated with NOPAP in the adjusted model. Insulin increased the odds of developing NOPAP by an OR (95% CI) of 1.99 (1.01, 3.92) ($P=0.046$) in the adjusted model (Table 2). Insulin was not significantly associated with APAP in either the unadjusted or the adjusted models (Table 2). The median (IQR) for glucagon was 14.32 ng/L (11.61, 23.61) in the NAP group, 22.43 ng/L (10.18, 24.31) in the APAP group, and 20.44 ng/L (15.29, 31.31) in the NOPAP group ($P=0.136$). Glucagon was not significantly associated with NOPAP in the unadjusted model but was significantly associated with NOPAP in the adjusted model. Glucagon increased the odds of developing NOPAP by an OR (95% CI) of 3.44 (1.06, 11.19) ($P=0.040$) in the adjusted model (Table 2). Glucagon was not significantly associated with APAP in the unadjusted and adjusted models (Table 2). The median (IQR) for amylin was 2.88 pmol/L (2.62, 3.14) in the NAP group, 3.14 pmol/L (1.96, 3.49) in the APAP group, and 2.81 pmol/L (1.89, 3.14) in the NOPAP group ($P=0.719$). Amylin was not significantly associated with

either NOPAP or APAP in the unadjusted and adjusted models (Table 2). The median (IQR) for C-peptide was 0.26 nmol/L (0.13, 0.49) in the NAP group, 0.24 nmol/L (0.13, 0.44) in the APAP group, and 0.39 nmol/L (0.25, 0.58) in the NOPAP group ($P=0.329$). C-peptide was not significantly associated with either NOPAP or APAP in the unadjusted and adjusted models (Table 2). The median (IQR) for GIP was 7.85 pmol/L (3.06, 15.84) in the NAP group, 10.16 pmol/L (4.07, 19.27) in the APAP group, and 8.32 pmol/L (6.76, 22.86) in the NOPAP group ($P=0.335$). The glucose-dependent insulinotropic peptide was not significantly associated with either NOPAP or APAP in the unadjusted and adjusted models (Table 2). The median (IQR) for GLP-1 was 41.56 pmol/L (25.03, 57.43) in the NAP group, 40.97 pmol/L (26.10, 69.53) in the APAP group, and 37.60 pmol/L (19.75, 53.08) in the NOPAP group ($P=0.789$). Glucagon-like peptide-1 was not significantly associated with either NOPAP or APAP in the unadjusted and adjusted models (Table 2). The median (IQR) for pancreatic polypeptide was 11.61 pmol/L (6.34, 21.81) in the NAP group, 18.75 pmol/L (9.38, 37.72) in the APAP group, and 21.51 pmol/L (11.25, 39.20) in the NOPAP group ($P=0.197$). The pancreatic polypeptide was not significantly associated with either NOPAP or APAP in the unadjusted and adjusted models (Table 2). The median (IQR) for peptide YY was 8.40 pmol/L (4.32, 10.60) in the NAP group, 9.95 pmol/L (4.32, 15.15) in the APAP group, and 9.95 pmol/L (8.40, 15.66) in the NOPAP group ($P=0.170$). Peptide YY was not significantly associated with either NOPAP or APAP in the unadjusted and adjusted models (Table 2).

## Predictive accuracy of pancreatic and gut hormones

The ROC curves for all the pancreatic and gut hormones at baseline as predictors of NOPAP are presented in Fig. 1. Insulin and glucagon, but not the other studied hormones, yielded statistically significant AUC. Insulin and glucagon combined had an AUC (95% CI) of 0.74 (0.60, 0.87), $P=0.012$. The cut-off value of 175 pmol/L for insulin had a sensitivity of 57.1%, a specificity of 76.9%, a negative predictive value of 83.3%, a negative likelihood ratio of 0.56, a positive predictive value of 47.1%, and a positive likelihood ratio of 2.47. The cut-off value of 21.4 ng/L for glucagon had a sensitivity of 46.1%, a specificity of 69.2%, a negative predictive value of 79.4%, a negative likelihood ratio of 0.78, a positive predictive value of 33.3%, and a positive likelihood ratio of a 1.50.

