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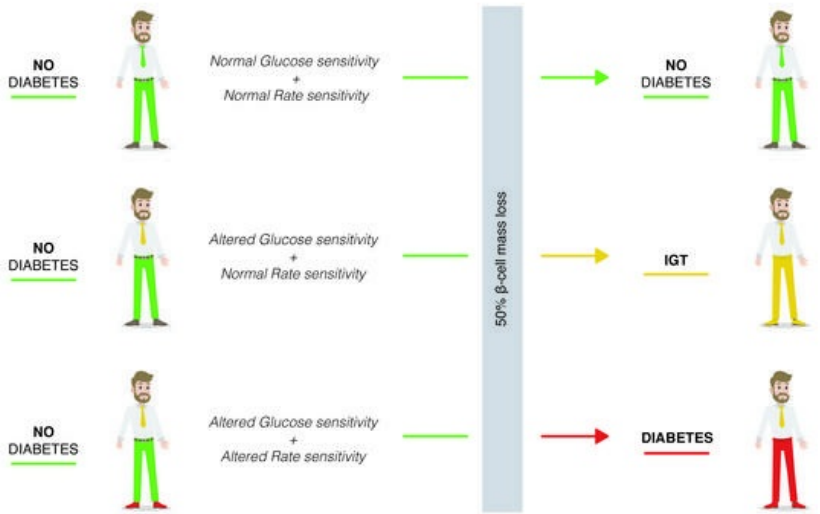
Graphical abstract



Duodenopancreatectomy as a model to demonstrate the fundamental role of dysfunctional β cell in predicting diabetes

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Abstract

Background: The appearance of hyperglycemia is due to insulin resistance, functional deficits in the secretion of insulin and a reduction of β-cell mass. There is a long-standing debate as to the relative contribution of these factors to clinically manifest β-cell dysfunction. The aim of this study was to verify the acute effect of one of these factors, the reduction of β-cell mass, on the subsequent development of hyperglycemia.

Methods: To pursue this aim, non-diabetic patients, scheduled for identical pancreaticoduodenectomy surgery, underwent oral glucose tolerance tests (OGTT) and hyperglycemic clamps (HC), followed by arginine stimulation before and after surgery. Based on post-surgery OGTT, subjects were divided into 3 groups depending on glucose tolerance: normal (post-NGT), impaired (post-IGT) or diabetic (post-DM).

Results: At baseline, the three groups showed similar fasting glucose and insulin levels, however, examining the various parameters, we found that reduced first phase insulin secretion and reduced glucose sensitivity and rate sensitivity were predictors of eventual post-surgery development of impaired glucose tolerance and diabetes.

Conclusion: Despite comparable functional mass and fasting glucose and insulin levels at baseline, and the very same 50% mass reduction, only reduced first phase insulin secretion and glucose sensitivity predicted the appearance of hyperglycemia. These functional alterations could be pivotal to the pathogenesis of type 2 diabetes (T2DM).

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INTRODUCTION

Beta-cell dysfunction and loss of beta-cell mass both contribute to the pathogenesis of hyperglycemia in type 2 diabetes(1). However, while some reports suggest that beta-cell mass loss occurs prior to beta-cell functional impairment, (2-4) others maintain that the decrease in beta-cell mass actually predisposes to impaired glucose homeostasis. (5, 6) Whereas cross-sectional autopsy studies have shown beta-cell loss and increased beta-cell apoptosis in type 2 diabetes, (7) studies examining the timing and relationship between changes in blood glucose, beta-cell morphology, insulin secretion and sensitivity, have identified the functional deficit as dominant and as requisite for the development of hyperglycemia. (8, 9) There is still, therefore, much debate as to whether insulin deficiency and consequent hyperglycemia are the results of compromised beta-cell function, reduced beta-cell mass, and/or a combination of both. (1, 5, 10)

Partial pancreatectomy is a standardized surgical procedure, and all patients undergoing pancreaticoduodenectomy receive virtually the same partial (50%) resection, maintaining almost the same remaining portion of the endocrine pancreas. It is well known that beta-cell mass may differ even within normal glucose tolerance (11), however, whatever the preexisting beta-cell mass may be, the surgical procedure always removes ~50% of it.

We had previously used this technique to demonstrate the extraordinary plasticity of islet and exocrine cells triggered by insulin resistance in order to maintain euglycemia in the pre-diabetic state(11). Although an increased beta-cell workload (insulin resistance) is a risk factor for hyperglycemia, in most individuals there is an adaptive increase in insulin and proinsulin secretion without apparent beta-cell failure(12). As beta-cell function declines in the presence of insulin resistance,(13) a sudden decrease in beta-cell mass compromises beta-cell function and secretory capacity and viability, leading to a progressive loss of beta-cell number and function. Several studies

have examined the metabolic changes occurring following partial pancreatectomy in humans (14, 15) and have suggested that the acute removal of beta-cell mass inevitably accelerates a decline in beta-cell functional capacity, already "stressed" by the attempt to compensate for increasing insulin demand.(7, 16)

In the present study we aim to evaluate: (1) the changes in beta-cell secretory capacity, modelled from an oral glucose tolerance test (OGTT) and hyperglycemic clamp (HC) in non-diabetic individuals before and after acute beta-cell reduction and (2) whether pre-existing insulin secretory defects can predict glucose disarrangements following a reduction in beta-cell mass.

