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Noradrenergic fibers are associated with beta-cell dedifferentiation and impaired beta-cell function in humans

F. Cinti [a,b,1], T. Mezza [a,b,1], I. Severi [c], M. Suleiman [d], C.M.A. Cefalo [a,b], G.P. Sorice [a,b], S. Moffa [a,b], F. Impronta [a,b], G. Quero [e,f], S. Alfieri [e,f], A. Mari [g], A. Pontecorvi [b], L. Marselli [d], S. Cinti [c], P. Marchetti [d], A. Giaccari [a,b,*]

[a] Centro per le Malattie Endocrine e Metaboliche, Fondazione Policlinico Universitario A. Gemelli IRCCS, Roma, Italy
[b] Dipartimento di Medicina e Chirurgia Traslazionale, Università Cattolica del Sacro Cuore, Rome, Italy
[c] Department of Clinical and Experimental Medicine, Center of Obesity, Università Politecnica delle Marche, Ancona, Italy
[d] Department of Clinical and Experimental Medicine, Islet Cell Laboratory, University of Pisa, Pisa, Italy
[e] Chirurgia Digestiva, Fondazione Policlinico Universitario A. Gemelli IRCCS, Roma, Italy
[f] Istituto di Semeiotica Chirurgica, Università Cattolica del Sacro Cuore, Roma, Italy
[g] Institute of Neuroscience, National Research Council, Padua, Italy

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ABSTRACT

Aims/hypothesis: Type 2 diabetes (T2D) is characterized by a progressive loss of beta-cell function, and the "disappearance" of beta-cells in T2D may also be caused by the process of beta -cell dedifferentiation. Since noradrenergic innervation inhibits insulin secretion and density of noradrenergic fibers is increased in type 2 diabetes mouse models, we aimed to study the relation between islet innervation, dedifferentiation and beta-cell function in humans.

Methods: Using immunohistochemistry and electron microscopy, we analyzed pancreata from organ donors and from patients undergoing pancreatic surgery. In the latter, a pre-surgical detailed metabolic characterization by oral glucose tolerance test (OGTT) and hyperglycemic clamp was performed before surgery, thus obtaining in vivo functional parameters of beta-cell function and insulin secretion.

Results: The islets of diabetic subjects were 3 times more innervated than controls (0.91 ± 0.21 vs 0.32 ± 0.10, n. fibers/islet; $p = 0.01$), and directly correlated with the dedifferentiation score ($r = 0.39$; $p = 0.03$). In vivo functional parameters of insulin secretion, assessed by hyperglycemic clamp, negatively correlated with the increase in fibers [beta-cell Glucose Sensitivity ($r = -0.84$; $p = 0.01$), incremental second-phase insulin secretion ($r = -0.84$, $p = 0.03$) and arginine-stimulated insulin secretion ($r = -0.76$, $p = 0.04$)]. Moreover, we observed a progressive increase in fibers, paralleling worsening glucose tolerance (from NGT through IGT to T2D).

Conclusions/interpretation: Noradrenergic fibers are significantly increased in the islets of diabetic subjects and this positively correlates with beta-cell dedifferentiation score. The correlation between in vivo insulin secretion parameters and the density of pancreatic noradrenergic fibers suggests a significant involvement of these fibers in the pathogenesis of the disease, and indirectly, in the islet dedifferentiation process.

1. Introduction

Type 2 diabetes is characterized by insulin resistance and a progressive loss of beta-cell function [1,2]. The latter is also ascribable to the process of dedifferentiation, which has been proposed as a contributing factor to the loss of functional beta-cell mass in diabetes, even though other mechanisms of beta-cell failure should also be taken into account [3–5]. The exhausted beta-cell, challenged by persistent glucotoxicity, seems to undergo dedifferentiation to escape cell death, exploiting this process to rest, while awaiting improved metabolic conditions to re-differentiate [6–10]. The fact that this process has been described in human type 2 diabetes and that its reversibility has been confirmed in mouse models, has shifted the spotlight onto the still unclear molecular mechanisms underlying it [8,9,11].

Besides morphological characteristics that allow us to quantify beta-cell mass deficits, we are also able to evaluate beta-cell function through highly sophisticated engineering modelling, even in humans [12,13]. beta-cell glucose sensitivity, for example, has been widely used as an in vivo parameter of beta-cell function, and the rare possibility of correlating this parameter with ex vivo morphological data currently

* Corresponding author at: Center for Endocrine and Metabolic Diseases, Fondazione Policlinico Universitario A. Gemelli IRCCS, Università Cattolica del Sacro Cuore, Largo Agostino Gemelli, 8 - 00168 Rome, – Italy.
E-mail address: andrea.giaccari@unicatt.it (A. Giaccari).
[1] Contributed equally.

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represents an opportunity to formulate a hypothesis on the pathogenesis of type 2 diabetes in humans [14,15].

