Increased β-Cell Workload Modulates Proinsulin-to-Insulin Ratio in Humans

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Increased proinsulin secretion, which characterizes type 2 diabetes and insulin resistance, may be due to an intrinsic, primitive defect in proinsulin processing or be secondary to increased demand on β-cells (hyperinsulinemia secondary to insulin resistance). An alternative way to investigate the relation between relative hyperproinsulinemia and increased secretory demand is to study the dynamic changes in the proinsulin-to-insulin ratio after partial pancreatectomy, a model of acute increased β-cell workload on the remaining pancreas. To pursue this aim, patients without diabetes, scheduled for partial pancreatectomy, underwent 4-h mixed-meal tests and hyperinsulinemic-euglycemic clamps before and after surgery. After acute β-cell mass reduction, no changes were observed in the fasting proinsulin-to-insulin ratio, whereas the fold change in the proinsulin-to-insulin ratio significantly increased over time after the meal. Further, our data demonstrate that whole-body insulin resistance is associated with underlying defects in proinsulin secretion, which become detectable only in the presence of increased insulin secretion demand.

Increased circulating levels of proinsulin, and a consequently elevated proinsulin-to-insulin ratio, is a well-known abnormality in type 2 diabetes (1–4). Disproportionate proinsulin secretion has been reported as a marker for β-cell dysfunction in both patients with diabetes and individuals at risk for diabetes (5,6). The exact mechanism behind this increase is unknown; although it has been hypothesized that an elevated proinsulin-to-insulin ratio is caused by increased secretory demand on β-cells due to insulin resistance and continuous hyperglycemia, which promotes the release of immature granules with a higher relative content of proinsulin and its conversion intermediates.

Experimental studies in nicotinic acid–induced insulin resistance in baboons (7) and humans (8), and observations in obese subjects without diabetes (9), which show unchanged proinsulin-to-insulin ratios, however, do not support the above hypothesis. Further, it has also been shown that an elevated proinsulin-to-insulin ratio in type 2 diabetes is highly correlated with the degree of decreased secretory capacity (10). However, it is still unclear how β-cell dysfunction per se or increased β-cell demand relates to the conversion of intact proinsulin to insulin, or whether a combination of insulin resistance, β-cell dysfunction, and increased β-cell demand has a differential effect on this conversion process.

To investigate the relation between relative hyperproinsulinemia and increased secretory demand, we examined the effects of acute ∼50% reduction of islet mass, and concomitant increased β-cell workload on the remaining ∼50% islet mass, using partial pancreatectomy as a human model. Several previous studies have examined the metabolic changes occurring after partial pancreatectomy in humans (11,12). Due to anatomical reasons (the head/right part of the pancreas is vascularized by branches of

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the gastroduodenal artery, and the tail is vascularized by branches of the splenic artery), all patients undergoing pancreaticoduodenectomy receive virtually the same partial (50%) resection, maintaining almost the same remaining portion of the endocrine pancreas. This surgery is therefore a unique way to examine the effects of a sudden increase in the workload of the remaining pancreas.

Furthermore, it is unclear which presurgery metabolic defects have a greater impact on postchallenge glucose tolerance and islet function. This issue is particularly relevant because metabolic response differs among subjects who undergo a 50% partial pancreatectomy, suggesting that underlying insulin resistance could play a major role.

Therefore, in the current study we aim to *1*) examine changes in proinsulin secretion and β-cell secretory capacity, modeled from a 4-h mixed-meal test (MMT), in individuals without diabetes before and after acute β-cell reduction and *2*) assess whether preexisting insulin resistance can modify proinsulin processing subsequent to increased β-cell workload.