**Table 1** Characteristics of the study groups.

| Characteristic | Group | | | p |
| --- | --- | --- | --- | --- |
| | NAP | APAP | NOPAP | |
| Age, *n* | | | | 0.739 |
|   Young adults | 11 | 4 | 3 | |
|   Middle-aged adults | 20 | 8 | 6 | |
|   Older adults | 7 | 5 | 5 | |
| Sex, *n* | | | | 0.948 |
|   Men | 17 | 8 | 7 | |
|   Women | 21 | 9 | 7 | |
| BMI, *n* | | | | 0.390 |
|   Normal | 14 | 3 | 5 | |
|   Overweight/obese | 24 | 14 | 9 | |
| Aetiology, *n* | | | | 0.382 |
|   Biliary | 21 | 9 | 8 | |
|   Alcohol-related | 6 | 1 | 4 | |
|   Other | 11 | 7 | 2 | |
| Recurrence, *n* | | | | 0.925 |
|   No | 29 | 14 | 11 | |
|   Yes | 9 | 3 | 3 | |
| Cholecystectomy, *n* | | | | 0.285 |
|   No | 20 | 12 | 6 | |
|   Yes | 18 | 5 | 8 | |
| Smoking, *n* | | | | 0.425 |
|   No | 25 | 12 | 8 | |
|   Yes | 8 | 5 | 6 | |

The age categories were as follows: young adults ≤ 36 years, middle-aged adults 37–64 years, older adults ≥ 65 years. The body composition categories were as follows: normal BMI ≤ 25 kg/m$^2$, overweight/obese BMI > 25 kg/m$^2$.

**Table 2** Associations between the study groups and pancreatic and gut hormones.

| Hormone | Model | NAP | APAP | | | | NOPAP | | | |
| | | | | 95% CI | | | | 95% CI | | |
| | | | OR | Lower | Upper | p | OR | Lower | Upper | p |
|---|---|---|---|---|---|---|---|---|---|---|
| Insulin | 1 | Reference | 1.62 | 0.91 | 2.86 | 0.099 | 1.72 | 0.94 | 3.15 | 0.078 |
| | 2 | | 1.66 | 0.92 | 2.99 | 0.094 | 1.99 | 1.01 | 3.92 | **0.046** |
| C-peptide | 1 | Reference | 1.21 | 0.77 | 1.90 | 0.409 | 1.48 | 0.84 | 2.63 | 0.175 |
| | 2 | | 1.24 | 0.78 | 1.97 | 0.357 | 1.52 | 0.83 | 2.79 | 0.172 |
| Amylin | 1 | Reference | 1.10 | 0.28 | 4.29 | 0.887 | 0.59 | 0.15 | 2.41 | 0.466 |
| | 2 | | 0.75 | 0.17 | 3.37 | 0.711 | 0.43 | 0.08 | 2.21 | 0.310 |
| Glucagon | 1 | Reference | 1.32 | 0.57 | 3.06 | 0.518 | 2.76 | 0.99 | 7.71 | 0.052 |
| | 2 | | 1.28 | 0.53 | 3.09 | 0.575 | 3.44 | 1.06 | 11.19 | **0.040** |
| GIP | 1 | Reference | 1.24 | 0.76 | 2.01 | 0.384 | 1.50 | 0.84 | 2.65 | 0.168 |
| | 2 | | 1.25 | 0.74 | 2.08 | 0.402 | 1.82 | 0.89 | 3.72 | 0.101 |
| GLP-1 | 1 | Reference | 1.16 | 0.42 | 3.19 | 0.771 | 0.76 | 0.27 | 2.12 | 0.597 |
| | 2 | | 1.21 | 0.40 | 3.60 | 0.736 | 0.66 | 0.20 | 2.14 | 0.484 |
| PPY | 1 | Reference | 1.45 | 0.77 | 2.71 | 0.248 | 1.82 | 0.89 | 3.73 | 0.100 |
| | 2 | | 1.46 | 0.75 | 2.84 | 0.264 | 1.83 | 0.84 | 3.98 | 0.128 |
| PYY | 1 | Reference | 1.36 | 0.69 | 2.71 | 0.377 | 2.37 | 0.90 | 6.24 | 0.081 |
| | 2 | | 1.32 | 0.65 | 2.69 | 0.447 | 2.48 | 0.78 | 7.86 | 0.123 |

Model 1: unadjusted; Model 2: adjusted for age, sex, BMI, and aetiology. Bold indicates statistical signficance, $P < 0.05$.
GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide-1; PPY, pancreatic polypeptide; PYY, peptide YY.