Recently, there has been much interest on the role of pancreatic innervation in the regulation of glucose metabolism and the beta-cell has been described as key in mediating islet neurotrophism in transplanted islet-cell models [16]. Parasympathetic, sympathetic and sensory neurons have been identified within the pancreas, although most of the data derive from murine models and differences with human models have been reported [17–21]. However, despite differences in terms of localization and quantification, the role of innervation in the regulation of glucose metabolism remains the same. Noradrenergic fibers exert a well-known inhibitory effect on insulin secretion [18,22–24], thus most of the literature is focused on pancreatic noradrenergic innervation.

In a previous study, in a genetically obese mouse model of type 2 diabetes, we found a significant increase in the density of noradrenergic fibers in diabetic mice compared to non-diabetic ones. Moreover, the fibers were in direct contact with beta-cells, suggesting a direct interplay between them [25].

At least in murine models, the presence of nerve fibers seems to accompany the beta-cell secretion/plasticity changes [25]. On the other hand, little is known about human islet innervation. The human pancreas has been described as sparsely innervated and the observed parasympathetic cholinergic axons and sympathetic fibers have been mostly associated with the regulation of blood flow [17] rather than the direct modulation of endocrine cell function or plasticity. A recent paper by Tang et al. provided an elegant 3D histology of human pancreas innervation suggesting that nerves enter the islet core and reside in the immediate microenvironment of islet cells [26]. However, they analyzed healthy donors without providing data on different metabolic conditions. Moreover, some studies even suggest that sympathetic nerve hyperactivity could be used as a predictor of diabetes, further increasing interest in this field [27–29].

Thus, the observation of a direct contact between fibers and beta-cells in humans, in different metabolic conditions, raises the possibility of an involvement of nerve fibers in beta-cell dysfunction and dedifferentiation.

We hypothesize that the direct contact of noradrenergic fibers with beta-cells could induce altered beta-cell secretion and dedifferentiation, and in the present study we aim: (1) to investigate relations between islet innervation and dedifferentiation in pancreata from a cohort of diabetic and non-diabetic organ donors, (2) to examine correlations between in vivo beta-cell function parameters and changes in pancreatic innervation in a cohort of metabolically well-characterized individuals.

2. Research design and methods

2.1. Subjects

2.1.1. Organ donors

We obtained pancreata from sixteen organ donors as previously described [30]. Eight had a history of type 2 diabetes with a duration of disease of 13.1 ± 2.6 years. The eight controls were organ donors without a known history of diabetes, with normal plasma glucose during their stay in the intensive care unit. The institutional review board at the University of Pisa approved all procedures. Characteristics of organ donors are summarized in Table 1.

2.2. Pancreatic surgery patients

Eleven patients due to undergo pylorus-preserving pancreatoduodenectomy were recruited at the Digestive Surgery Unit and studied at the Center for Endocrine and Metabolic Diseases (both at the A. Gemelli University Hospital, Rome, Italy). The study protocol (ClinicalTrials.gov Identifier: NCT02175459) was approved by the local ethics committee (P/656/CE2010 and 22573/14) and all participants provided written informed consent, after which a comprehensive medical evaluation was carried out. Indication for surgery was low-grade malignancy periampullary tumor. 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 [31].

All subjects underwent an oral glucose tolerance test (OGTT) (75 g glucose). In a subgroup of subjects we also performed a hyperglycemic clamp with arginine stimulation to evaluate insulin secretion (from C-peptide deconvolution), as described below ($n = 7$).

Clinical and metabolic characteristics of patients are shown in Table 1 and Supplementary Table 1.

2.3. Metabolic evaluation of surgery group

2.3.1. Oral glucose tolerance test

A standard 75 g oral glucose tolerance test was performed with measurements of glucose and insulin at 0, 30, 60, 90, 120 min after glucose

Table 1
Clinical and metabolic characteristics of subjects. Data are mean ± SE or n. (sex distribution and clinical diagnoses).

Subject characteristics – pancreatic surgery	NGT	IGT	T2D
Gender (M:F)	3:2	1:2	1:2
Mean age (y)	57 ± 6.14	70.6 ± 4.33	65.3 ± 2.60
BMI (kg/m^2)	23.0 ± 2.2	21.3 ± 0.45	24.0 ± 0.72
Fasting glucose (mg/dl)	83.8 ± 4.31	83.6 ± 0.48	109 ± 28.53
Fasting insulin (μUI/ml)	8.83 ± 0.16	7.00 ± 0.30	8.30 ± 1.60
2-h glucose during OGTT (mg/dl)	111 ± 7.05	158 ± 7.00	242 ± 14.3
AUC glucose during OGTT ($mg \cdot dl^{-1} \cdot min^{-1} \cdot 10^{-3}$)	15.5 ± 0.45	18.4 ± 0.93	27.3 ± 8.02
AUC Insulin during OGTT ($\mu l \cdot ml^{-1} \cdot min^{-1} \cdot 10^{-3}$)	6.90 ± 0.74	7.98 ± 0.32	6.50 ± 1.35

Subject characteristics – organ donors	Nondiabetic	Diabetic	*p*
Gender (M:F)	4:4	5:3	NS
Mean age (y)	64.8 ± 3.78	76.0 ± 2.61	0.048
BMI (kg/m^2)	24.8 ± 0.9	25.3 ± 1.4	NS
Years from diagnosis	N/A	13.1 ± 2.6	N/A

load. Based on the results of the OGTT, we classified the patients as normal glucose tolerant (NGT = 5), impaired glucose tolerant (IGT = 3) and diabetic (T2D = 3).