Table 1—Clinical and metabolic characteristics of patients before and after surgery

Subject characteristics	Before surgery	After surgery	*P* value
Mean age (year)	55 ± 7.0	—	—
Sex (female/male)	7/2	—	—
BMI (kg/m^2)	27.8 ± 1.63	25.2 ± 1.32	0.17
Fasting glucose (mg/dL)	88.0 ± 6.00	117 ± 12.0	0.01
Fasting insulin (μIU/mL)	5.56 ± 0.79	5.23 ± 1.77	0.92
Fasting C-peptide (ng/mL)	2.55 ± 0.66	2.03 ± 0.22	0.36
Total cholesterol (mg/dL)	203 ± 23.4	144 ± 19.9	0.03
Triglycerides (mg/dL)	142 ± 12.6	102 ± 13.5	0.06
HbA_{1c}, % (mmol/mol)	5.61 ± 0.19 (38.0 ± 1.83)	6.76 ± 0.4 (50.0 ± 4.36)	0.008

Data are means ± SE or *n*. $P < 0.05$ is considered statistically significant.

RESEARCH DESIGN AND METHODS

Subject Selection and Protocols

Nine patients (seven female and two male; mean age 55 ± 7 years ± SE) undergoing pylorus-preserving pancreatoduodenectomy were recruited from the Digestive Surgery Unit and studied in the Center for Endocrine and Metabolic Diseases Unit (both at the Agostino Gemelli University Hospital). The study protocol (identifier NCT02175459, clinicaltrials.gov) was approved by the local ethics committee (P/656/CE2010 and 22573/14), and all participants provided written informed consent, which was followed by a comprehensive medical evaluation. Indication for surgery was tumor of the ampulla of Vater. None of the enrolled patients had a known history of diabetes. Patients underwent both fasting glucose and HbA_{1c} testing to exclude diabetes, according to the American Diabetes Association criteria (13). 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 morphological criteria for pancreatitis, were considered exclusion criteria. Patients with severe obesity (BMI >40 kg/m^2), uncontrolled hypertension, and/or hypercholesterolemia were also excluded. Clinical and metabolic characteristics of patients are shown in Table 1.

All subjects underwent a 2-h euglycemic clamp (insulin infusion rate 40 mIU · min^{-1} · m^{-2}) hyperglycemic clamp with arginine stimulation and a 4-h MMT to evaluate insulin secretion (from C-peptide deconvolution) and proinsulin-to-insulin ratio, as described below, before and ~40 days after surgery. The adequacy of the recovery period was determined on the basis of the normalization of inflammatory parameters such as C-reactive protein, erythrocyte sedimentation rate, stability of weight, and absence of symptoms of abnormal intestinal motility or exocrine pancreatic deficiency.

Hyperinsulinemic-Euglycemic Clamp Procedure

The hyperinsulinemic-euglycemic clamp test was performed after a 12-h overnight fast using insulin 40 mIU · min^{-1} · m^{-2} of body surface according to DeFronzo et al. (14). 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 · kg^{-1} · 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 5-g arginine bolus was administered to measure maximum C-peptide secretory capacity at a steady-state blood glucose concentration of 250 mg/dL. Arginine-stimulated β-cell secretory capacity was calculated as the delta of 130-min C-peptide and 120-min C-peptide levels.

MMT

An MMT was performed as previously described (15). Patients were instructed to consume a meal of 830 kcal (107 kcal from protein, 353 kcal from fat, and 360 kcal from carbohydrates) within 15 min. Blood samples were drawn twice in the fasting state and at 30-min intervals over the following 240 min (sample time 0′, 30′, 60′, 90′, 120′, 150′, 180′, 210′, and 240′) for the measurement of plasma glucose, insulin, C-peptide, and proinsulin. Blood samples for proinsulin were sampled in tubes containing EDTA; after centrifugation (1,000 rpm for 10 min at 4°C), they were stored at −80°C until analysis. Insulin levels were determined using a commercial RIA kit (Medical System; Immulite DPC, Los Angeles, CA). Plasma glucose concentrations were determined by the glucose oxidase technique using a glucose analyzer (Beckman Instruments, Palo Alto, CA). Plasma C-peptide was measured by Auto-DELFIA automatic fluoroimmunoassay (Wallac, Turku, Finland), with a detection limit of 17 pmol/L. Proinsulin was measured by ELISA kit no. 10-1118-01 (Mercodia), which reacts specifically with total proinsulin.