## Effects of covariates on pancreatic and gut hormones in the study groups

Aetiology, sex, and smoking had a significant positive association with insulin, whereas BMI and recurrence had a negative association with insulin in the NOPAP group. Specifically, women, being overweight/obese, smokers, others aetiology, and recurrent episodes of AP collectively explained 65% of the variance in insulin levels (Table 3). Age had a significant positive association with glucagon in the NOPAP group. Specifically, young age explained 57% of the variance in glucagon levels (Table 3). Age and aetiology had a significant positive association with GIP in the NOPAP group. Specifically, old age and individuals with non-alcohol and non-biliary aetiology collectively explained 69% of the variance in GIP levels. Recurrence had a significant negative association with C-peptide in the NOPAP group. Specifically, recurrent episodes of AP explained 45% of the variance in C-peptide levels. None of the studied covariates had a statistically significant association with amylin, GLP-1, pancreatic polypeptide, and peptide YY in the NOPAP group (Table 3). The associations between the covariates and pancreatic and gut hormones in the APAP and NAP groups are presented in Table 3.

## Discussion

The present study was the first prospective longitudinal study to investigate the relationship between fasting levels of eight pancreatic and gut hormones at baseline and the progression from normoglycaemia to NOPAP during follow-up of individuals with non-necrotising AP. Elevated levels of fasting insulin and glucagon in normoglycaemic individuals at baseline were the strongest predictors of subsequent NOPAP. Specifically, insulin increased the odds of progressing to NOPAP by an OR of 1.99 ($P=0.046$) and glucagon by an OR of 3.44 ($P=0.040$), after adjustment for patient-related and pancreatitis-related factors. Further, insulin and glucagon combined showed a good predictive accuracy (AUC=0.74) in predicting individuals at a high risk of future NOPAP. Importantly, the study purposely included the APAP group (i.e. patients with AP and co-existing prediabetes at baseline) and showed that neither insulin nor glucagon was significantly associated with the APAP group.

The presence of hyperinsulinaemia during a state of normoglycaemia is an established risk factor for the development of abnormal glucose metabolism in the general population (24, 25, 26, 27, 28, 29). A population-based study showed that high fasting levels of insulin (≥ 75th percentile group in that cohort) at baseline were associated with approximately two-times increased risk of progressing to incident prediabetes during nine years of follow-up (24). Another long-term population-based study conducted over a follow-up period of 24 years also showed that normoglycaemic individuals with fasting insulin levels in the topmost quintile (≥ 176.4 pmol/L in that cohort) were two times more likely to develop prediabetes than individuals with lower insulin levels (25). While hyperinsulinaemia is well recognised as a
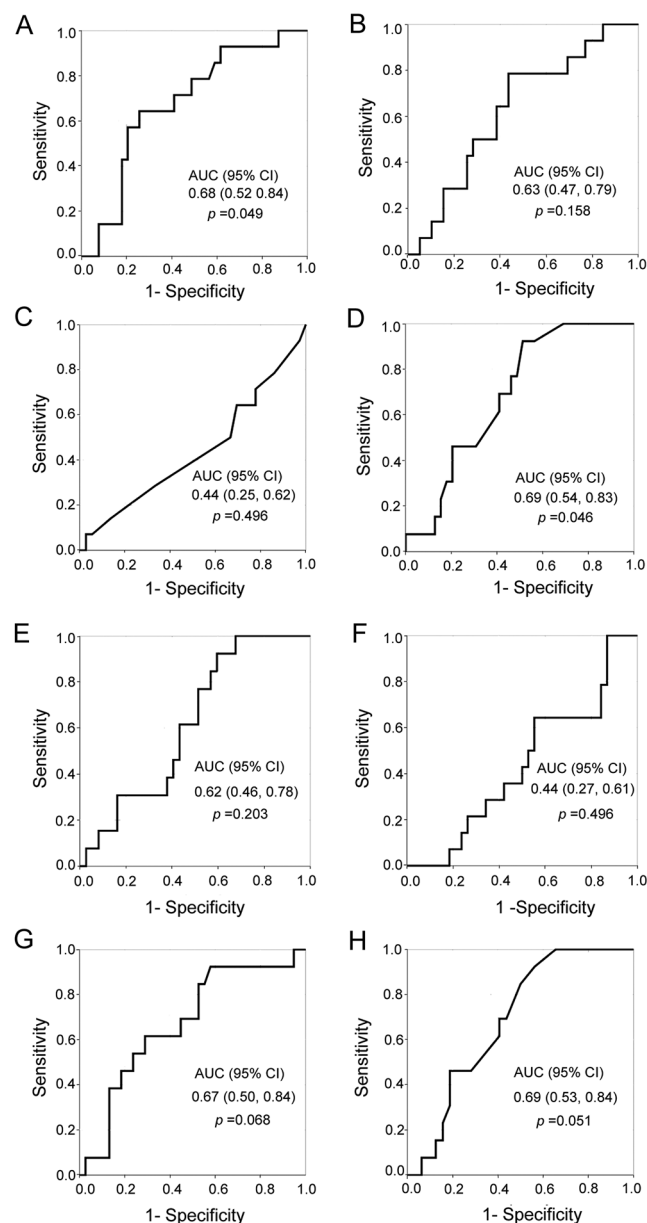
**Figure 1**