2.3.2. Hyperglycemic clamp procedure

On a different day, 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 an 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 (SALF s.p.a. Cenate Sotto, BG, Italy) was administered to measure maximum C-peptide secretory capacity at a steady-state blood glucose concentration of 250 mg/dl [32].

2.4. Calculations

During the hyperglycemic clamp, insulin secretion rate was derived from C-peptide levels by deconvolution [33]. 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 above basal insulin secretion during the last 20 min of the hyperglycemic clamp. Beta-cell Glucose Sensitivity (βCGS), 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 in insulin secretion and glucose concentration from baseline [15]. Arginine-stimulated beta-cell secretory capacity was calculated as delta of 130 min C-peptide and 120 min C-peptide levels.

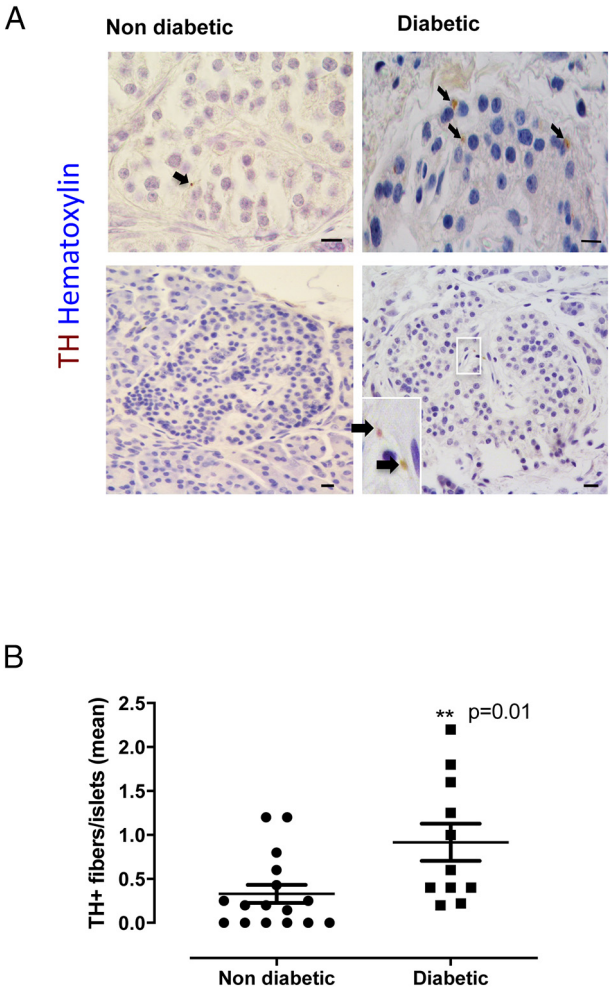

Fig. 1. Significant increase in tyrosine hydroxylase positive parenchymal fibers (TH+) in islets of human diabetic compared to nondiabetic subjects. a) Immunohistochemistry on pancreatic sections using TH (brown) and Hematoxylin (blue). Black arrows indicate TH positive parenchymal fibers. Enlargement of fibers within islets (white square). Scale bars 20 μm. b) Quantitative analysis of TH+ fibers/islet. Data are means ± SEM. ** $p = 0.01$ by Student's t-test.

2.5. Immunohistochemical and morphometric analyses

We fixed and processed tissue for immunohistochemistry as previously described [34]. We focused the study on the head and neck region of the pancreas [35]. Paraffin sections, each 3 μm-thick, were obtained for hematoxylin and eosin staining, and for immunohistochemistry. Tissue sections were imaged with a Nikon Eclipse E800 light microscope using a 40× objective and digital images were captured with a Nikon DXM 1200 camera. We performed histochemical reactions for the entire cohort at the same time. We controlled each reaction by omitting primary or secondary antibodies to determine signal specificity. For the immunofluorescence analysis we applied antigen retrieval at pH 9.0 (Nacalai USA) to facilitate antigen retrieval detection. We used Alexa-conjugated donkey secondary antibodies (Jackson Immunoresearch Laboratories and Molecular Probes) as described [8]. We used confocal microscopy and Laser Scanning Microscope Software (LEICA) to study dedifferentiation and capture images. We performed quantification in a blinded fashion using ImageJ software to analyze individual cells in whole-slide fluorescent and optical images.

To perform quantitative analyses, we scored at least one random section per donor and 10 random islets. We scored the dedifferentiation as the percentage of cells positive for the endocrine marker synaptophysin but negative for the 4 major endocrine hormones (insulin, glucagon, somatostatin and pancreatic polypeptide) as previously described [8]. We quantified the number of tyrosine hydroxylase positive (TH+) fibers/islets by immunohistochemistry with a Nikon Eclipse E800 light microscope using a 100× objective.