Surgical Procedures

Pancreatoduodenectomy was performed according to the pylorus-preserving technique (16). In brief, 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 invaginated pancreato-jejunostomy. Further downstream, an end-to-side hepaticojejunostomy and an end-to-side pylorojejunostomy were performed. The volume of pancreas removed during the surgery was constant (~50%), as previously reported by Schrader et al. (17).

Calculations

To further characterize the relation between insulin resistance and changes in the proinsulin-to-insulin ratio, we divided subjects according to their insulin sensitivity, as measured by the euglycemic-hyperinsulinemic clamp procedure before surgery. As previously described (18), the cutoff for insulin sensitivity was the median value of glucose uptake in the overall cohort (4.9 mg · kg^{-1} · min^{-1}); therefore, subjects whose glucose uptake was above the median value were classified as "more insulin sensitive" than subjects whose glucose uptake was below the median; for ease of comprehension, we defined the subjects as "insulin sensitive" (n = 5) or "insulin resistant" (n = 4).

During the MMT, insulin secretion was derived from C-peptide levels by deconvolution (19). β-Cell glucose sensitivity (βCGS), i.e., the slope of the relationship between insulin secretion and glucose concentration, was estimated from the mixed meal by modeling, as previously described (20,21).

Statistics

All data are expressed as mean ± SEM, unless otherwise indicated. Because samples did not deviate significantly from normal, differences in means were tested by two-tailed Student t test. The relationship between variables was derived with linear regression analysis. For measures of insulin, C-peptide, proinsulin, and proinsulin-to-insulin ratio, we compared the effects of time and pancreatectomy using linear mixed model for repeated measures, with each parameter as the dependent variable and time (analyzed as a continuous variable), pancreatectomy, and the product term of time × pancreatectomy to investigate interaction effects. For the proinsulin-to-insulin ratio, we evaluated third-level interaction by including a product term of time × pancreatectomy × insulin sensitivity in the model. The area under the curve (AUC) of the proinsulin-to-insulin ratio was calculated during the 0–240 interval of the MMT using the trapezoidal method. The proinsulin-to-insulin ratio was also calculated as fold change in the proinsulin-to-insulin ratio, dividing each time point by the basal level. P values <0.05 were considered significant. Analysis was performed using Stata (StataCorp, College Station, TX).

RESULTS

Clinical and metabolic characteristics of study subjects are provided in Table 1.

Hemipancreatectomy Induced a Marked Reduction in Basal and Total Insulin Secretion Rate, With Concomitant Reduction in Glucose Sensitivity

Subjects were evaluated 1 week before surgery and 40 ± 7 days (range 34–48 days) after surgery, as stated in the study design. As expected, both the basal insulin secretion rate (ISR) (presurgery 85.3 ± 10.3 vs. postsurgery 57.7 ± 7.59 pmol · min^{-1} · m^{-2}, P = 0.04) (Fig. 1*A*) and total ISR during the meal test (presurgery 69.6 ± 10.9 vs. postsurgery 34.0 ± 4.43 nmol · m^{-2}, P = 0.01) (Fig. 1*B*) decreased significantly after surgery. Further, partial pancreatectomy also caused a significant reduction in βCGS (presurgery 187 ± 46.1 vs. postsurgery 51.7 ± 18.7 pmol · $min^{-1}m^{-2}$ · $mmol/L^{-1}$, $P < 0.01$) (Fig. 1*C*) and in arginine-stimulated insulin secretion (indirect measure of β-cell mass) (presurgery 1,691 ± 366.5 vs. postsurgery 632.5 ± 265.8 pmol/L, P = 0.02) (Fig. 1*D*). To provide further proof of increased β-cell workload, we also normalized all insulin secretory parameters for arginine-stimulated insulin secretion. We observed a significant increase in basal ISR (presurgery 0.045 ± 0.005 vs. postsurgery 0.262 ± 0.099, $P < 0.04$) (Supplementary Fig. 1*A*), total ISR (presurgery 0.040 ± 0.005 vs. postsurgery

