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Original Article

Beta-cell function and glucose metabolism in patients with chronic pancreatitis

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ABSTRACT

Aims: Chronic pancreatitis (CP) is – along with acute pancreatitis - the most frequent cause of diabetes of the exocrine pancreas (DEP). Although insulin deficiency is widely accepted as the major feature of DEP, it is still unclear whether diabetes associated with CP is characterized by additional or different functional defects of the insulin secretory machinery. To identify possible functional defects specifically induced by CP, we performed a cross-sectional study in individuals with normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and diabetes mellitus (DM) comparing patients with and without CP (CP vs. NCP).

Methods: We administered an oral glucose tolerance test (OGTT) to all participants and, according to their glucose tolerance, classified them as NGT, IGT and DM. Insulin sensitivity and beta-cell functional parameters were derived from OGTT, hyperglycemic clamp and hyperinsulinemic euglycemic clamp.

Results: Studying 146 subjects, we found that beta-cell function and insulin secretion were significantly lower in CP compared to NCP patients. However, when we classified the subjects according to OGTT-derived glucose tolerance, we found no differences in beta-cell function or in insulin sensitivity between CP and NCP with the same glucose tolerance status. Of note, we found that arginine-stimulated insulin secretion is reduced only in subjects with CP and DM compared to NCP subjects with DM.

Conclusions: Patients with CP had no specific alterations in insulin secretion and beta-cell function. However, in patients diagnosed with diabetes, we found a lower arginine-stimulated insulin secretion, a marker of reduced functional mass.

1. Introduction

Diabetes of the exocrine pancreas (DEP), - also defined as pancreatic, pancreatogenic, or type 3c diabetes - arises from the structural or functional loss of insulin secretion secondary to exocrine pancreatic diseases [1,2]. The most common etiologies of DEP are acute and chronic pancreatitis (CP) followed by pancreatic ductal adenocarcinoma [2]. The widely accepted pathophysiology for DEP is insulin deficiency,

Abbreviations: CP, chronic pancreatitis; DEP, diabetes of the exocrine pancreas; DM, diabetes mellitus; HC, hyperglycemic clamp; HEC, hyperinsulinemic euglycemic clamp; IGT, impaired glucose tolerance; NCP, without chronic pancreatitis; ISR, insulin secretion rate; GS, glucose sensitivity; NGT, normal glucose tolerant; OGTT, oral glucose tolerance test; RS, rate sensitivity.

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with two potential mechanisms responsible for relative or absolute insulin deficit: the inflammatory environment and extensive fibrosis of the exocrine pancreas [2]. Although insulin deficiency is widely accepted as the major feature of DEP, it is still unclear whether diabetes caused by CP is characterized by additional or different functional defects of the insulin secretory machinery. Studies investigating endocrine pancreatic deficiency during CP often lack accurate metabolic evaluation and/or appropriate control groups [3–6]. Further, in patients without CP (NCP), impaired glucose tolerance (IGT) or diabetes mellitus (DM) are associated with specific changes in insulin secretion, namely, altered glucose sensitivity, rate sensitivity (among others), which explain disease progression [7–15]. However, it is unknown whether patients with CP share these specific alterations in insulin secretion or, as generally believed, their progression to diabetes is solely due to insufficient insulin. Since in NCP, defects in insulin secretion are progressive and differ according to the degree of glucose tolerance, it is fundamental to compare patterns of insulin secretion in CP and NCP patients with the same glucose tolerance. Identifying any differences, will allow us to pinpoint the specific mechanism through which chronic pancreatitis causes diabetes, and eventually treat it.

In this study, using oral glucose tolerance test, hyperinsulinemic euglycemic clamp, hyperglycemic clamp and model-derived measures of beta-cell function and insulin secretion *in vivo*, we assessed pancreatic endocrine function in individuals with (CP) and without (NCP) chronic pancreatitis at different metabolic states.

2. Methods

One hundred and forty-six patients (73 females, 73 males) with pancreatic diseases and indication for surgery were recruited from the Digestive Surgery Unit and studied at the Centre for Endocrine and Metabolic Diseases Unit at the Agostino Gemelli University Hospital, from January 2017 to December 2022. Indications for surgery were pancreatic adenocarcinoma, periampullary tumors, biliary tract tumors, pancreatic intraductal papillary tumors, mucinous cystic neoplasm of the pancreas, and nonfunctional pancreatic neuroendocrine tumors (Table 1).

The study protocol (ClinicalTrials.gov NCT02175459) was approved by the Ethical Committee Fondazione Policlinico Universitario Agostino Gemelli IRCCS – Università Cattolica del Sacro Cuore (P/656/CE2010 and 22,573/14), and all participants provided written informed consent, followed by a comprehensive medical evaluation.

Only patients with normal cardiopulmonary and kidney function, as determined by medical history, physical examination, electrocardiography, estimated glomerular filtration rate and urinalysis were included. We excluded subjects taking medications that affect glucose metabolism.