RESULTS

Seventy-eight patients (41 females; 38 males; mean age 64±12 yrs. ±SE) undergoing pylorus-preserving pancreatoduodenectomy were recruited from January 2017 to April 2019 at the Digestive Surgery Unit and studied at the Centre for Endocrine and Metabolic Diseases unit (both at the Agostino Gemelli University Hospital, Rome, Italy). Indications for surgery were: periampullary tumors, pancreatic intraductal papillary tumors, mucinous cystic neoplasm of the pancreas, non-functional pancreatic neuroendocrine tumors. None of the patients enrolled had a known history of diabetes. Only patients with normal cardiopulmonary and kidney function, as determined by medical history, physical examination, electrocardiography, estimated glomerular filtration rate, and urinalysis were included. Altered serum lipase and amylase levels prior to surgery, as well as morphologic criteria for pancreatitis, were considered exclusion criteria. Patients with severe obesity (BMI> 40), uncontrolled hypertension and/or hypercholesterolemia were also excluded. Patients underwent both OGTT and HbA1c testing to exclude diabetes, on the basis of the American Diabetes Association criteria and subjects with fasting glucose above 126 mg/dl, or with 2-hour post-load glucose at baseline above 200 mg/dl, or with HbA1c $\geq$ 48 mmol/mol (6.5%) were excluded from the

study. As can be seen in Fig. 1 CONSORT diagram, only 33 of the 78 non-diabetic subjects underwent both an OGTT and a hyperglycemic clamp with arginine stimulation to evaluate insulin secretion (from C-peptide deconvolution), as described below, both before and ~40 days after surgery. The adequacy of the recovery period was determined by the normalization of inflammatory parameters, such as C-reactive protein, erythrocyte sedimentation rate, stability of weight, absence of symptoms of abnormal intestinal motility, or exocrine pancreatic deficiency. Clinical and metabolic characteristics of study subjects are provided in Table 1.

Identical hemipancreatectomy has different results after surgery

All groups displayed similar levels of fasting glucose (Table 1, p=0.12) and mean glucose at pre-surgery OGTT (Figure 2A); while post-surgery OGTT showed that fasting and mean glucose levels increased significantly only in subjects who went on to develop diabetes ($p<0.01$ for interaction, Figure 2A), as expected according to classification. Moreover, insulin sensitivity, as assessed by the hyperinsulinemic euglycemic clamp (Table 1), fasting and mean insulin levels at OGTT (Figure 2B) were also similar among groups before surgery. Insulinogenic index (Supplementary figure 2, p=0.77) and disposition index (Supplementary figure 3, p=0.27) were also comparable among the groups before surgery.

Although the Matsuda index was not validated in this model, we observed similar Matsuda indexes in all groups before surgery (Supplementary figure 2A, p=0.61) and an increase in Matsuda index in all groups after surgery (Supplementary figure 2A, $p<0.05$), while the insulinogenic index decreased significantly only in IGT and post-DM groups after surgery (Supplementary Figure 2B, p=0.01). Post-surgery OGTT showed that insulin and C-peptide levels had decreased significantly for all groups (Figure 2B and 2C). Although 50% pancreatectomy led to decreased insulin and C-peptide levels in the entire cohort, its effect on change over time in glucose, insulin and C-peptide levels across the 3

groups was significantly different (P<0.01 for the interaction between pancreatectomy, time and glucose tolerance of glucose, insulin and C-peptide levels) (Figure 2).

Functional defects predict diabetes occurrence after hemipancreatectomy

After surgery, beta-cell glucose sensitivity, as a model-based index of beta-cell function derived from OGTT, was significantly reduced across the three groups (post-NGT: 88.2±22.7, post-IGT: 32.1±10.4 and post-DM: 11.3±2.8 pmol·min^{-1}m^{-2}·mM^{-1}, p=0.02; Figure 3A). The comparison of groups before surgery revealed that beta-cell glucose sensitivity was significantly worse in the post-IGT and post-DM groups compared to the post-NGT group (p=0.01 post-NGT vs post-IGT group; p<0.01 post-NGT vs post-DM group, Figure 3A),

We also assessed the model-based rate sensitivity parameter, representing early phase insulin release. We found that rate sensitivity was already reduced before surgery in those subjects who developed diabetes following pancreaticoduodenectomy, (post-NGT: 993±225, post-IGT: 1111±289 and post-DM: 87.2±48.8 pmol·min^{-1}m^{-2}·mM^{-1}, p=0.02; Figure 3B). Post-surgery rate sensitivity differed significantly among the three groups (p<0.01, Figure 3B). Further, we observed that the post-IGT group showed a greater reduction in rate sensitivity compared to post-NGT and post-DM groups (p<0.01 for rate sensitivity post-surgery adjusted for rate sensitivity pre-surgery), suggesting that acute beta-cell mass reduction had a considerable effect on early insulin release only in the post-IGT group.

Impaired first phase insulin secretion, rather than reduced beta-cell mass, predicts diabetes appearance.

To further investigate changes in the different phases of insulin secretion in response to 50% pancreatectomy, we also performed a 2-h hyperglycemic clamp followed by an arginine bolus, which is the gold standard technique to measure insulin secretion, and an indirect measure of beta-cell mass.