Receiver-operating characteristic curves of pancreatic and gut hormones for predicting new-onset prediabetes after acute pancreatitis at baseline (A) insulin; (B) C-peptide; (C) amylin; (D) glucagon; (E) glucose-dependent insulinotropic peptide; (F) glucagon-like peptide-1; (G) pancreatic polypeptide; (H) peptide YY. AUC, area under the curve. 262 × 137 mm.

predictor of incident prediabetes/type 2 diabetes, the relationship between hyperinsulinaemia and other types of diabetes (such as post-pancreatitis diabetes mellitus) is established to a lesser extent. Post-pancreatitis diabetes mellitus is less common than type 2 diabetes but has a considerably heavier burden than type 2 diabetes (30). Yet, there is a dearth of studies investigating biomarkers that could predict which patients with AP are at risk of

post-pancreatitis diabetes mellitus. Earlier cross-sectional studies by the COSMOS group (the DORADO project) and others showed that increased insulin resistance and the resulting compensatory hyperinsulinaemia are some of the key mechanisms in the pathogenesis of post-acute pancreatitis diabetes mellitus (31, 32, 33, 34). In the present longitudinal cohort study, 20% of AP individuals with normoglycaemia who had elevated fasting insulin levels at baseline progressed to NOPAP during prospective follow-up. Further, baseline hyperinsulinaemia was associated with two-times increased risk of developing NOPAP after accounting for several covariates (such as age, sex, and BMI, and aetiology). These findings provide the strongest evidence to date implicating hyperinsulinaemia in the development of NOPAP. Our results suggest that elevated fasting insulin levels in normoglycaemic AP individuals can be considered as an early predictor of a high risk of future derangements of blood glucose metabolism in this setting. Given that the present study showed no significant association of C-peptide (a marker for insulin secretion) with NOPAP, the increase in insulin levels at baseline may not be due to increased insulin secretion but rather due to reduced insulin clearance. This and other possible mechanisms of hyperinsulinaemia (i.e. downregulation of insulin receptors, differences in β-cell size and mass, altered hypothalamic and parasympathetic signalling pathways) warrant investigations in purposely designed mechanistic studies (31, 35, 36, 37, 38, 39).

The other notable finding was that baseline fasting glucagon was significantly associated with progression from normoglycaemia to NOPAP during follow-up. An earlier cross-sectional study (as a part of the DORADO project) did not find a statistically significant association between fasting glucagon and post-pancreatitis diabetes mellitus (40, 41). Hence, the present longitudinal study takes the field further by demonstrating, for the first time, that elevated levels of fasting glucagon at baseline significantly increase the odds of developing NOPAP by more than three times (after accounting for patient-related and pancreatitis-related characteristics). Earlier studies showed that fasting glucagon levels are higher in individuals with prediabetes (and type 2 diabetes) compared with individuals with normal glucose tolerance (42, 43, 44), making it plausible that elevated baseline plasma glucagon in AP individuals represent an early pathogenic event prior to the onset of prediabetes. In the general population, elevated glucagon levels are typically attributed to impaired glucagon-insulin sensitivity relationship (42) or α-cell compensatory changes (45). In patients with AP, elevated glucagon levels could relate to a stress-induced counter-regulatory and

inflammatory response during acute illness (46). However, this would hold true for all AP patients, regardless of their prediabetes status. Moreover, the baseline blood samples were collected at a mean of 0.9 months after diagnosis of non-necrotising AP, suggesting that any transient inflammation-induced increase in glucagon would have resolved by the time of blood collection. Future studies will provide detailed mechanistic insights into the role of glucagon as an early predictor of NOPAP.