All subjects underwent metabolic evaluation by standard 75 g oral glucose tolerance test (OGTT), seventy-four subjects underwent hyperglycemic clamp (HC) and sixty-eight subjects underwent hyperinsulinemic euglycemic clamp (HEC) (Fig. S1).

To define the relationship between CP and endocrine pancreas function, subjects were divided into two groups based on medical history: with chronic pancreatitis CP ($n = 50$) vs no chronic pancreatitis NCP ($n = 96$). All patients with chronic pancreatitis were seen in consultation and clinical history was reviewed by a multi-disciplinary team. Chronic pancreatitis diagnosis was based on at least one of the following, as previously described [16,17]: (1) documented episodes of recurrent acute pancreatitis (amylase/lipase >3x ULN) with progression to characteristic chronic abdominal pain; (2) functional studies supporting chronic pancreatitis (low fecal elastase); (3) imaging studies supporting chronic pancreatitis (according to Cambridge Classification) [18], details reported in Table S1.

Patients with chronic pancreatitis and exocrine pancreas deficiency were treated with pancreatic enzyme replacement therapy, as previously described (Table S1) [19].

Diabetes mellitus was diagnosed according to American Diabetes Association Guidelines [20], while for the NCP group we selected only subjects who had been diagnosed with diabetes at least two years prior to enrolment, to avoid any confounders related to pancreatic tumors. In the CP group, subjects with diabetes had been diagnosed either concomitantly with chronic pancreatitis or within two years from chronic pancreatitis diagnosis.

Table 1
Prevalence of pancreatic disease with indications for surgery in CP vs NCP.

Indications for surgery	CP ($n = 50$)	NCP ($n = 95$)
Pancreatic adenocarcinma	68 % ($n = 34$)	61 % ($n = 58$)
Periampullary tumors	8 % ($n = 4$)	12.6 % ($n = 12$)
Biliary tract tumors	4 % ($n = 2$)	5.3 % ($n = 5$)
Pancreatic intraductal papillary tumors	6 % ($n = 3$)	6.4 % ($n = 6$)
Mucinous cystic neoplasm of the pancreas	2 % ($n = 1$)	2.1 % ($n = 2$)
Nonfunctional pancreatic neuroendocrine tumors	12 % ($n = 6$)	12.6 % ($n = 12$)

2.1. Oral glucose tolerance test

A standard 75 g oral glucose tolerance test was administered after a 12 h overnight fast with measurements of glucose, insulin and C-peptide at 0, 30, 60, 90, 120 min after the glucose load.

Based on OGTT, we classified the population as normal glucose tolerant (NGT) if 2-hour post load glucose was below 140 mg/dl, impaired glucose tolerant (IGT) if 2-hour post load glucose was 140–199 mg/dl, and diabetes mellitus (DM) if 2-hour post load glucose was higher or equal to 200 mg/dl [20].

During OGTT, insulin secretion was derived from C-peptide levels by deconvolution. Beta-cell glucose sensitivity (GS), i.e., the slope of the relationship between insulin secretion and glucose concentration, was estimated from the OGTT by modeling, as previously described [21,22]. Rate sensitivity (RS), also estimated from OGTT modeling, is a beta-cell functional parameter that represents the dependence of the insulin secretion rate (ISR) on the rate of change in glucose concentration and is related to early insulin release.

2.2. Hyperinsulinemic euglycemic clamp

The HEC test was administered after a 12 h overnight fast using an insulin infusion of 40 mIU/min/m^2 of body surface according to DeFronzo and colleagues [23]. A primed-constant infusion of insulin was administered (Actrapid HM, Novo Nordisk, Copenhagen, Denmark). The variable priming insulin infusion lasted 10 min; in the meantime, a variable infusion of 20 % glucose was started via a separate infusion pump and the rate was adjusted, based on plasma glucose samples drawn every 5 min, to maintain plasma glucose concentration at each participant's fasting plasma glucose level. Whole-body peripheral glucose utilization was calculated during the last 30 min period of the steady-state insulin infusion and was measured as the mean glucose infusion rate (as mg·Kg^{-1}·min^{-1}).

2.3. Hyperglycemic clamp

In the HC procedure plasma glucose was clamped at a stable level of 125 mg/dl above fasting blood glucose concentration. The HC was started with a bolus dose of 200 mg/mL dextrose (150 mg/kg) administered via the antecubital vein. Blood was drawn from a cannulated dorsal hand vein on the opposite arm. Every 5 min, venous plasma glucose was measured with a glucose analyzer and the infusion of 20 % glucose was adjusted to achieve a stable glucose level of 125 mg/dl above the fasting value. Serum samples for insulin and C-peptide were drawn at 0, 2.5, 5, 7.5, 10, 15, 30, 60, 90, 120, 130, 140, and 150 min. At 120 min, a 5 g arginine bolus was administered to measure maximum C-peptide secretory capacity at a steady-state blood glucose concentration

of 125 mg/dl above the fasting value. Arginine-stimulated beta-cell secretory capacity was calculated as the difference between 130-minute C-peptide and 120-minute C-peptide levels. ISR was derived from C-peptide levels by deconvolution [21].