Comparison among the three groups revealed that pancreatectomy had a significantly different effect on time-dependent change in insulin and C-peptide levels during hyperglycemic clamp ($P<0.01$ for the interaction between pancreatectomy, time and glucose tolerance of glucose, insulin and C-peptide levels) (Supplementary Figure 4).

Further, we also used mathematical modelling to calculate incremental first and second phase of insulin secretion rate and arginine-stimulated insulin secretion. We observed that the first phase insulin secretion rate (ISR) was significantly lower in post-IGT and post-DM group compared to post-NGT, both before surgery (Figure 4A, $p=0.02$), and after surgery (Figure 4A, $p<0.01$). Despite different levels of first phase ISR both before and after surgery, all groups experienced a similar rate of reduction following surgery ($p=0.20$ for first phase ISR post-surgery adjusted for first phase ISR pre-surgery). Second phase ISR was comparable among groups before surgery (Figure 4B), while significant differences were observed after surgery ($p=0.03$). Further, the post-surgery change in second phase ISR differed significantly among the three groups ($p=0.05$ for second phase ISR post-surgery adjusted for second phase ISR pre-surgery), suggesting that pancreatectomy had a significantly different impact on the second phase ISR.

Despite comparable levels among the three groups before surgery, arginine-stimulated insulin secretion (indirect measure of beta-cell mass) was significantly different among the three groups after surgery (Figure 4C, $p<0.01$). Further, while all groups experienced a reduction after surgery, a greater decrease was observed in the IGT and DM groups compared to NGT (Figure 4C, $p=0.01$). Thus, even though subjects had comparable basal levels of arginine-stimulated insulin secretion and the same proportion of surgical beta-cell mass reduction, arginine proxy of beta-cell mass was different after surgery. Impairment of beta-cell function, rather than decrease in beta-cell mass, is the main predictor of insulin deficiency.

DISCUSSION

Our study demonstrates that pre-existing defects in beta-cell function in non-diabetic subjects predict the risk of developing hyperglycemia after partial pancreatectomy, a model of acute beta-cell mass reduction. We found that only subjects with impaired first-phase insulin secretion and reduced beta-cell glucose sensitivity became diabetic or IGT after acute beta-cell mass reduction, and only subjects with impaired rate sensitivity before surgery became diabetic, suggesting that these are the pre-existing functional defects in the beta-cell secretory machinery that lead to hyperglycemia. Our results do not imply that loss of beta-cell mass is less important than beta-cell function. In fact, reduction of beta-cell mass is the trigger that reveals the pivotal role of first phase insulin secretion in predicting the appearance of impaired glucose metabolism after partial pancreatectomy.

Our data are in line with previous studies reporting a rate of post-operative diabetes of about 10% to 25%, we found that diabetes rate was about 27%, which demonstrates that reduction of beta-cell mass is not the dominant variable determining diabetes occurrence and that pre-existing defects in the functional mass could be responsible for progression to diabetes.

Based on the insulin response to acute glucose stimuli before surgery, we observed three functional and clinical trajectories after acute reduction in beta-cell mass. Subjects with the highest beta-cell glucose sensitivity and incremental first phase insulin secretion before surgery remained NGT despite the reduction in beta-cell mass, and reduction of first and second phase insulin secretion (evaluated following intravenous administration of glucose). Subjects who had lower beta-cell glucose sensitivity and incremental first phase insulin secretion developed impaired glucose tolerance or became diabetic after surgery. Only subjects who had lower beta-cell glucose sensitivity and incremental first phase insulin secretion, and more importantly, reduced rate sensitivity (a measure

of early phase insulin release) developed diabetes after surgery. This supports the hypothesis that the decrease in early phase insulin release is the key defect leading to hyperglycemia when competent functional islet mass is insufficient.

Our study design presents several advantages. Firstly, the only variable modified was that of an acute and significant reduction in beta-cell mass. Secondly, none of our subjects had DM before surgery. Thirdly, before surgery all individuals were evaluated not only through anamnesis and HbA1c but also using the gold standard OGTT thus allowing us to exclude unknown diagnosis of diabetes (see Supplementary Fig. 1). Interestingly, all had comparable glucose and insulin levels during OGTT before surgery; therefore, OGTT per se is not sufficient to truly identify the metabolic and hormonal effects of partial pancreatectomy. Only the mathematical modelling of first phase insulin secretion rate and glucose sensitivity allowed us to trace three different trajectories and to distinguish functional defects in a homogenous group of non-diabetic humans undergoing the same beta-cell mass reduction(7).

The importance of the role of first phase insulin secretion in the pathogenesis of hyperglycemia is well known in both Type 1 and Type 2 diabetes (17, 18). Aging may exert a role in beta-cell dysfunction;(19) and could have contributed to our results, however, there were no differences in age in the three groups studied. Earlier studies have been inconclusive regarding the reduction of first phase insulin release in prediabetes, mainly due to difficulties in defining the 'first phase' during OGTT (20, 21). Only studies performed with intravenous glucose can actually measure first phase insulin secretion, while complex mathematical models are needed to estimate it during OGTT. Interestingly, Ferrannini et al (22) found that rate sensitivity is not significantly reduced in IGT compared to normal individuals, suggesting that rate sensitivity is not the ideal parameter for determining initial loss of glucose tolerance. However, our data show that only subjects with

decreased rate sensitivity before surgery became diabetic after surgery, suggesting that loss of rate sensitivity is a marker of worsened glucose tolerance after surgery.