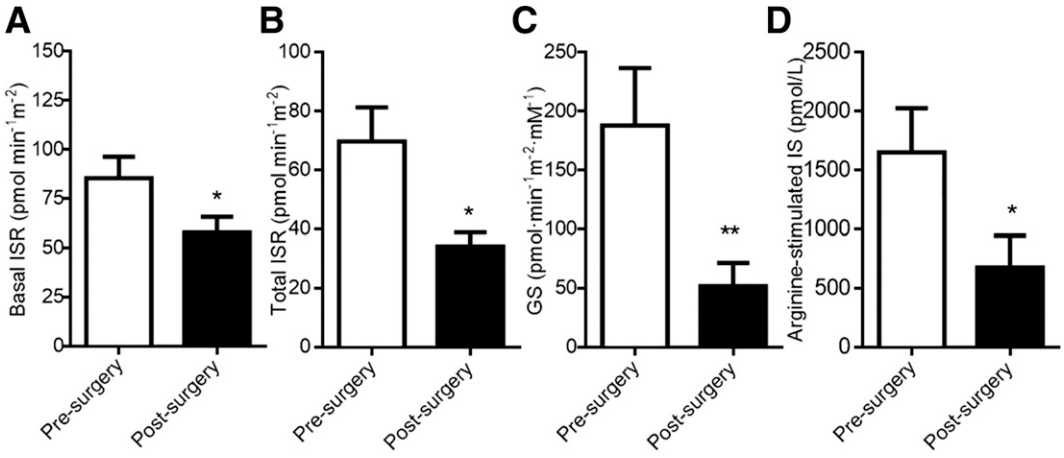

Figure 1—MMT-derived basal ISR (*A*), total ISR (*B*), and glucose sensitivity (GS) (*C*). Clamp-derived arginine-stimulated insulin (IS) (*D*) before (white bars) and after (dark bars) partial pancreatectomy. *$P < 0.05$, **$P < 0.01$.

0.157 ± 0.050, $P < 0.03$) (Supplementary Fig. 1*B*), and βCGS (presurgery 0.080 ± 0.014 vs. postsurgery 0.233 ± 0.046, $P < 0.01$) (Supplementary Fig. 1*C*) per arginine-stimulated insulin secretion after acute islet cell mass reduction, which indicates an enhanced β-cell workload in the remaining islet cell mass. Conversely, no changes were observed in insulin sensitivity, as assessed by the hyperinsulinemic-euglycemic clamp (18), after pancreatectomy (presurgery 5.08 ± 0.26 vs. postsurgery 5.04 ± 0.17 mg · kg^{-1} · min^{-1}, $P = 0.95$). Fasting and plasma glucose concentrations during MMT increased significantly after surgery ($P < 0.01$ for pancreatectomy) (Fig. 2*A*), whereas insulin and C-peptide secretion were significantly reduced ($P < 0.01$ and $P < 0.01$ for pancreatectomy, respectively) (Fig. 2*B* and *C*).

Hemipancreatectomy Induces an Increase in the Proinsulin-to-Insulin Ratio, Whereas Its Dynamic Secretion Is Preserved

Proinsulin concentration changes over time tended to increase after surgery ($P = 0.08$ for pancreatectomy) (Fig. 2*D*). Insulin-to-proinsulin ratios showed increased time-dependent response both before and after surgery ($P = 0.01$ for time), and no differences due to surgery were detectable. Fold change in proinsulin-to-insulin ratios increased considerably over time before and after surgery, and the increase over time was significantly greater after surgery (P value for interaction = 0.01) (Fig. 2*E*).