The first-phase insulin secretion response was calculated as the mean incremental insulin secretion between 0 and 5 min, when ISR had fallen from the initial peak to a nadir in all subjects. Second-phase insulin secretion was calculated as the increment in insulin secretion during the last 20 min of the HC above basal insulin secretion. Beta-cell Glucose Sensitivity (GS), i.e., the slope of the relationship between insulin secretion and glucose concentration, was estimated from the hyperglycemic clamp as the ratio of the increments from baseline of insulin secretion and glucose concentration [21,22].

2.4. Statistical analysis

Categorical variables were described by absolute frequencies and percentages, while the continuous variables were synthesized through mean and standard errors and/or median and interquartile range, as appropriate. Normal distribution of the data was evaluated using the Shapiro-Wilk test and Q-Q plot (quantile-quantile plot). Quantitative variables were compared by using T-test and/or non-parametric Mann-Whitney U test, as appropriate. To investigate the differences between groups, a further subgroup analysis was performed.

Thus, logistic regression analysis was performed to evaluate the associations between beta-cell function indexes (RS, GS, arginine-stimulated insulin secretion and glucose uptake) and chronic pancreatitis (CP vs NCP). The outcome variable in the logistic regression model is the factor (dichotomous variable) chronic pancreatitis (composed of two levels CP and NCP) so both CP and NCP groups were considered in this analysis. Firstly, ordinary models were fitted and effects of the predictors on the outcome variable were evaluated in terms of odds ratio (OR), P values and 95 % confidence intervals (95 % CI). Subsequently, multivariable model was also fitted by adjusting for clinical and demographic variables such as age, gender, and BMI. At this stage, the effects of the predictors were conditional, i.e., by providing the expected outcome variation (in OR terms) per unit increase of predictor, while keeping the others in the built-in model fixed. In addition, logistic regression models were fitted for each stratum of glucose tolerance (NGT, IGT, DM) to evaluate the association between chronic pancreatitis (CP vs NCP) and beta-cell function indexes, adjusting for age, gender and BMI.

P values under 0.05 were considered statistically significant. Analyses were performed using R version 4.2.1 (R cran, R Core Team, 2022).

3. Results

3.1. Subjects with CP had lower basal insulin secretion rate and reduced OGTT-derived beta-cell glucose sensitivity compared to controls

We evaluated anthropometric characteristics for the entire population enrolled (Table 2), and found that NCP subjects were older than CP (**$p = 0.011$**) but comparable for gender and BMI. Prevalence of underlying disease/neoplasms was similar between the two groups (Table 1). Table 3 shows indexes of beta-cell function and insulin sensitivity derived by OGTT, HC and HEC in individuals with and without chronic pancreatitis (CP vs NCP), while baseline characteristics of the population based on presence or absence of chronic pancreatitis are shown in Table S2.

OGTT derived glucose (Fig. S2a) insulin (Fig. S2b) and C-peptide (Fig. S2c) curves are shown in Fig. S2. At OGTT, total ISR was higher in NCP than CP (CP 30.17 (24.42; 43.07) vs NCP 43.98 (28.79; 57.22) nmol·m^{-2}; **$p = 0.004$**) but there were no differences in basal ISR (CP 57.76 (46.02; 89.67) vs NCP 71.34 (48.04; 92.94) pmol·min^{-1}·m^{-2}; $p = 0.374$). NCP participants had better insulin secretion during OGTT (Table 3) and increased beta-cell GS compared to the CP group (CP 48.89 (18.81; 66.92) vs NCP 67.14 (35.21; 93.71) pmol·min^{-1}·m^{-2}·mM^{-1}; **$p = 0.017$**), but no differences were found in

Table 3
Parameters of beta-cell function and insulin sensitivity derived from oral glucose tolerance test (OGTT) ($n = 146$), hyperglycemic clamp (HC) ($n = 74$) and hyperinsulinemic euglycemic clamp ($n = 68$), in presence or absence of chronic pancreatitis (CP vs NCP).