It should be noted that preserved first phase insulin secretion after pancreatic duodenectomy could be due to an increase in GLP1. In fact, in a previous study, we demonstrated that circulating GLP1 levels increase after pancreaticoduodenectomy,(23) and several other reports have suggested that the increase in GLP1 following surgery is one of the factors leading to diabetes remission after bariatric surgery.(24) Though the role of GLP1 could be important in maintaining first phase insulin secretion, it should be underlined that all our patients were subjected to the same type of surgery, therefore similar changes in GLP1 would be expected. However, a different incretin effect among the three groups cannot be excluded.

Some insights can be derived from the comparison between our model and procedures for diabetes remission, e.g., bariatric surgery and diets. It has been found that even if diabetic patients undergo the very same bariatric surgery, only those with higher first phase insulin secretion experience diabetes remission. (25) Interestingly, in this study, only patients with short diabetes duration had higher first phase insulin secretion, again confirming that preservation of first phase insulin secretion is more important than GLP1. The role of the preservation of first phase insulin secretion has also been confirmed in diabetes remission induced by diet in which the changes in GLP1 secretion induced by bariatric surgery are not present. In addition, the different post-operative diabetes rate could also be influenced by differences in the ability to implement compensatory mechanisms of regeneration and neogenesis in the remaining pancreas. This has been demonstrated in rats after pancreatectomy where beta-cell regeneration and/or appearance of new small islets seem to compensate for decreasing beta-cell mass. Exploring this possibility in humans is, however, unfeasible.

An elegant study by Ferrannini et al analyzed the changing parameters of beta-cell function during OGTT to explain the variability of 2-h plasma glucose levels, shedding light on beta-cell dysfunction

in the early stages of the natural history of diabetes. They concluded that there is a decrease in beta-cell glucose sensitivity even in the NGT range and that this is associated with rising 2-h plasma glucose concentrations. Being cross-sectional, the study could not distinguish the relative contribution of each of the determinants of glucose tolerance to the risk of diabetes occurrence. On the contrary, our model allows us to establish the relative contribution of beta-cell mass and function to the prediction of diabetes.

In this context, trying to identify strategies to restore beta-cell function (namely, first phase insulin secretion), rather than increasing beta-cell mass, could be pivotal in preventing and treating diabetes in a personalized medicine approach.

Although surgically induced hyperglycemia cannot be considered a true representation of the pathogenesis of type 2 diabetes, we believe that our study can provide an understanding of the functional defects underlying this disease. Type 2 diabetes is the result of 3 main factors: insulin resistance, functional deficits in the secretion of insulin and a reduction of beta-cell mass. Since changes in these variables are interrelated and change continuously over the course of the disease, studying their time-course adaptation is unlikely to provide a solution to this debate, as we will only observe the variables changing interactively and adapting to new situations in order to maintain euglycemia, also as a response to other variables (ageing, glucose toxicity, genes, etc.). We believe that a possible way to overcome this problem could be to modify only one of these variables to evaluate the effect on the others. With this method, we here demonstrate that beta-mass reduction is not sufficient to invariably determine hyperglycemia. On the contrary, only subjects who already showed changes in first phase insulin secretion developed diabetes. As previously reported, the acute removal of beta-cell mass inevitably accelerates a decline in beta-cell functional capacity, previously "stressed" by an attempt to compensate for increasing insulin demand. Since the surgical procedure is the same in all subjects, but only patients with previous islet remodeling and impaired beta-cell

function develop hyperglycemia and diabetes, the true determinant of the appearance of diabetes is the already present 'pre-diabetic' functional milieu (i.e., loss of first phase), rather than surgery.

In conclusion, beta-cell mass reduction per se is not responsible for the appearance of hyperglycemia. In our study, we found that in non-diabetic humans, beta-cell function and patterns of secretion differed significantly, and that these differences were further amplified following acute beta-cell mass reduction. Therefore, only pre-existing impairments in beta-cell function, i.e., reduced first-phase insulin release, predict impairment in glucose tolerance and diabetes after partial pancreatectomy.

RESEARCH DESIGN AND METHODS

Oral Glucose Tolerance Test

A standard 75 g oral glucose tolerance test was performed with measurement of glucose, insulin and C-peptide at 0, 30, 60, 90, 120 min after glucose load. Based on the post-surgery OGTT results, we classified the patients as normal glucose tolerant (post-NGT, n.11), impaired glucose tolerant (post-IGT, n. 13), and diabetic (post-DM, n.9).

Hyperinsulinemic euglycemic clamp procedure

The hyperinsulinemic euglycemic clamp test was performed after a 12h overnight fast using insulin 40 $mIU \cdot min^{-1} \cdot m^{-2}$ of body surface according to DeFronzo and colleagues.(26) A primed-constant infusion of insulin was administered (Actrapid HM, Novo Nordisk, Copenhagen, Denmark). The constant rate for the insulin infusion was reached within 10 min to achieve steady-state insulin levels; in the meantime, a variable infusion of 20% glucose was started via a separate infusion pump and the rate was adjusted, on the basis of plasma glucose samples drawn every 5 min, to maintain plasma glucose concentration at each participant's fasting plasma glucose level. During the last 20 min of the clamp procedure, plasma samples from blood drawn at 5–10 min intervals were used to determine

glucose and insulin concentrations. 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 $mg \cdot Kg^{-1} \cdot min^{-1}$).