In the present study, we started to gain insights into the effect of several common covariates on circulating levels of glucagon and insulin (and the other studied hormones) in the post-acute pancreatitis setting. Young age (36 years or younger, based on the 25th percentile of the IQR) showed a significant association with glucagon in the NOPAP group, explaining 57% of the variance in glucagon levels. Several recent population-based studies showed that the age-specific risk of post-pancreatitis diabetes mellitus is the highest among young adults with AP (6, 47, 48, 49). A study from the UK showed that the risk of newly diagnosed diabetes in individuals aged 30–39 was significantly higher in those with a history of AP than in the general population without a history of AP (OR = 1.68) (47). A study from Taiwan showed that the age-specific risk of post-pancreatitis diabetes mellitus (irrespective of the severity of AP) was the highest in men aged <45 years (adjusted hazard ratio = 7.46) (6). A study from Israel showed that AP individuals under the age of 40 years had the highest risk of developing diabetes (adjusted OR = 4.65) compared with the general population (48). Further, a study from New Zealand showed that young adults aged 30–34 with post-pancreatitis diabetes mellitus had the greatest loss in life expectancy than young adults with other types of diabetes (49). The present study provides initial data that designate elevated levels of glucagon as a possible mechanistic basis for the higher burden of post-pancreatitis diabetes mellitus in young adults. However, given that the number of young adults in the NOPAP group was limited, the effect of age on glucagon in the context of metabolic derangements after pancreatitis warrants further investigations. Also, our study showed that 65% of the variance in insulin levels was explained by factors such as normal BMI (≤ 25 kg/m²), sex (women), active tobacco smoking, first episode of AP, and non-biliary non-alcohol-related cause for AP. The above findings justify larger prospective studies into the independent effect of the above covariates on pancreatic hormones after an attack of AP.

Overall, our findings suggest that elevated insulin and glucagon levels could be reasonably accurate predictors of NOPAP among normoglycaemic people after an attack

**Table 3** Effect of covariates on pancreatic and gut hormones in the study groups[a].

| Hormone | NAP Covariate | B (95% CI) | $R^2$ | APAP Covariate | B (95% CI) | $R^2$ | NOPAP Covariate | B (95% CI) | $R^2$ |
|---|---|---|---|---|---|---|---|---|---|
| Insulin | Cholecystectomy | 0.91 (0.18, 1.64) | 0.187 | Recurrence (yes) | 1.25 (0.02, 2.48) | 0.331 | Sex (women) | 1.05 (0.38, 1.73) | 0.650 |
| | | | | | | | BMI (overweight/obese) | −0.81 (−1.60, −0.02) | |
| | | | | | | | Smoking (yes) | 1.22 (0.39, 2.05) | |
| | | | | | | | Recurrence (yes) | −0.95 (−1.76, −0.13) | |
| | | | | | | | Aetiology (other) | 1.66 (0.56, 2.75) | |
| C-peptide | – | | | – | | | – | | |
| Amylin | Sex (women) | 1.30 (0.19, 2.41) | 0.213 | Aetiology (alcohol-related) | −1.15 (−2.06, −0.25) | 0.317 | Recurrence (yes) | −1.48 (−2.42, −0.54) | 0.453 |
| Glucagon | – | | | – | | | Age (young adults) | 0.97 (0.46, 1.49) | 0.574 |
| GIP | – | | | – | | | Age (older adults) | 0.97 (0.36, 1.59) | 0.694 |
| | | | | | | | Aetiology (other) | 1.20 (0.41, 1.98) | |
| GLP-1 | – | | | Cholecystectomy | −0.70 (−1.38, −0.02) | 0.205 | – | | |
| PPY | Age (young adults) | −0.86 (−1.56, −0.17) | 0.223 | Aetiology (biliary) | −0.89 (−1.68, −0.09) | 0.225 | – | | |
| | Recurrence (yes) | −0.95 (−1.80, −0.11) | | | | | | | |
| PYY | Age (young adults) | −0.97 (−1.72, −0.22) | 0.185 | – | | | – | | |