Insulin Resistance Induces Amplified Proinsulin-to-Insulin Ratio After Hemipancreatectomy

To further characterize changes in the proinsulin-to-insulin ratio after removal of ~50% of the pancreas, we investigated the correlations between insulin sensitivity (glucose infusion rate during the euglycemic-hyperinsulinemic clamp) and the proinsulin-to-insulin ratio after pancreatectomy. The analysis of the entire cohort revealed strong inverse correlations, both in the fasting state (basal proinsulin-to-insulin ratio $r = -0.78$, $P = 0.01$) (Fig. 3*A*) and as AUC after the meal test (AUC proinsulin-to-insulin ratio 0–240 min $r = -0.73$, $P = 0.02$) (Fig. 3*B*).

In order to better characterize the relation between insulin sensitivity and changes in the proinsulin-to-insulin ratio, we compared insulin-resistant and insulin-sensitive subjects. A trend to increase in basal ISR, total ISR, and βCGS per arginine-stimulated insulin secretion was observed in both insulin-sensitive and insulin-resistant subjects after acute islet cell mass reduction (Supplementary Fig. 2).

Interestingly, basal proinsulin-to-insulin ratio (insulin sensitive 0.39 ± 0.10 vs. insulin resistant 0.33 ± 0.08, $P = 0.65$) (Supplementary Fig. 3*B*) and total proinsulin-to-insulin ratio (AUC proinsulin-to-insulin ratio 0–240 min insulin sensitive 68.2 ± 20.3 vs. insulin resistant 59.0 ± 17.2, $P = 0.80$) (Supplementary Fig. 1*C*) were comparable before surgery, whereas insulin secretion was significantly greater in insulin-resistant subjects ($P = 0.03$) (Supplementary Fig. 4*A*).

Although 50% pancreatectomy led to an increased proinsulin-to-insulin ratio in the entire cohort, pancreatectomy had a significantly different and opposite effect on time-dependent change in the proinsulin-to-insulin ratio in insulin-sensitive compared with insulin-resistant subjects ($P < 0.001$ for the interaction between pancreatectomy, time, and insulin sensitivity) (Supplementary Fig. 3*A*).

After partial pancreatectomy, all subjects experienced a significant reduction in insulin secretion, and insulin levels were comparable in insulin-sensitive compared with insulin-resistant subjects ($P = 0.83$) (Supplementary Fig. 4*B*), but only the insulin-sensitive subjects showed a significant decrease in the basal proinsulin-to-insulin ratio (insulin sensitive before surgery 0.39 ± 0.10 vs. after surgery 0.20 ± 0.07, $P = 0.02$) (Supplementary Fig. 3*A* and *B*) and a numerical decrease in total proinsulin-to-insulin ratio (AUC proinsulin-to-insulin ratio 0–240 min insulin sensitive before surgery 68.2 ± 20.3 vs. after