	CP	NCP	p-value
basal ISR OGTT (pmol·min^{-1}·m^{-2})	57.76 (46.02; 89.67)	71.34 (48.04; 92.94)	0.374
total ISR OGTT (nmol·m^{-2})	30.17 (24.42; 43.07)	43.98 (28.79; 57.22)	**0.004**
GS OGTT (pmol·min^{-1}·m^{-2}·mM^{-1})	48.89 (18.81; 66.92)	67.14 (35.21; 93.71)	**0.017**
RS OGTT (pmol·m^{-2}·mM^{-1})	105.5 (0.00; 570.9)	211.6 (0.00; 641.2)	0.382
ISRb (pmol·min^{-1}·m^{-2})	66.88 (43.09; 78.99)	63.01 (41.35; 74.75)	0.666
ISR1abs (pmol·min^{-1}·m^{-2})	181 (88.77; 339.1)	198.6 (112.9; 269.7)	0.817
ISR1inc (pmol·min^{-1}·m^{-2})	103.6 (28.33; 283.5)	123.85 (53.84; 206.73)	0.882
ISR2abs (pmol·min^{-1}·m^{-2})	295 (190.8; 374.6)	341 (227.19; 462.32)	0.284
ISR2inc (pmol·min^{-1}·m^{-2})	238 (152.7; 305.6)	275 (169.60; 389.73)	0.306
GS (pmol·min^{-1}·m^{-2}·mM^{-1})	38.7 (17.99; 64.76)	42.8 (24.40; 69.15)	0.541
ARG (pmol/l)	894 (397.2; 1721.2)	1125 (761.3; 1688.1)	0.246
Glucose uptake (mg·Kg^{-1}·min^{-1})	3.61 (2.87; 4.77)	3.63 (2.70; 5.63)	0.698

Variables are expressed as median and interquartile range and comparisons were performed using Mann-Whitney test.
ISR: insulin secretion rate; GS: glucose sensitivity; RS: rate sensitivity; ISRb: basal insulin secretion rate; ISR1abs: insulin secretion rate of absolute 1st phase response; ISR1inc: insulin secretion rate of incremental 1st phase response; ISR2abs: insulin secretion rate of absolute 2nd phase response; ISR2inc: insulin secretion rate of incremental 2nd phase response; ARG: arginine-stimulated insulin secretion.

Table 2
Characteristics of study population in CP vs NCP stratified according to their glucose tolerance (NGT, IGT, DM). Data are expressed as numbers and percentages (glucose tolerance and gender) or mean± standard deviation (age, BMI).

$N = 146$	CP (50)			P-value	NCP (96)			P-value
Glucose tolerance	NGT 13 (26 %)	IGT 12 (24 %)	DM 25 (50 %)		NGT 24 (25 %)	IGT 34 (35 %)	DM 38 (40 %)	
Age	61 (±14.39)	62.50 (±13.91)	67.12 (±7.59)	0.237	64.38 (±10.81)	64.15 (±12.34)	70.82 (±7.38)	**0.011**
Gender	M; F 7; 6	M; F 5; 7	M; F 13; 12	0.798	M; F 8; 16	M; F 18; 16	M; F 22; 16	0.154
BMI	24.35 (±3.32)	28.44 (±9.68)	26.15 (±4.66)	0.243	24.28 (±3.54)	24.92 (±4.24)	25.20 (±4.67)	0.718

NGT: normal glucose tolerance; IGT: impaired glucose tolerance; DM: diabetes mellitus; BMI: body mass index (kg/m^2).

RS, demonstrating partial alteration of beta-cell function in the CP group, compared with NCP (Table 3).

No differences were detected in basal ISR, and first and second phase response of ISR, evaluated during HC (Table 3). Further, there were no differences between the two groups in GS and arginine-stimulated insulin secretion (Table 3).

Finally, as shown in Table 3, there were no significant differences in insulin sensitivity at HEC, expressed as glucose uptake, between the two groups.

The analysis of the entire cohort revealed a significantly reduced beta-cell function compared to the NCP group only in response to glucose load, with no differences in insulin sensitivity, in ISR, GS and arginine-stimulated insulin secretion during HC.

Severity of chronic pancreatitis was scored according to Cambridge Classification [18] and prevalence of mild, moderate and severe chronic pancreatitis in CP group is shown in Table S1.

To specifically examine the impact of chronic pancreatitis at any metabolic status, we classified the population according to their glucose tolerance (NGT, IGT, DM) and compared parameters of beta-cell function, insulin secretion and insulin sensitivity for the same glucose tolerance class.

3.2. Chronic pancreatitis does not alter beta-cell function in patients with prediabetes

Among NGT individuals, glucose (Fig. S3a), insulin (Fig. S3b) and C-peptide (Fig. S3c) levels during OGTT were similar in CP compared to NCP group. At OGTT there were no differences in ISR, in GS or in RS, as shown in Table 4. The HC yielded similar results: no differences emerged between CP and NCP in basal ISR, first-phase response and second-phase response of ISR, in GS or in arginine-stimulated insulin secretion (Table 4).

At HEC, no differences were found in insulin sensitivity between the two groups (Table 4).

These results demonstrated that there were no differences in beta-cell function and in insulin sensitivity in normal glucose tolerant subjects with and without diagnosis of exocrine pancreas disease.

Among IGT group, glucose (Fig. S4a), insulin (Fig. S4b) and C-peptide (Fig. S4c) levels during OGTT were comparable. At OGTT no difference was found in ISR between the two groups, and beta-cell function indexes were comparable both for GS and RS, as shown in Table 4. At HC, basal ISR was similar in CP and NCP subjects as first-phase response and second-phase response of ISR (Table 5). Moreover, GS was comparable in the two groups as arginine-stimulated insulin secretion, demonstrating no differences in beta-cell function and beta-cell mass at HC (Table 5).