[a]Only covariates that showed a significant association (P < 0.05) with the studied hormones in the respective groups are presented.
GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide-1; PPY, pancreatic polypeptide; PYY, peptide YY.

of AP. A key strength of our study is that we purposely included a group of AP patients with antecedent prediabetes (defined as HbA1c between 39 and 47 mmol/mol (5.7–6.4%)) at baseline and who did not develop new-onset diabetes during follow-up. Contrary to the NOPAP group, fasting insulin and glucagon were not statistically significantly associated with the APAP group. This means that the two hormones are positioned well to predict incident, not prevalent, prediabetes in people with a history of AP. Further, a ROC curve analysis showed that insulin and glucagon combined had an AUC (95% CI) of 0.74 (0.60, 0.87) in predicting NOPAP. This suggests that the two hormones are accurate enough to consider their use in a routine clinical settings (following external validation of our findings) (50). That is why we calculated cut-off thresholds for insulin and glucagon. It is argued that the most clinically useful accuracy metric in the context of predicting NOPAP is negative predictive value (NPV) – the proportion of individuals without the disease (i.e. NOPAP) identified correctly (23). In the present study, the cut-off value of 175 pmol/L for insulin yielded an NPV of 83%. This means that more than four out of five normoglycaemic AP patients with baseline insulin values <175 pmol/L will be correctly identified as those who will not develop NOPAP. An important practical point is that, although the above cut-off value for insulin was derived empirically, it is very similar to previously reported diagnostic thresholds for hyperinsulinaemia (25), indicating that diagnosing hyperinsulinaemia in a normoglycaemic AP patient heralds the future risk of NOPAP. Glucagon yielded a predictive accuracy similar to the one of insulin (NPV of 79%). Specifically, four out of five normoglycaemic AP patients with baseline glucagon values < 21.4 ng/L will be correctly identified as those who will not develop NOPAP. The practical application of the above findings is that, once externally validated, insulin and glucagon measurements can be adopted for use in routine clinical practice, enabling health care professionals to triage AP patients according to the risk of future blood glucose derangements and to offer apposite strategies to prevent NOPAP and its associated metabolic abnormalities. It is also worth noting that, although pancreatic polypeptide and peptide YY did not show a statistically significant association with the NOPAP group, the ROC curves for these hormones were not dissimilar from the ROC curves for glucagon (Fig. 1). While our findings did not provide conclusive evidence on the role of pancreatic polypeptide and peptide YY as predictors of NOPAP, evidence from earlier cross-sectional studies suggests that the gut-brain axis plays a functional role in post-pancreatitis diabetes mellitus (31, 51). Additional research in larger prospective cohorts is required to investigate the role of these hormones in NOPAP.

Several limitations of the study need to be acknowledged. First, the study sample size was relatively small. Yet, significant findings in the NOPAP group suggest that the effect size of the associations of insulin and glucagon is large. At the same time, it is possible that the effect size of the associations between some of the other hormones (e.g. pancreatic polypeptide and peptide YY) and the NOPAP group might have not been large enough to be detected in the present study. Further research is warranted to investigate the above associations in sufficiently powered large-scale studies. Second, one could argue that pancreatitis-related characteristics (e.g. severity of AP) might have affected the studied associations. However, by design, we only included participants with non-necrotising AP. Third, we excluded participants who progressed to new-onset diabetes during follow-up. This was done on purpose to ensure that participants were in the early stages of their disease progression and the associated (patho)physiological changes were relatively homogeneous. The present study sets the stage for investigating changes in pancreatic and gut hormones as predictors of new-onset diabetes after AP. Last, all laboratory measurements were done in the fasted state only. This is because the study was designed keeping in mind the practicality of its findings to be implemented in routine clinical practice. However, it is appreciated that deeper insights into the pathogenesis of blood glucose derangements in the post-pancreatitis setting will be gained in studies that employ oral glucose tolerance test or euglycaemic-hyperinsulinaemic clamp test.

In conclusion, elevated fasting levels of insulin and glucagon in normoglycaemic individuals with non-necrotising AP at baseline portend a heightened risk for NOPAP. The specific thresholds for insulin and glucagon reported in the present study pave the way for operationalising the measurements of plasma insulin and glucagon in routine clinical practice with a view to identifying AP patients who are at high risk of future derangements of glucose metabolism.

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