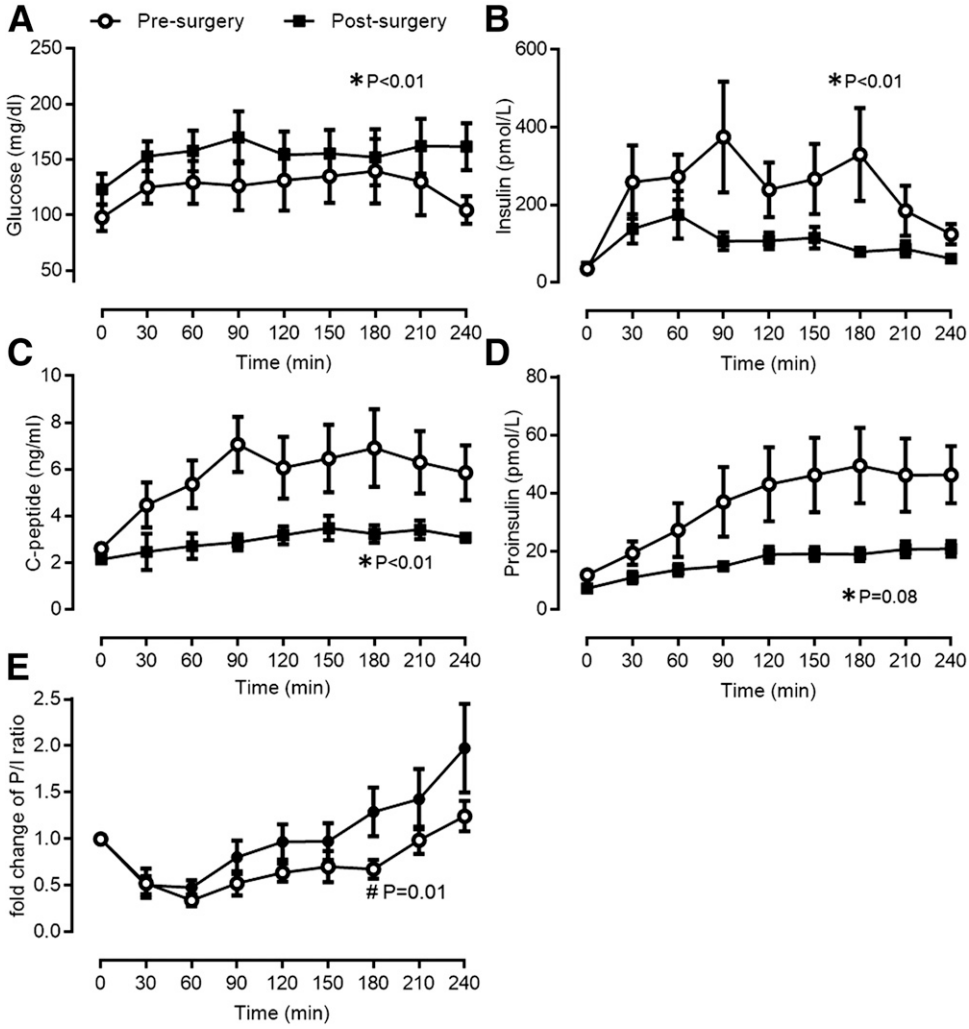

Figure 2—Glucose (*A*), insulin (*B*), C-peptide (*C*), and proinsulin (*D*) levels before (white circles) and after (dark squares) partial pancreatectomy. *E*: Fold change in proinsulin-to-insulin (P/I) ratio during MMT before (white circles) and after (dark squares) partial pancreatectomy. **P* < 0.01 for pancreatectomy (*A*–*C*); **P* = 0.08 for pancreatectomy (*D*); #*P* value for interaction = 0.01 (*E*).

surgery 47.9 ± 10.2, *P* = 0.18) (Supplementary Fig. 3). Conversely, in the insulin-resistant subjects, the basal proinsulin-to-insulin ratio (insulin sensitive before surgery 0.33 ± 0.08 vs. after surgery 0.46 ± 0.08, *P* = 0.04) (Supplementary Fig. 3*A* and *B*) and total proinsulin-to-insulin ratio increased after surgery (AUC proinsulin-to-insulin ratio 0–240 min insulin sensitive before surgery 59.0 ± 16.2 vs. after surgery 78.1 ± 21.4, *P* = 0.04) (Supplementary Fig. 3).

DISCUSSION

Our findings suggest that an acute per cell insulin secretion demand alters insulin secretion processes (proinsulin-to-insulin ratio) mainly in the presence of insulin resistance. The key result is that insulin sensitivity after partial pancreatectomy was strongly and inversely correlated with the proinsulin-to-insulin ratio, measured either in the fasting state or after the mixed meal.

To reproduce a condition of acute increased insulin secretory demand due to loss of islets of Langerhans, we used subjects undergoing a 50% pancreatectomy as a model. After a ~50% removal of islets of Langerhans, we observed a twofold time-dependent increase in the proinsulin-to-insulin ratio (expressed as a fraction of the basal value) during a meal, suggesting that this increased β-cell workload affects proinsulin processing.