At HEC, insulin sensitivity, measured as glucose uptake, was comparable in the two groups, as shown in Table 5.

These results demonstrate that CP subjects with impaired glucose tolerance have similar alterations in insulin secretion and insulin sensitivity to NCP subjects with the same glucose tolerance, suggesting that CP does not significantly impact islet function.

3.3. Patients with CP and diabetes had a decreased arginine-stimulated insulin secretion compared to NCP

In DM patients, evaluation of OGTT responses showed no differences in glucose (Fig. S5a), insulin (Fig. S5b) and C-peptide (Fig. S5c) levels. HbA_{1c} was also comparable in the two groups (CP 48±3 mmol/ml vs NCP 50±3 mmol/ml; $p = 0.658$). Prevalence of diabetes treatments in the two groups is shown in Table S3.

At OGTT, the two groups had comparable basal ISR and total ISR

Table 4
Parameters of beta-cell function and insulin sensitivity derived from oral glucose tolerance test (OGTT), hyperglycemic clamp (HC) and hyperinsulinemic euglycemic clamp, in presence or absence of chronic pancreatitis (CP vs NCP), in NGT group.

	CP	NCP	p-value
basal ISR OGTT ($pmol \cdot min^{-1} \cdot m^{-2}$)	48.51 (41.46; 59.84)	59.54 (45.30; 78.49)	0.371
total ISR OGTT ($nmol \cdot m^{-2}$)	35.43 (27.42; 43.05)	43.39 (29.50; 55.59)	0.169
GS OGTT ($pmol \cdot min^{-1} \cdot m^{-2} \cdot mM^{-1}$)	65.18 (57.91; 91.60)	93.93 (76.85; 112.7)	0.084
RS OGTT ($pmol \cdot m^{-2} \cdot mM^{-1}$)	557.73 (0.39; 966.08)	359.28 (36.86; 962.57)	0.826
ISRb ($pmol \cdot min^{-1} \cdot m^{-2}$)	54.12 (42.20; 70.52)	51.74 (32.17; 66.13)	0.646
ISR1abs ($pmol \cdot min^{-1} \cdot m^{-2}$)	202.8 (159.2; 348.6)	259.89 (203.77; 339.3)	0.799
ISR1inc ($pmol \cdot min^{-1} \cdot m^{-2}$)	111.4 (105.1; 306.4)	201.02 (164.9; 286.7)	0.574
ISR2abs ($pmol \cdot min^{-1} \cdot m^{-2}$)	307.5 (286.7; 339.2)	391.7 (327.5; 445.2)	0.129
ISR2inc ($pmol \cdot min^{-1} \cdot m^{-2}$)	244.5 (237.0; 247.8)	349.9 (287.5; 399.5)	0.063
GS ($pmol \cdot min^{-1} \cdot m^{-2} \cdot mM^{-1}$)	54.73 (43.91; 64.77)	67.93 (56.63; 82.30)	0.442
ARG (pmol/l)	1622 (1556; 1820)	1257.8 (943.4; 2217.7)	0.532
Glucose uptake ($mg \cdot Kg^{-1} \cdot min^{-1}$)	3.220 (3.030; 4.170)	5.135 (3.550; 6.385)	0.505

Variables are expressed as median and interquartile range and comparisons were performed using Mann-Whitney test.
ISR: insulin secretion rate; GS: glucose sensitivity; RS: rate sensitivity; ISRb: basal insulin secretion rate; ISR1abs: insulin secretion rate of absolute 1st phase response; ISR1inc: insulin secretion rate of incremental 1st phase response; ISR2abs: insulin secretion rate of absolute 2nd phase response; ISR2inc: insulin secretion rate of incremental 2nd phase response; ARG: arginine-stimulated insulin secretion.

Table 5
Parameters of beta-cell function and insulin sensitivity derived from oral glucose tolerance test (OGTT), hyperglycemic clamp (HC) and hyperinsulinemic euglycemic clamp, in presence or absence of chronic pancreatitis (CP vs NCP), in IGT group.

