

Taurine supplementation ameliorates glucose homeostasis, prevents insulin and glucagon hypersecretion, and controls β , α , and δ -cell masses in genetic obese mice

Junia C. Santos-Silva¹ · Rosane Aparecida Ribeiro² · Jean F. Vettorazzi¹ · Esperanza Irles³ · Sarah Rickli¹ · Patrícia C. Borck¹ · Patricia M. Porciuncula¹ · Ivan Quesada³ · Angel Nadal³ · Antonio C. Boschero¹ · Everardo M. Carneiro¹

Received: 3 February 2015 / Accepted: 15 April 2015 / Published online: 5 May 2015
© Springer-Verlag Wien 2015

Abstract Taurine (Tau) regulates β -cell function and glucose homeostasis under normal and diabetic conditions. Here, we assessed the effects of Tau supplementation upon glucose homeostasis and the morphophysiology of endocrine pancreas, in leptin-deficient obese (ob) mice. From weaning until 90-day-old, C57Bl/6 and ob mice received, or not, 5 % Tau in drinking water (C, CT, ob and obT). Obese mice were hyperglycemic, glucose intolerant, insulin resistant, and exhibited higher hepatic glucose output. Tau supplementation did not prevent obesity, but ameliorated glucose homeostasis in obT. Islets from ob mice presented a higher glucose-induced intracellular Ca^{2+} influx, NAD(P)H production and insulin release. Furthermore, α -cells from ob islets displayed a higher oscillatory Ca^{2+} profile at low glucose concentrations, in association with glucagon hypersecretion. In Tau-supplemented ob mice, insulin and glucagon secretion was attenuated, while Ca^{2+} influx tended to be normalized in β -cells and Ca^{2+}

oscillations were increased in α -cells. Tau normalized the inhibitory action of somatostatin (SST) upon insulin release in the obT group. In these islets, expression of the glucagon, GLUT-2 and TRPM5 genes was also restored. Tau also enhanced MafA, Ngn3 and NeuroD mRNA levels in obT islets. Morphometric analysis demonstrated that the hypertrophy of ob islets tends to be normalized by Tau with reductions in islet and β -cell masses, but enhanced δ -cell mass in obT. Our results indicate that Tau improves glucose homeostasis, regulating β -, α -, and δ -cell morphophysiology in ob mice, indicating that Tau may be a potential therapeutic tool for the preservation of endocrine pancreatic function in obesity and diabetes.

Keywords Glucagon secretion · Insulin secretion · Obesity · Somatostatin · Taurine supplementation

Introduction

Obesity is a chronic metabolic disorder that predisposes to the development of insulin resistance and type 2 diabetes (T2D) (Kahn et al. 2006). Pancreatic islets are heterogeneous cell aggregates that contain β , α , δ , ϵ and pancreatic polypeptide (PP) cells (Cabrera et al. 2006). Pancreatic β and δ -cells release insulin and somatostatin (SST), respectively, in response to an increase in blood glucose (Hellman et al. 2009; Hauge-Evans et al. 2012). However, α -cells secrete glucagon when glycemia decreases, preventing the hypoglycemia that can occur in the fasting state (Quesada et al. 2008). Although, the establishment of T2D is often associated with β -cell dysfunction, in parallel with the lower insulin action (Cerf 2013), impairments in α and δ -cell functions also contribute to glucose homeostasis disruption in this disease (Hauge-Evans et al.