2.6. Transmission electron microscopy

Tissue fragments measuring about 1 mm^3 were immersed in a fixative consisting of 2% glutaraldehyde and 2% paraformaldehyde in 0.1 mol/l PB, pH 7.4, for 4 h. They were then washed with PB, post-fixed in 1% OsO4 for 60 min at 4 °C, dehydrated in acetone and embedded in an Epon-Araldite mixture. Thin sections were obtained with an MTX ultramicrotome (RMC, Tucson, AZ, USA), stained with lead citrate, and examined with a Philips CM 10 transmission electron microscope (Philips, Eindhoven, The Netherlands). Synaptic structures were defined as structures filled with vesicles of different electron density, dimensions and shape, and with post-synaptic densities.

2.7. Statistical methods

We used two-tailed Student's *t*-test for data analysis and the customary threshold of $p < 0.05$ for statistically significant differences. We present quantitative data as mean ± SEM. The relationship between variables was derived with linear regression analysis (Spearman's r) using Prism version 7 software. Multiple comparisons were performed using a univariate analysis of variance (ANOVA with Brown-Forsythe post hoc test).

3. Data and resource availability

3.1. Data sharing

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

3.2. Resource sharing

3.2.1. Antibodies

We used the following primary antibodies: Insulin (Agilent Cat# A056401-2, RRID:AB_2617169, dilution 1:10000), Somatostatin (Agilent Cat# A0566, RRID:AB_2688022, dilution 1:50), TH (Millipore Cat# AB1542, RRID:AB_90755, dilution 1:400), Synaptophysin (LsBio Cat# LS-C174787, RRID:AB_2811021, dilution 1:100), PP (DAKO Cat# A0619, dilution 1:2000), Glucagon (LifeSpan Cat# LS-B4738-100, RRID:AB_10801031, dilution 1:1000).

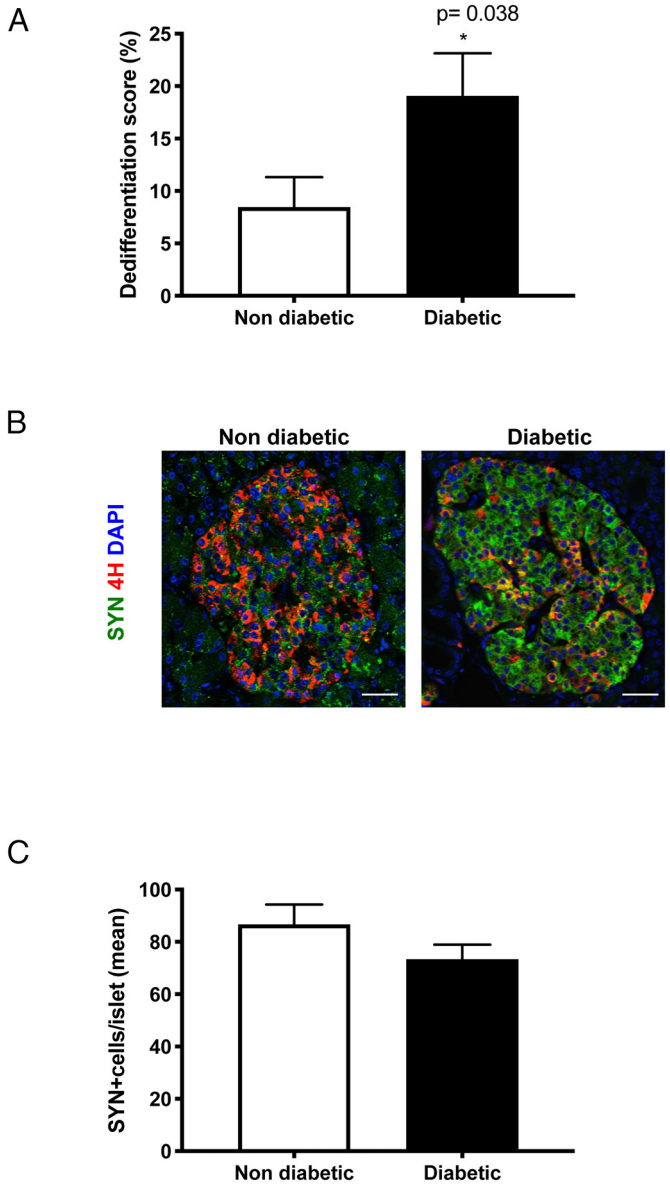

Fig. 2. Quantification of dedifferentiation score a) Dedifferentiation score calculated as Synaptophysin+/4 hormones- (SYN+/4H− cells/islet) (%). Data are means ± SEM. $p = 0.038$ by Student's t-test. b) Immunofluorescent histochemistry on pancreatic sections using combined insulin, glucagon, somatostatin and pancreatic polypeptide (4H) (red) and synaptophysin (Syn) (green), nuclei (DAPI)(blue). Scale bars 20 μm. c) Synaptophysin+ cell/islet. Data are means ± SEM. $p = 0.3$ by Student's t-test.