Hyperglycemic clamp procedure

Plasma glucose was clamped at a stable level of 125 mg/dl above fasting blood glucose concentration. The hyperglycemic clamp was started with a bolus dose of dextrose 200 mg/mL (150 mg/kg) administered into the antecubital vein. Blood was drawn from a cannulated dorsal hand vein on the opposite arm. Every 5 min, venous plasma glucose was analyzed 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 5g arginine bolus was administered to measure maximum C-peptide secretory capacity at a steady-state blood glucose concentration of 250 mg/dl. Arginine-stimulated beta-cell secretory capacity was calculated as delta of 130 min C-peptide and 120 min C-peptide levels.

Surgical procedures

Pancreatoduodenectomy was performed according to the pylorus preserving technique. Briefly, the pancreatic head, the entire duodenum, common bile duct, and gallbladder were removed en-bloc, leaving a functioning pylorus intact at the gastric outlet. All adjacent lymph nodes were carefully removed. The continuity of the gastrointestinal tract was restored by an end-to-side pancreatojejunostomy. Further downstream, an end-to-side hepaticojejunostomy and an end-to-side pylorojejunostomy were performed. The volume of pancreas removed during the surgery is constant (~50%), as previously reported by Schrader et al.(27)

Calculations

To better define the relation between glucose metabolism after acute islet mass reduction and pre-existing metabolic defects, we divided subjects according to their glucose tolerance after surgery, as determined by post-surgery OGTT. According to the ADA classification, subjects whose 2-h post-load glucose was below 140 mg/dl were defined as normal glucose tolerant (post-NGT), subjects whose 2-h post-load glucose was 140–199 mg/dl were defined as impaired glucose tolerant (post-IGT) and subjects whose 2-h post-load glucose was higher than 200 mg/dl were defined as diabetic (post-DM).

During the OGTT and hyperglycemic clamp, insulin secretion was derived from C-peptide levels by deconvolution. Beta-cell Glucose Sensitivity (βCGS), i.e. the slope of the relationship between insulin secretion and glucose concentration, was estimated from the OGTT by modelling, as previously described.(13, 28) Rate sensitivity, also estimated from OGTT modelling, is a beta-cell functional parameter which represents the dependence of the insulin secretion rate on the rate of change in glucose concentration and is related to early insulin release.

Matsuda indexes (29) were calculated as indexes of whole body insulin sensitivity based on insulin and glucose values obtained from the OGTT, while beta-cell function was evaluated by calculating the insulinogenic index as the change in insulin over the first 30 min divided by the change in glucose over the first 30 min.

Integrated beta-cell function was also measured using the oral disposition index, which provides an assessment of insulin secretion in relation to insulin sensitivity, calculated as the product of the insulinogenic index and the Matsuda index(30).

During the hyperglycemic clamp, the first phase insulin secretion response was calculated as the mean incremental insulin secretion between 0 and 5 min, when insulin secretion rate 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 hyperglycemic clamp above basal insulin secretion.

Statistics

Continuous variables were summarized as mean ± SEM and categorical variables as frequencies and percentages, unless otherwise indicated. Normality of distribution was assessed by generation of histograms and quantile-quantile plots. Since samples did not deviate significantly from normal, differences in means across groups at baseline were tested by ANOVA. The relationship between variables was derived by linear regression analysis. Variables derived from OGTT and clamp were regressed against glucose tolerance status by using i) pre-pancreatectomy values ii) post-pancreatectomy values iii) post-pancreatectomy values adjusted for pre-pancreatectomy values. For measurement of glucose, insulin, C-peptide, we evaluated third-level interactions by including a product term of time x pancreatectomy x glucose tolerance in the model. We compared the effects of time and pancreatectomy using a linear mixed model for repeated measures, with each parameter as the dependent variable and time (analyzed as a categorical variable), pancreatectomy and the product term of time x pancreatectomy x glucose tolerance to investigate interaction effects. Linear and quadratic fits were used to explore the relationships between insulinogenic index and Matsuda index. A two-tailed p-value <0.05 was considered statistically significant. Analyses were performed using Stata 15.1 (StataCorp, TX, USA).

Study approval

The study protocol (ClinicalTrials.gov Identifier: NCT02175459 - Fig S1) was approved by the local ethics committee (P/656/CE2010 and 22573/14) (Rome, Italy) and all participants provided written informed consent, which was followed by a comprehensive medical evaluation.

AUTHOR CONTRIBUTION

T.M. generated the data and wrote the manuscript. P.M.F. performed statistical analysis. A.M., S.A. and A.G. reviewed/edited manuscript. U.C., G.Q., S.M., F.C., F.I. and A.P. contributed to discussion, reviewed/edited manuscript. C.M.A.C., G.Q. and G.D.G. researched data, A.M. generated data. T.M. and A.G. 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.