	CP	NCP	p-value
basal ISR OGTT ($pmol \cdot min^{-1} \cdot m^{-2}$)	74.64 (65.39; 87.32)	77.39 (51.52; 87.76)	0.883
total ISR OGTT ($nmol \cdot m^{-2}$)	39.01 (31.58; 49.35)	47.58 (32.49; 65.25)	0.520
GS OGTT ($pmol \cdot min^{-1} \cdot m^{-2} \cdot mM^{-1}$)	59.83 (48.41; 78.32)	78.55 (46.57; 94.91)	0.445
RS OGTT ($pmol \cdot m^{-2} \cdot mM^{-1}$)	158.4 (0.0; 617.9)	211.8 (0.00; 709.1)	0.779
ISRb ($pmol \cdot min^{-1} \cdot m^{-2}$)	69.40 (51.93; 77.69)	61.61 (45.91; 72.14)	0.645
ISR1abs ($pmol \cdot min^{-1} \cdot m^{-2}$)	310.8 (255.0; 399.0)	222.68 (144.51; 308.9)	0.091
ISR1inc ($pmol \cdot min^{-1} \cdot m^{-2}$)	255.3 (208.19; 326.58)	150.76 (81.05; 231.20)	0.057
ISR2abs ($pmol \cdot min^{-1} \cdot m^{-2}$)	386.3 (307.3; 668.5)	396.9 (277.2; 496.6)	0.498
ISR2inc ($pmol \cdot min^{-1} \cdot m^{-2}$)	316.9 (255.4; 579.3)	302.8 (214.2; 422.7)	0.431
GS ($pmol \cdot min^{-1} \cdot m^{-2} \cdot mM^{-1}$)	57.35 (39.51; 111.25)	53.10 (31.07; 88.47)	0.355
ARG (pmol/l)	1721.2 (827.5; 2184.6)	1341 (1101; 1713)	0.975
Glucose uptake ($mg \cdot Kg^{-1} \cdot min^{-1}$)	3.775 (2.877; 4.947)	3.730 (2.930; 5.495)	0.974

Variables are expressed as median and interquartile range and comparisons were performed by Mann-Whitney test.
ISR: insulin secretion rate; GS: glucose sensitivity; RS: rate sensitivity; ISRb: basal insulin secretion rate; ISR1abs: insulin secretion rate of absolute 1st phase response; ISR1inc: insulin secretion rate of incremental 1st phase response; ISR2abs: insulin secretion rate of absolute 2nd phase response; ISR2inc: insulin secretion rate of incremental 2nd phase response; ARG: arginine-stimulated insulin secretion.

Table 6
Parameters of beta-cell function and insulin sensitivity derived from oral glucose tolerance test (OGTT), hyperglycemic clamp (HC) and hyperinsulinemic euglycemic clamp, in presence or absence of chronic pancreatitis (CP vs NCP), in DM group.

	CP	NCP	p-value
basal ISR OGTT ($pmol \cdot min^{-1} \cdot m^{-2}$)	55.40 (43.23; 107.26)	73.36 (49.26; 116.09)	0.469
total ISR OGTT ($nmol \cdot m^{-2}$)	26.57 (18.73; 30.43)	44.15 (21.33; 51.14)	0.080
GS OGTT ($pmol \cdot min^{-1} \cdot m^{-2} \cdot mM^{-1}$)	17.97 (14.96; 29.62)	33.83 (15.03; 59.14)	0.061
RS OGTT ($pmol \cdot m^{-2} \cdot mM^{-1}$)	38.69 (0.00; 187.91)	0.00 (0.00; 249.4)	0.892
ISRb ($pmol \cdot min^{-1} \cdot m^{-2}$)	66.09 (45.92; 78.99)	71.57 (45.36; 87.43)	0.879
ISR1abs ($pmol \cdot min^{-1} \cdot m^{-2}$)	87.18 (60.01; 135.04)	116.15 (73.48; 168.69)	0.531
ISR1inc ($pmol \cdot min^{-1} \cdot m^{-2}$)	21.45 (15.93; 64.50)	48.74 (19.96; 72.84)	0.350
ISR2abs ($pmol \cdot min^{-1} \cdot m^{-2}$)	199.89 (132.65; 261.66)	262.67 (178.75; 362.83)	0.448
ISR2inc ($pmol \cdot min^{-1} \cdot m^{-2}$)	133.43 (60.10; 212.68)	184.66 (107.74; 288.53)	0.350
GS ($pmol \cdot min^{-1} \cdot m^{-2} \cdot mM^{-1}$)	16.71 (8.78; 24.88)	21.51 (12.42; 39.92)	0.621
ARG (pmol/l)	397.2 (132.4; 662.0)	794.4 (604.1; 1133.7)	**0.023**
Glucose uptake ($mg \cdot Kg^{-1} \cdot min^{-1}$)	3.240 (2.565; 3.985)	3.305 (2.450; 4.202)	0.898

Variables are expressed as median and interquartile range and comparisons were performed using Mann-Whitney test.
ISR: insulin secretion rate; GS: glucose sensitivity; RS: rate sensitivity; ISRb: basal insulin secretion rate; ISR1abs: insulin secretion rate of absolute 1st phase response; ISR1inc: insulin secretion rate of incremental 1st phase response; ISR2abs: insulin secretion rate of absolute 2nd phase response; ISR2inc: insulin secretion rate of incremental 2nd phase response; ARG: arginine-stimulated insulin secretion.

(Table 6). Furthermore, GS and RS also did not differ significantly in the two groups (Table 6), thus there were no actual differences in beta-cell function evaluated at OGTT.