4. Results

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

All morphological analyses were performed in the entire cohort of patients (organ donors + pancreatic surgery patients).

4.1. Increase of noradrenergic tyrosine hydroxylase positive fibers (TH+) in Type 2 diabetes

We measured mean islet area (mean of Synaptophysin positive area/islet) and found no differences between the two groups (mean islet area 860.38 ± 272.07 μ^2 in diabetes vs 872.51 ± 257.91 μ^2 in controls, $p = 0.9$). Then we investigated TH expression, a widely used marker for noradrenergic fibers [17], in our population using immunohistochemistry. Quantification of TH positive fibers density within islets, either calculated as number of fibers per islet (0.91 ± 0.2 vs 0.32 ± 0.1 fibers/islet; $p = 0.01$) as well as number of fibers per unit cross-sectional area (0.13 ± 0.04 vs 0.04 ± 0.01 fibers/100 μ^2, $p = 0.03$), showed a 3-fold increase in diabetic samples compared to nondiabetic ones (Fig. 1 A–B). The data were in line with those observed in mouse models [25] but with a remarkable difference in quantitative terms (much higher in mice) (positive controls are shown in Supplementary Fig. 1).

4.2. The increase in TH+ fibers within islets positively correlates with the dedifferentiation score

We confirmed a significant percentage increase in beta-cell dedifferentiation, assessed as the percentage of cells retaining endocrine features (synaptophysin immunoreactivity) but no longer containing the four endocrine hormones [6,8], in diabetic samples, which was over 3 times greater than in nondiabetic samples (19.08 ± 4.05 vs 8.46 ± 2.86%; $p = 0.038$) (Fig. 2A–B). As expected, there was no difference in synaptophysin positive cells between the two groups (86.74 ± 7.55 vs 73.38 ± 5.5 cells/islet; $p = 0.20$)(Fig. 2C) [8,36]. We found a significant positive correlation between the dedifferentiation score and the average of TH+ fibers within the islets ($r = 0.39$, $p = 0.039$) (Fig. 3 A). Studying samples using electron microscopy and serial consecutive sections stained with specific antibodies, we confirmed that fibers are in direct contact with beta-cells (Fig. 3 B and Supplementary Fig. 2). Diameter of the synaptic vesicles of these fibers had an average size of 13.90 ± 1.70 nm (a size-range common to empty noradrenergic vesicles found in terminals or varicosities of efferent sympathetic nerve fibers [37]).

4.3. The increase of noradrenergic fibers correlates with an impairment of beta-cell function and shows a progressive increase from non-diabetic to diabetic condition

In order to strengthen the results on the potential functional role of noradrenergic fibers in the pathogenesis of T2D we correlated morphological analyses with in vivo parameters of patients who were metabolically characterized before sample collection (during pancreatoduodenectomy). Detailed metabolic data of these patients are shown in Table 1 and Supplementary Table 1.

We evaluated beta-cell secretory function, i.e. beta-Cell Glucose Sensitivity (βCGS) using a 2-hour hyperglycemic clamp. We observed a strong inverse correlation between TH+ fibers and βCGS ($r = -0.84$; $p = 0.01$; Fig. 4 A), which suggests that beta-Cell dysfunction might be related to innervation. Further, we also observed significant inverse correlations between TH+ fibers and the incremental second-phase insulin secretion ($r = -0.84$, $p = 0.03$) and arginine-stimulated insulin secretion ($r = -0.76$, $p = 0.04$) (Fig. 4 B–C), while no relations were