In line with a previous study in which the surgical removal of two-thirds of the pancreas in dogs failed to reproduce a surgically induced change in the proinsulin-to-insulin ratio (1), no statistically significant changes in fasting proinsulin-to-insulin ratio were observed after acute β-cell mass reduction. This suggests that the imbalance in the insulin secretory machinery due to increasing β-cell demand occurs under stimulus. These data differ from those obtained in a study on pancreas donors after partial pancreatectomy (22), in which an increased fasting

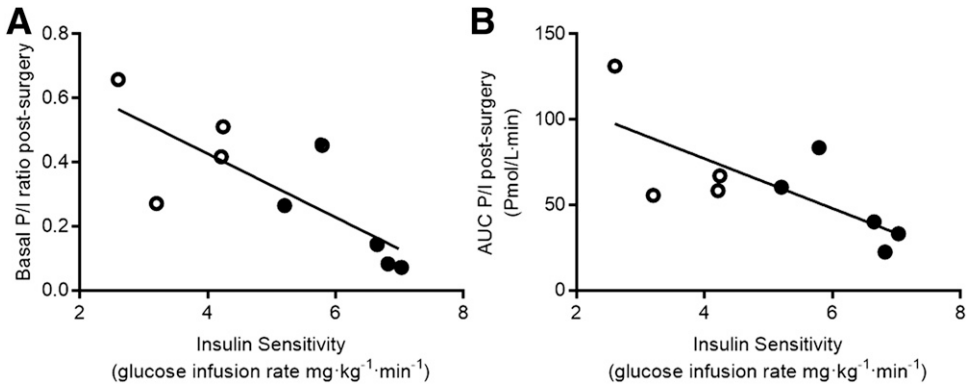

Figure 3—*A*: Correlation between glucose uptake (insulin sensitivity index) and basal proinsulin-to-insulin (P/I) ratio after partial pancreatectomy in all subjects. $r = -0.78$; $P = 0.01$. *B*: Correlation between glucose uptake (insulin sensitivity index) and total proinsulin-to-insulin ratio after partial pancreatectomy in all subjects. $r = -0.73$; $P = 0.02$. Black circles, insulin-sensitive subjects; white circles, insulin-resistant subjects.

proinsulin-to-insulin ratio was observed after surgery, and this was directly correlated with fasting glucose levels and measures of maximal β-cell reserve capacity. This controversial result could be explained by the different timing of the postsurgical evaluation. Indeed, whereas Seaquist et al. (22) evaluated donors no earlier than 12 months and up to 96 months after hemipancreatectomy, we performed a follow-up after a mean of 45 days. It seems that increased proinsulin-to-insulin ratio in the fasting state needs a chronic adaptation to increasing β-cell workload, which requires a long-term evaluation.

Further, we also demonstrated that the proinsulin-to-insulin ratio increases physiologically in a time-dependent manner in the 4 h after a meal and that this time-dependent secretion in response to meals is preserved after acute β-cell mass reduction.

We must also account for the effect of hyperglycemia, as it is the major factor increasing insulin secretory demand, and the changes observed in our study are also mediated by worsening glucose control.

We used an MMT, which reproduces physiological stimulus to insulin secretion and represents an appropriate method to investigate the proinsulin response. This finding highlights the important role of appropriate stimuli in investigating insulin secretion dynamics.

Despite preservation of the time-dependent proinsulin-to-insulin ratio after β-cell mass reduction, overall secretion increased after partial pancreatectomy, in the presence of a concomitant worsening in β-cell secretion rate and glucose sensitivity, suggesting that the remaining β-cell mass is under stress due to the increased workload (likely due to a combination of insulin resistance and reduced β-cell mass).

These data support our hypothesis that increased β-cell demand, rather than preexisting β-cell dysfunction per se, determines a rapid change in the proinsulin-to-insulin secretion ratio, in line with earlier studies showing that increased proinsulin levels, which are the consequence of impaired proinsulin conversion to insulin, predict worsening glucose tolerance (23,24).

Furthermore, both basal and stimulated levels of the proinsulin-to-insulin ratio were comparable in insulin-sensitive and insulin-resistant subjects at baseline (before surgery), suggesting that underlying insulin resistance does not affect proinsulin secretion in normal physiological conditions.