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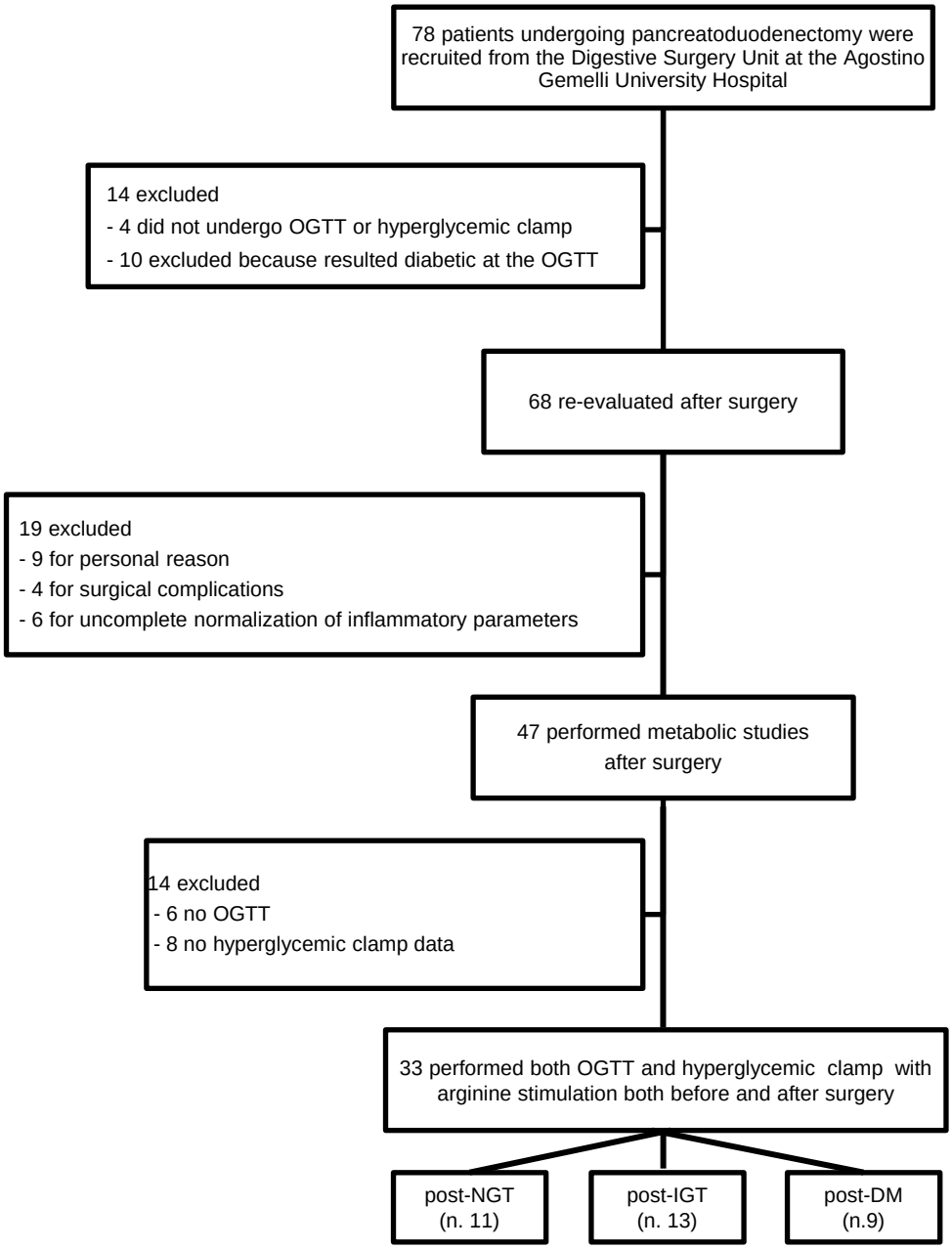