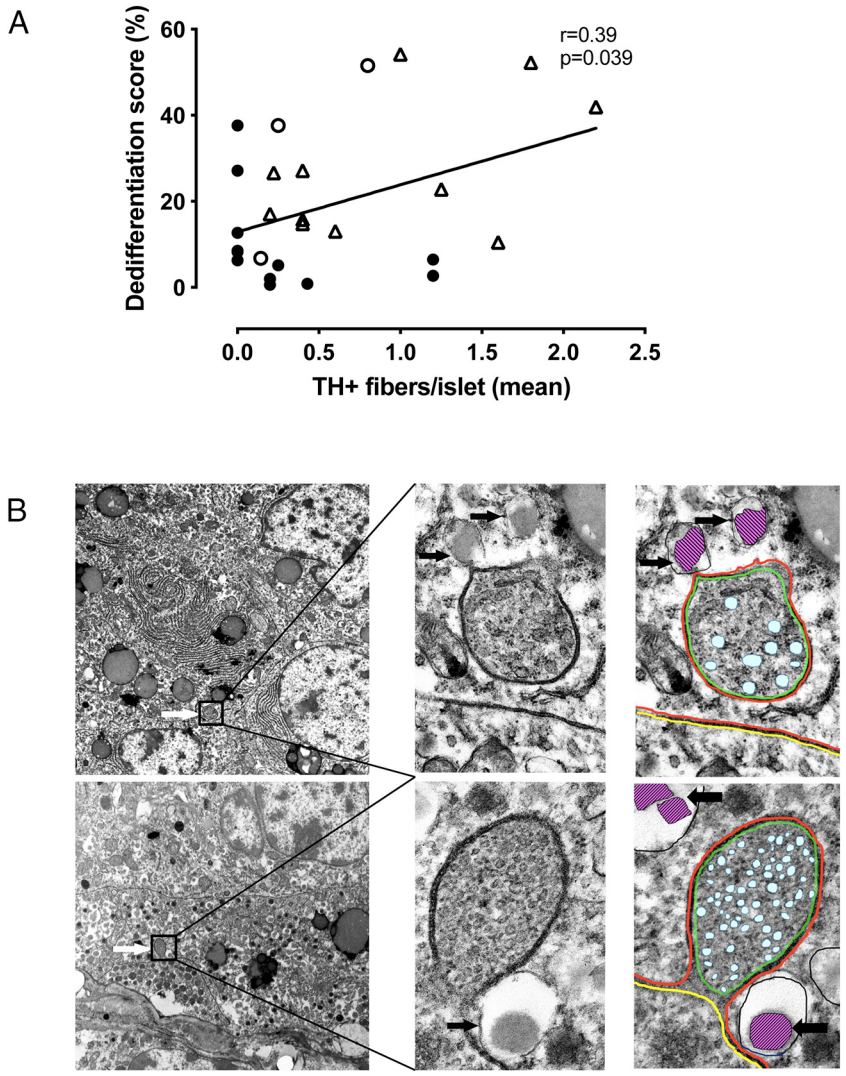

Fig. 3. The increase of TH+ fibers within islets positively correlates with the dedifferentiation score. a) We plotted linear correlation analyses (Spearman's r) between the dedifferentiation score, calculated as % ratio of SYN+4H−/SYN cells, and average of TH + fibers/islet. R = 0.39; $p = 0.039$ [n = 16 nondiabetic - circles (open circles represent IGT) and n = 11 diabetic - triangles]. b) Transmission electron microscopy of islets of diabetic patient showing fibers in direct contact with beta-cells. Upper panel shows a degranulated beta-cell. White arrows: synaptoid structure; enlargement of synaptoid structures (inset). Black arrow: typical insulin granules (some indicated) of beta-cells. Right panel schemas: green lines indicate membrane of synaptoid structure with synaptic vescicles in light blue; red lines indicate beta cell membrane with insulin like granules within the cytoplasm in purple; yellow lines indicate non beta cells membrane;

observed between the number of TH fibers and other insulin secretion parameters assessed by 2-h hyperglycemic clamp, i.e. basal insulin secretion ($r = -0.21$, $p = 0.64$), and incremental first-phase insulin secretion ($r = -0.65$, $p = 0.11$).

We found a significant positive correlation between 2-hour glucose during OGTT and the average of TH + fibers/islet ($R = 0.61$; $p = 0.046$) (Fig. 5 A). We also observed a progressive numerical increase in TH+ fibers in the islets of these patients from NGT through IGT to T2D, (one-way ANOVA $p = 0.057$) (Fig. 5 B).

Moreover, we found no statistically significant correlation between TH+ fibers and donor age, body mass index (BMI), or, when present, duration of diabetes. There was a weak positive trend for an association between TH+ fibers and duration of the disease, in line with data described in the mouse model [25] (Supplementary Fig. 3).

5. Discussion

Despite wide consensus on the role played by insulin resistance and beta-cell dysfunctions in the natural history of type 2 diabetes, many other aspects of its pathogenesis are still unclear [1,38]. Of all the complex mechanisms involved in beta-cell dysfunctions, dedifferentiation has recently been identified as one of the primary processes responsible for beta-cell failure [7,39]. Moreover, besides the "morphological" aspect of beta-cells, we are now able to determine in vivo beta-cell function parameters even in humans, e.g. beta-cell glucose sensitivity (a measure of the amount of secreted insulin for any specific glucose concentration) [13,14] which allow us to formulate pathogenic hypotheses when we link ex vivo with in vivo data.

Another mechanism involved in beta-cell dysfunction is islet innervation. It is well known that noradrenergic fibers inhibit insulin secretion [18,22,24] and an alteration of sympathetic activity has been proposed as a predictor of diabetes [29]. In a genetic mouse model of type 2 diabetes [25], we demonstrated a significant increase in noradrenergic fibers in parallel with the worsening of the disease. These fibers were in direct contact with beta-cells, suggesting a direct interplay between them.

Thus, we wanted to explore the possibility that noradrenergic innervation could be involved in the process of dedifferentiation and the beta-cell dysfunctions observed in Type 2 diabetes.