These data were confirmed at HC, as no differences were found in ISR, with no significant difference in insulin secretion in CP vs NCP with diabetes (Table 5). Beta-cell GS also did not differ between the two groups (CP 16.71 (8.78; 24.88) vs NCP 21.51 (12.42; 39.92) $pmol \cdot min^{-1} \cdot m^{-2} \cdot mM^{-1}$; $p = 0.621$), while arginine-stimulated insulin secretion was significantly lower in the CP group (CP 397.2 (132.4; 662.0) vs NCP 794.4 (604.1; 1133.7) pmol/l; $\boldsymbol{p = 0.023}$) (Table 5), likely demonstrating a lower beta-cell response to a threshold stimulation in subjects with CP.

At HEC, insulin sensitivity did not differ between the two groups, as shown in Table 6.

3.4. Arginine-stimulated insulin secretion was the main predictor of diabetes in individuals with chronic pancreatitis

To investigate the associations between beta-cell function indexes (RS, GS, arginine-stimulated insulin secretion and glucose uptake) and presence of chronic pancreatitis, we performed a logistic regression analysis (Table S4). While there was no significant relationship between GS, RS and glucose uptake with CP in the glucose tolerance classes, by adjusting for confounding factors such as age, gender and BMI, we found that arginine-stimulated insulin secretion was significantly associated with diabetes diagnosis in CP patients (Table S4). Arginine-stimulated insulin secretion was thus the main predictor of diabetes occurrence in subjects with chronic pancreatitis.

4. Discussion

We found that, in individuals in the same glucose tolerance class, chronic pancreatitis does not significantly impact beta-cell function and insulin secretion. Specifically, in non-diabetic subjects with or without exocrine pancreas disease, we found no differences in beta-cell functional parameters or in insulin sensitivity. In subjects with diabetes mellitus (DM), however, although beta-cell function and insulin sensitivity were similar, arginine-stimulated insulin secretion, (an indirect marker of functioning beta-cell mass) was lower only in subjects with diabetes and chronic pancreatitis.

It is well known that the presence of impaired glucose tolerance (IGT) or DM is associated with specific changes in beta-cell function, namely, altered glucose sensitivity and rate sensitivity (among others) [7–12,24], which explains why, in patients without chronic pancreatitis, insulin secretion is reduced or inappropriate, causing disease progression. Our main aim was to attempt to identify additional or different alterations in beta-cell function and insulin secretion induced by chronic pancreatitis, which could explain a different progression induced by the latter (compared with similar patients without chronic pancreatitis). Since alterations of beta-cell function and insulin secretion are different according to the class of glucose tolerance, to identify any specific alterations eventually induced by chronic pancreatitis we needed to compare patients with CP vs. NCP within the same glucose tolerance class (NGT, IGT, DM).

As already evidenced in previous studies [4–6], when comparing CP vs NCP, independently of glucose tolerance, defects in insulin secretion and beta-cell function were greater in subjects with CP. This difference could also be due to the higher percentage of DM subjects in the CP group compared to the NCP group, since the cohort studied demonstrated a higher prevalence of diabetes in CP subjects. When taking glucose tolerance into account, we found no difference in beta-cell functional parameters and in insulin sensitivity in the non-diabetic (NGT) and prediabetic states (IGT). In CP and NCP subjects with diabetes, we again found comparable altered beta-cell function and insulin sensitivity. However, a lower arginine-stimulated insulin secretion was also present in CP. Arginine is a potent physiologic stimulus for insulin secretion [25] and arginine-stimulated insulin secretion provides a clinical measure of beta-cell functional mass and secretory capacity [26–29]. It has been demonstrated that arginine-stimulated insulin secretion remains present even after glucose stimulated insulin secretion is reduced [30,31]. We found a lower insulin secretion in response to arginine stimulation in subjects with diabetes and CP than in those with diabetes but without CP. Consequently, this measure of functional beta-cell mass seems to be reduced to a greater extent in subjects with DM and CP than in NCP subjects with diabetes.

Our data are in line with previous studies reporting impaired insulin secretion and alteration of insulin sensitivity in CP patients. In a 1968 study [4], patients with CP with different metabolic status had an impaired insulin reserve compared to NGT controls in response to a provocation test with glucagon and tolbutamide after oral glucose load. Some years later, Kalk et al. [32], using the same metabolic investigation, demonstrated that patients with CP had similar incremental insulin response compared to controls, but impaired response at glucose intravenous injection, suggesting a reduced first-phase insulin response [32]. These findings suggest that defects in insulin secretion and/or impaired beta-cell function are the main features of DEP, but whether these defects are different in subjects with the same glucose tolerance has not been investigated.

When comparing CP vs. NCP, we found no differences in insulin secretion and insulin sensitivity in the NGT and IGT glucose tolerance classes. These data are in line with previous studies suggesting that insulin sensitivity is not altered in subjects with chronic pancreatitis [33], but differ from the results obtained by Lundberg R et al. [3], which suggest that CP patients without diabetes are more insulin resistant and have a lower first-phase response compared to controls. In line with a

previous study [32], we found no impaired response to IV arginine stimulation when we compared the overall population or only NGT and IGT subjects.