Figure 1. flow diagram of study participants

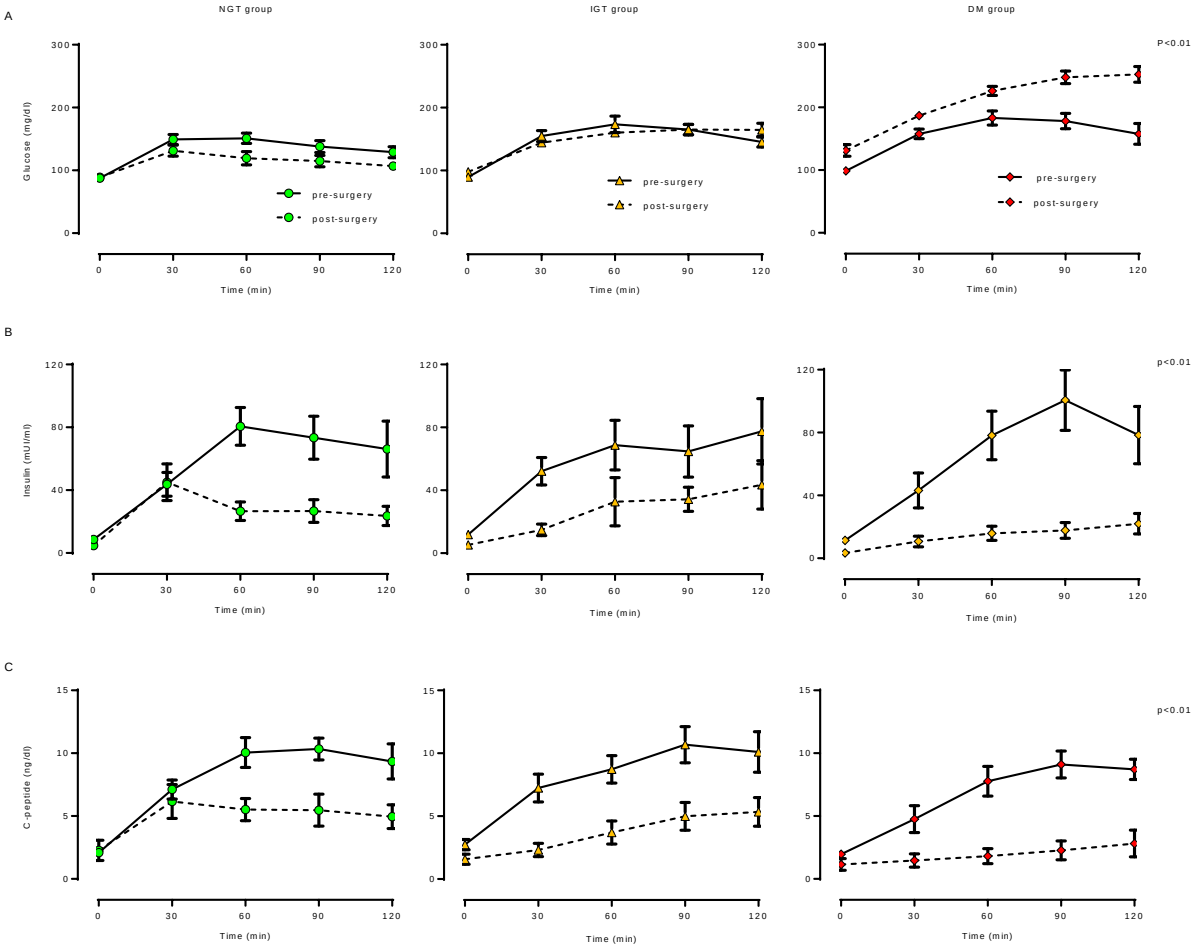
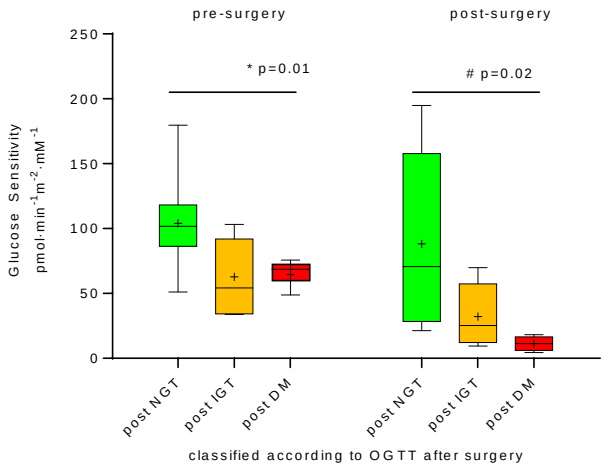


Figure 2. Glucose (A), insulin (B) C-peptide (C) levels during OGTT before (solid lines) and after (dotted lines) partial pancreatectomy in NGT (first column – green circle), IGT (second column – orange triangle), DM (third column – red diamonds). p-value <0.05 was considered statistically significant for glucose, insulin and c-peptide levels for third-level interactions by including a product term of time x pancreatectomy x glucose tolerance in the model.

A



B

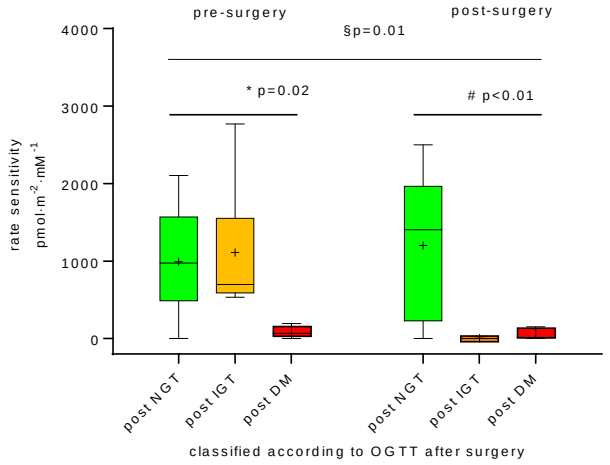


Figure 3: OGTT-derived glucose sensitivity (A) and rate sensitivity (B) before and after partial pancreatectomy in normal glucose tolerant (green box), impaired glucose tolerant (orange box) and diabetic subjects (red box). The relationship between variables was derived by linear regression analysis. Variables were regressed against glucose tolerance status by using i) pre-pancreatectomy values ii) post-pancreatectomy values iii) post-pancreatectomy values adjusted for pre-pancreatectomy values. * $p<0.05$ pre-surgery, # $p<0.05$ post-surgery and § $p<0.05$ pre-surgery adjusted for post-surgery. Box plots indicate median and interquartile range and whiskers indicate 2.5th - 97.5th percentile, + = mean value.