We studied pancreata from two different populations: organ donors and patients who prior to pancreatic surgery had undergone an in-

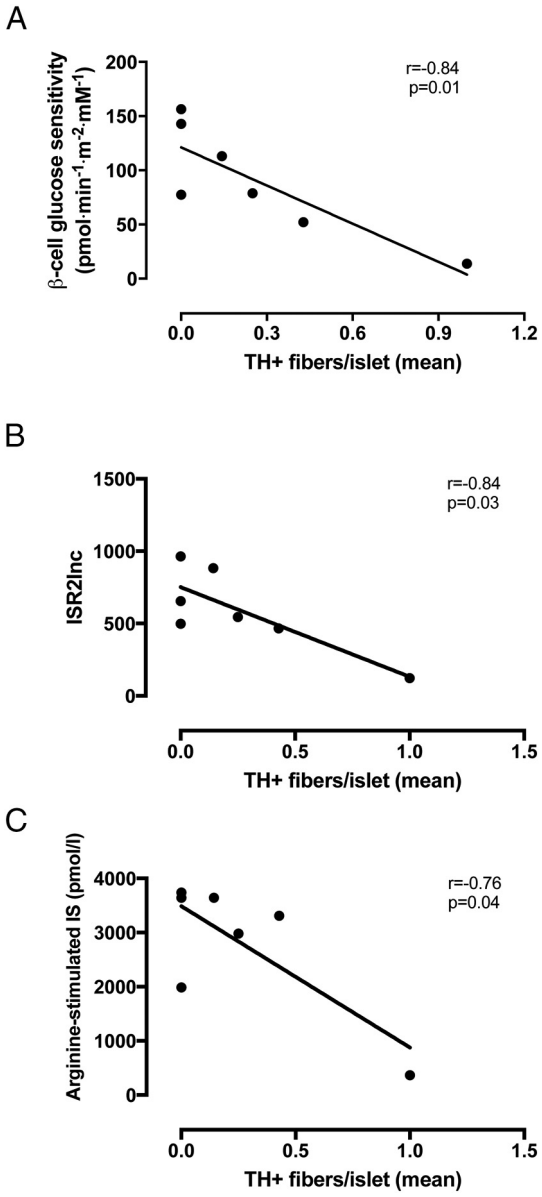

Fig. 4. Noradrenergic fibers negatively correlate with beta-cell functions Linear correlation analyses (Spearman's r) between average of TH + fibers/islet and beta-cell Glucose Sensitivity (A) ($R = -0.84$; $p = 0.01$), incremental second-phase insulin secretion (ISR2Ins) ($R = -0.84$; $p = 0.03$) (B) and arginine stimulated insulin secretion ($R = -0.76$; $p = 0.04$) (C).

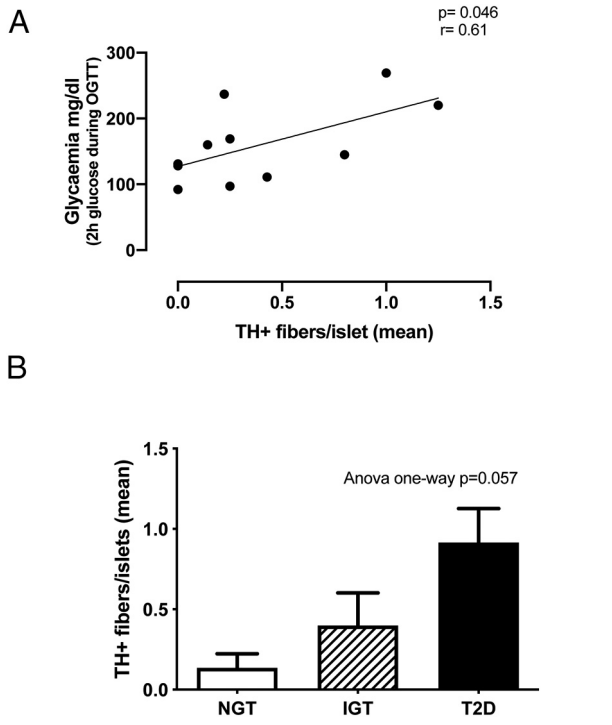

Fig. 5. a) Increase in noradrenergic fibers positively correlates with 2-h glucose during OGTT: Linear correlation analyses (Spearman's r) between 2- h glucose (mg/dl) during OGTT and average of TH + fibers/islet. $R = 0.61$; $p = 0.046$. b) Progressive increase in noradrenergic fibers from nondiabetic to diabetic condition: Data are means ± SEM. $p = 0.057$ by ANOVA one-way test.

depth metabolic evaluation. Taking advantage of the opportunity to study both populations, we were able to link important morphological observations to metabolic functions. We found a nearly threefold increase in the quantity of noradrenergic fibers in samples from diabetic compared to those from nondiabetic subjects. We report a significant positive correlation between noradrenergic fibers and dedifferentiation score, assessed as the percentage of cells retaining endocrine features (synaptophysin immunoreactivity) but no longer containing the four endocrine hormones [6,8]. Human islets have been previously described as sparsely innervated and the potential role of noradrenergic fibers in diabetes onset has been ascribed to the regulation of blood flow within the islet [17].Using electron microscopy we were instead able to observe that these fibers were in direct contact with endocrine cells bearing typical insulin granules and morphology [40,41], thus bona fide beta-cells, suggesting a direct interplay between innervation and beta-cells.