Earlier studies have been inconclusive regarding the relative fasting proinsulin levels in prediabetes, mainly because proinsulin and insulin were analyzed only at baseline, i.e., nonstimulated samples. Several studies have shown an increase in the proinsulin-to-insulin ratio in impaired glucose tolerance, obese, and insulin-resistant individuals without diabetes, suggesting that proinsulin conversion might be a useful biomarker for the prediction of diabetes risk and β-cell dysfunction (25–27), whereas others have found normal levels compared with control subjects (28–31).

Despite the small population, it is worth noting that our data overcome the limits of previous studies on this relevant topic, and we did our best to strengthen the study with a stronger statistical analysis. We therefore performed a mixed-measures repeated statistical analysis, which includes every single time point in the outcome evaluation without violating the assumption of data independence while accounting for random effects.

As there are differences in the half-lives of insulin and proinsulin (32), the circulating concentrations during the fasting state do not reflect what is actually released by the β-cell granules. Instead, measuring insulin secretion directly after acute stimulation provides a better estimate of the granule content of insulin and proinsulin. Larsson and Ahrén (33) have shown an increase in the proinsulin-to-insulin ratio after acute stimulation of insulin secretion with arginine in women with impaired glucose tolerance, but we were unable to demonstrate the same trend when comparing insulin-sensitive and insulin-resistant subjects

before surgery, probably due to the use of a different stimulus.

However, in response to increased β-cell workload after acute β-cell mass reduction, insulin-resistant subjects showed an increase in both fasting and AUC proinsulin-to-insulin ratio, whereas the proinsulin-to-insulin ratio showed no increase in insulin-sensitive subjects. Further, it seems less likely that intrinsic processing defects are involved, as otherwise one would expect the same response in the more and less insulin-sensitive subgroups.

Despite the acute onset of hyperglycemia (40 days), we cannot exclude that worsening insulin sensitivity, leading to glucose toxicity effects and structural modifications in the β-cells (as observed after many years of chronic hyperglycemia) may play a role in the proinsulin-to-insulin changes observed or that hyperglycemia and other metabolic parameters may also impair proinsulin processing. Further, we do not have functional follow-up reevaluation before 40 days, and we cannot exclude that a different condition could be present a few hours/days after surgery. Nevertheless, we purposely chose to conduct follow-up reevaluation 40 days after surgery to ensure full recovery after this complex surgery and limit additional confounders due to the short period of recovery from the surgical procedure.

Besides, in type 2 diabetes, it has been demonstrated that the proinsulin-to-insulin ratio is increased both in the fasting state (34–38) and after acute stimulation (39). It seems, therefore, that insulin resistance is a feature of prediabetes, in which there are compensatory attempts to cope with the various aspects of the β-cell dysfunction seen in diabetes, such as defective proinsulin processing in the granules.

Furthermore, we suggest here that morphological and functional modifications induced by insulin resistance directly impact the β-cell secretory system, but that their negative effects become evident only in response to supraphysiological β-cell demand, when probably no further compensation for increased β-cell workload is possible.

In this context, the improvement of proinsulin to insulin conversion recently observed after lifestyle intervention and at long-term follow-up is an important finding (40), as it suggests that in the early β-cell dysfunction phase, the defect in proinsulin processing could be partially reversed by improving insulin sensitivity. Investigating the mechanisms underlying defects in proinsulin secretion could reveal new potential therapeutic targets to slow or cure β-cell dysfunction, by applying personalized strategies for each single aspect of β-cell dysfunction.

In summary, the proinsulin-to-insulin ratio increases after physiological stimulation of insulin secretion in humans without diabetes, and this is further amplified after acute β-cell mass reduction. This indicates a physiological regulation in the proinsulin secretion process, which is significantly impaired after prolonged stimulation and when β-cell demand is increased after β-cell mass reduction. Thus, insulin resistance directly impacts proinsulin processing, leading to increased relative, both basal and stimulated, proinsulin release, detectable only in the presence of increased insulin secretion demand, due to acute β-cell mass reduction.

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