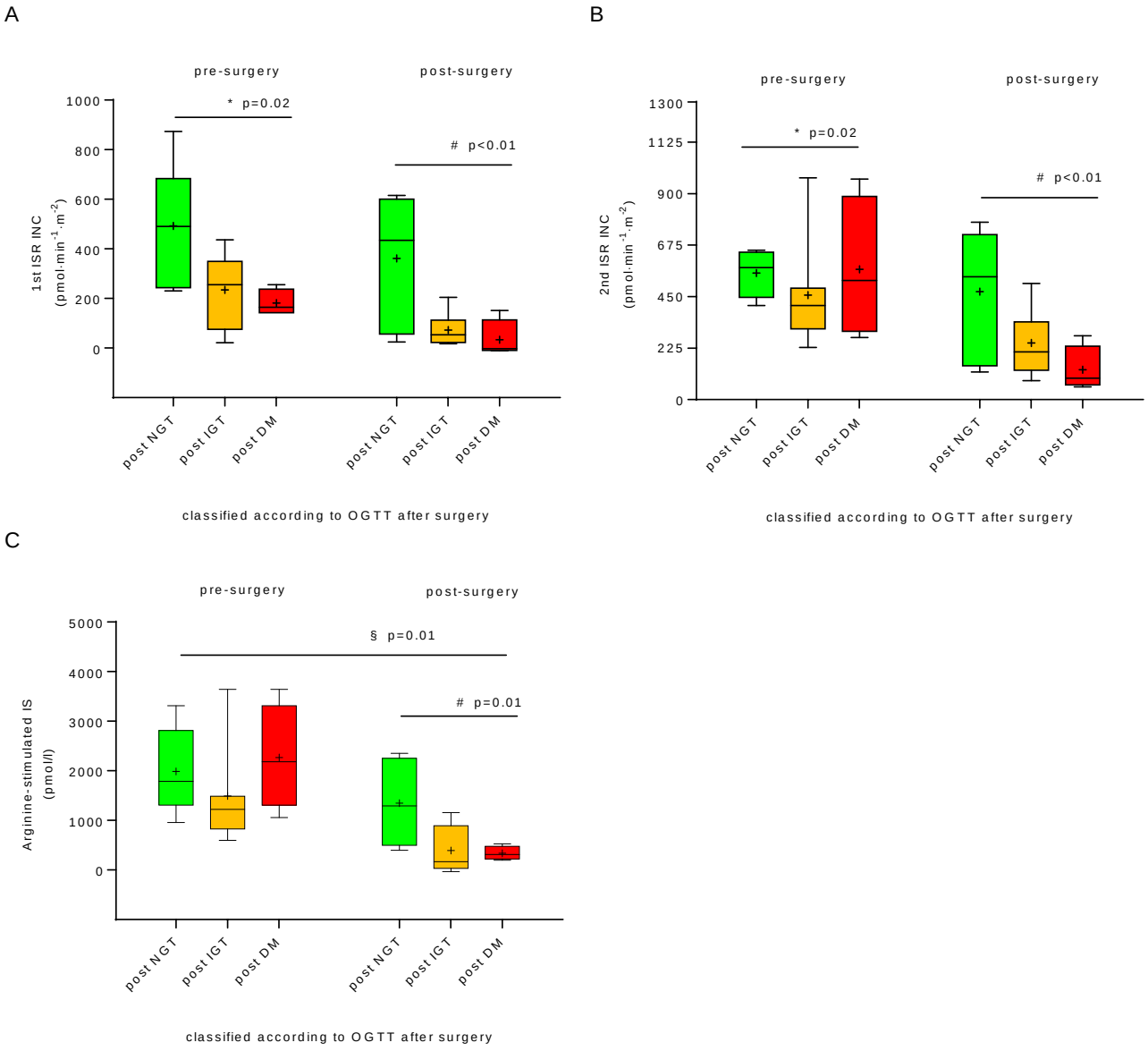


Figure 4: clamp-derived first phase insulin secretion (A), second phase insulin secretion (B) and arginine stimulated insulin secretion (C) before and after partial pancreatectomy in normal glucose tolerant (green box), impaired glucose tolerant (orange box) and diabetic subjects (red box). The relationship between variables was derived by linear regression analysis. Variables were regressed against glucose tolerance status by using i) pre-pancreatectomy values ii) post-pancreatectomy values iii) post-pancreatectomy values adjusted for pre-pancreatectomy values. * $p<0.05$ pre-surgery, # $p<0.05$ post-surgery and § $p<0.05$ pre-surgery adjusted for post-surgery. Box plots indicate median and interquartile range and whiskers indicate 2.5^{th} -97.5^{th} percentile, + = mean value.

Table 1

Subject characteristics	Post NGT (n. 11)	Post IGT (n. 13)	Post DM (n. 9)	P Value
Mean age (y)	57.6 ± 5.17	65.6 ± 3.62	61.6 ± 4.07	___
Gender (female/male)	7/4	9/4	4/5	___
Glucose uptake ($mg \cdot Kg^{-1} \cdot min^{-1}$)	5.79±0,79	4.92±0.59	4.21±0.74	0.22
Fasting glucose (mg/dl)	87.6 ± 3.70	89.6 ± 3.78	98.8 ± 3.42	0.12
Fasting insulin (µIU/ml)	8.57 ± 1.21	11.8 ± 2.62	11.3 ± 1.66	0.38
Fasting C-peptide (ng/ml)	2.09 ± 0.24	2.74 ± 0.45	1.97 ± 0.23	0.21
HbA1c (%)	37.0±2.12	31.5±4.93	38.0±5.66	0.46

Table 1. Clinical and metabolic characteristics of non-diabetic subjects before surgery, classified according to glucose tolerance after surgery into normal glucose tolerant, impaired glucose tolerant and diabetic. Data are means ± SE, * P- value <0.05 is considered statistically significant.