Consistent with our clinical findings, previous studies investigating pancreas morphology in CP have also suggested that depletion of exocrine tissue is more pronounced than the loss of endocrine islets [34, 35]. Islet cells seem to be less vulnerable to the autodigestive process than exocrine cells, justifying a preferential destruction of acinar tissue during CP. Further, the reduction of pancreas volume, beta-cell area and islet density detected in pancreas samples of CP subjects [36] does not necessarily reflect an impairment in endocrine function.

Although the present study is only cross-sectional, our data suggest that in subjects with CP there are no specific alterations in insulin secretion and beta-cell function, even though we found a reduced functional mass (arginine-stimulated insulin secretion) in patients diagnosed with diabetes. Therefore, the preexisting dysfunctional milieu (independent of pancreatitis) is responsible for the majority of the insulin secretion defects. In other words, in line with our previous results in a human model of acute beta-cell mass reduction [12,37,38], chronic pancreatitis can induce diabetes only in subjects that have an underlying defect in beta-cell function and a concomitant reduction in functioning beta-cell mass. An in-depth metabolic evaluation of patients with exocrine pancreas disease is useful in identifying those subjects with underlying beta-cell dysfunction who are more prone to insulin deficiency.

Even though we cannot exclude different pathophysiological events and disease progression, our study suggests that both type 2 diabetes and diabetes in the context of chronic pancreatitis are characterized by a defect in insulin secretion against a background of insulin resistance. As for type 2 diabetes, a full metabolic evaluation of individuals with DEP may be helpful in assessing the degree of insulin deficiency and the actual need for insulin treatment.

Our study design presents several advantages. Firstly, all individuals enrolled in CP and NCP groups were evaluated not only through medical history, fasting glucose and HbA1c, but also using the gold standard OGTT, HC and HEC, thus allowing us to evaluate insulin secretion, insulin resistance and beta-cell function *in vivo*. Secondly, we also performed the mathematical modelling of insulin secretion, which allowed us to investigate beta-cell function. Finally, stratifying the individuals according to their glucose tolerance, we were able to identify three different conditions and distinguish endocrine functional defects in a homogenous group of non-diabetic, pre-diabetic and diabetic humans with and without exocrine pancreas disease.

Our study also presents some limitations, such as the cross-sectional design, that did not allow us to evaluate the decline in beta-cell function over time. Even though the stratification of individuals according to their glucose tolerance was very useful in analyzing differences in beta-cell function, there were relatively few subjects in each subgroup (CP_NGT, NCP_NGT, CP_IGT, etc.). Also, while all subjects were administered the OGTT, not all subjects were administered the hyperglycemic clamp (68/146) and the hyperinsulinemic euglycemic clamp (74/146), thus reducing the amount of data for each group. Furthermore, as subjects were all recruited from the Digestive Surgery Unit, there could be a potential selection bias. Finally, the relationship between pancreatic adenocarcinoma and diabetes is very complex, exhibiting bidirectional links. We cannot exclude that the presence of diabetes may represent one of the factors that contribute to the development of pancreatic adenocarcinoma in our population.

5. Conclusion

We found that the presence of chronic pancreatitis per se does not induce different or additional alterations in insulin secretion. Patients with chronic pancreatitis and diabetes, however, were also characterized by a reduced functional beta-cell mass, combined with the beta-cell functional defects usually found in type 2 diabetes. A better understanding of the different trajectories leading to endocrine pancreas deficiency will allow us to understand which functional step fails in chronic pancreatitis, and at what stage this progression towards insulin insufficiency is still reversible. Thus, therapeutic strategies aimed at improving the dysfunctional milieu and reducing beta-cell workload may prevent beta-cell failure, and consequently delay insulin deficiency in diabetes of the exocrine pancreas.

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Data availability

The data that support the findings of this study are not openly available due to privacy issues and are available from the corresponding author upon reasonable request.

Authors' contribution statement

GC curated the data and wrote the original draft. GDG curated the data and revised the manuscript. LS curated the data and analyzed the data. GQ curated the data and assessed the methodology. ECN curated the data and revised the manuscript. MB curated and analyzed thedata. FC revised the manuscript. SM curated the data and revised the manuscript. UC curated the data and revised the manuscript. VT assessed methodology and revised the manuscript. AM acquired, analyzed and interpreted data and revised the manuscript. AG validated the data and revised the manuscript. AP revised the manuscript. SA curated data and revised the manuscript. AG analyzed and interpreted data and revised the manuscript. TM conceptualized the study design, obtained funding, analyzed data, supervised the study and revised the manuscript.

GC, AG, and TM are guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of data analyses.

Declaration of competing interest

All authors declare that they have no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejim.2024.06.007.

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