Using the extraordinary advantage of the pancreatic surgery group and in vivo insulin secretion study [42], we linked innervation to beta-cell function and glucose tolerance. We verified whether noradrenergic fibers were related to altered insulin secretion. We found a significant negative correlation between the number of noradrenergic fibers within islets and beta-cell glucose sensitivity, second phase insulin secretion and arginine-stimulated insulin secretion, which are all hallmarks of type 2 diabetes, thus further substantiating the significant role played by noradrenergic fibers in the pathogenesis of beta-cell dysfunction in human diabetes. Finally, we observed that the increment of the fibers was progressive, in parallel with worsening glucose tolerance, from normal glucose tolerance to impaired glucose tolerance to diabetes, and perfectly in line with the data described in the mouse model [25].

A potential interpretation of the reason why the sympathetic nervous system causes an increase in pancreatic noradrenergic fibers, inducing beta cell dedifferentiation and therefore blocking insulin secretion, could be that this branch of the nervous system is particularly sensitive to insulin [43,44]. It is difficult to comprehend why, at a time when insulin secretion is indispensable to prevent diabetes onset, the body reduces its secretion. On the other hand hyperinsulinemia is a well-known risk factor for many diseases, including cancer [45–47]. Thus noradrenergic fibers could represent a defense mechanism against a deleterious hypersecretion of insulin.

In vitro experiments are needed to find and study potential molecular mechanisms linking noradrenergic innervation to beta cell dedifferentiation. The decrease of intracellular cAMP, induced by the activation of the alpha 2 receptor by noradrenergic fibers, inhibits beta cell insulin release [48]. When this stimulus becomes chronic, the reduction of cAMP could potentially mediate the dedifferentiation of beta cells by leading to an alteration of gene transcription, such as a reduction of FOXO1 transcription, the main transcription factor involved in curbing the process of beta cell dedifferentiation [6–8].

Our study is the first to describe a comparison of islet innervation, associated with in vivo metabolic studies, in patients with and without diabetes. However, our study has some limitations. We assumed that the described fibers contain noradrenalin, due to its well-known

inhibitory effect on insulin secretion [49], but we cannot exclude an involvement of the dopaminergic system because tyrosine hydroxylase is the enzyme responsible for catalyzing the conversion of amino acids into the precursors of either dopamine or noradrenalin [50,51]. Finally, as in any human morphologic study, all our results are correlations, without a true "morphologic" follow-up or linear tracing, obviously not feasible in humans. Despite these limitations, we can affirm that our data strongly suggest the hypothesis that beta-cell dedifferentiation and dysfunction may be modulated by noradrenergic innervation, providing a new potential explanation for the induction of beta-cell failure. We found that the progressive increase in fibers paralleled the worsening of glucose tolerance, suggesting the need to act on pre-diabetes, before dedifferentiation is too advanced to be reversed. The reversibility of beta-cell dedifferentiation has already been proven in rodents [11,52–54]; thus innervation might represent a new target to prevent diabetes. The new frontier will be to investigate and provide clinical, molecular and genetic pathways linking innervation and beta-cell dedifferentiation in line with the new therapeutic approaches of a personalized medicine.

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Author contributions

F.C. and T.M. designed, executed, analyzed the experiments and wrote the manuscript. G.P.S., C.M.A.C, S.M. and F.I. performed in vivo experiments. S.A. and G.Q. performed surgeries from which donor samples were obtained. A.M. generated data. L.M., and M.S. selected patients and obtained pancreata. I.S. and S.C. performed EM data. P.M., A.P., S.C. contributed to the discussion and edited the manuscript. A.G. reviewed/edited the manuscript. F.C. and A.G. are the 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 the data analysis.

CRediT authorship contribution statement

Francesca Cinti: Conceptualization, Investigation, Writing - Original Draft, Writing - Review & Editing, Funding acquisition.
Teresa Mezza: Conceptualization, Investigation, Writing - Original Draft, Writing - Review & Editing, Funding acquisition.
Ilenia Severi: Investigation, Writing - Review & Editing.
Mara Suleiman: Investigation.
Chiara Maria Assunta Cefalo: Investigation.
Gian Pio Sorice: Investigation.
Simona Moffa: Investigation.
Flavia Impronta: Investigation.
Giuseppe Quero: Investigation.
Sergio Alfieri: Investigation.
Andrea Mari: Investigation.
Alfredo Pontecorvi: Investigation.
Lorella Marselli: Investigation.
Saverio Cinti: Supervision, Writing - Review & Editing.
Piero Marchetti: Supervision, Writing - Review & Editing.
Andrea Giaccari: Conceptualization, Supervision, Writing - Review & Editing, Funding acquisition.

Declaration of competing interest

No potential conflicts of interest relevant to this article were reported.

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