✉ Rosane Aparecida Ribeiro
rosaneribeirobio@gmail.com

✉ Everardo M. Carneiro
emc@unicamp.br

¹ Departamento de Biologia Estrutural e Funcional, Instituto de Biologia, e Centro de Pesquisa em Obesidade e Comorbidades, Universidade Estadual de Campinas (UNICAMP), C.P. 6109, Campinas, SP CEP 13083-970, Brazil
² Universidade Federal do Rio de Janeiro, NUPEM, Campus UFRJ-Macaé, Avenida São José do Barreto, 764, Barreto, Macaé, RJ CEP 27965-045, Brazil
³ Instituto de Bioingeniería y Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas, CIBERDEM, Universidad Miguel Hernández de Elche, Elche, Alicante, Spain

2009; Walker et al. 2011; Unger and Orci 2010). Among the situations in which β , α and δ -cell dysfunction may manifest (Walker et al. 2011; Cerf 2013), disruption of the paracrine interactions between insulin, SST and glucagon within the islet can seriously compromise absolute and pulsatile islet secretory function, impairing glucose homeostasis (Quesada et al. 2008; Hellman 2009; Unger and Orci 2010).

Taurine (Tau) is an amino acid involved in several biological processes (Ripps and Shen 2012). Tau supplementation prevents the development of obesity (Tsuboyama-Kasaoka et al. 2006; Nardelli et al. 2011) and ameliorates glycemia, the action of insulin, and dyslipidemia in T2D (Tsuboyama-Kasaoka et al. 2006; Nardelli et al. 2011; Kim et al. 2012; Batista et al. 2013). Tau regulates insulin secretion, improving islet Ca^{2+} handling, in response to high glucose concentrations (Carneiro et al. 2009; Ribeiro et al. 2009). In diet-induced obesity, Tau treatment prevents hyperinsulinemia (Ribeiro et al. 2012; Batista et al. 2013; Vettorazzi et al. 2014), insulin hypersecretion and islet hypertrophy (Ribeiro et al. 2012). However, in pancreatic islets, Tau is concentrated mainly in the α and δ -cells (Bustamante et al. 2001), which indicates that this amino acid may also regulate glucagon secretion. Here, using leptin-deficient obese (ob) mice that display morbid obesity (Lindstrom 2010), insulin resistance, and insulin hypersecretion (Tomita et al. 1992; Chen et al. 1993), we assessed the effects of Tau supplementation upon obesity, glucose homeostasis, and islet-cell morphophysiology. Tau treatment prevented insulin and glucagon hypersecretion, and ameliorated β -cell responsiveness to SST in ob islets. Additionally, Tau decreased islet hypertrophy, increased δ -cell mass, regulated Ca^{2+} handling in β and α -cells, and modified the expression of transcription factors and genes that participate in islet morphofunction.

Materials and methods

Experimental groups

All experimental procedures were developed in accordance with the ethics committee in animal experimentation, UNICAMP (certificate no.: 2018-1). From weaning to 90 days of age, male C57Bl/6 (C group) or leptin-deficient obese (ob) mice were distributed into four groups: C; C that received 5 % Tau (Ribeiro et al. 2012) in their drinking water (CT); ob or obT. All mice groups were maintained on a 12 h light–dark cycle (lights 8:00–20:00 h) with controlled humidity and temperature ($21 \pm 2^\circ\text{C}$), and allowed free access to standard laboratory chow (Nutrilab, Colombo, PR, BRA) and water.

Obesity evaluation and biochemical nutritional parameters

At the end of the supplementation period, the final body weight (BW) and nasoanal length were measured in all mice groups to obtain the Lee index [from the ratio of $\text{BW}(\text{g})^{1/3}/\text{nasoanal length (cm)} \times 1000$] (Bernardis and Patterson 1968). Afterwards, mice were euthanized and the retroperitoneal and perigonadal fat pads were collected and weighed. Blood samples were collected and the plasma was used for insulin and glucagon measurement by radioimmunoassay (RIA) (Ribeiro et al. 2010). Plasma glucose concentrations were measured using a glucose analyzer (Accu-Chek Perfoma, Roche Diagnostic, Switzerland). Total cholesterol (CHOL), triglycerides (TG) and non-esterified fatty acids (NEFAs) levels were measured using standard commercial kits, according to the manufacturer's instructions (Roche/Hitachi®; Indianapolis, USA, and Wako®; Richmond, USA, respectively).

Tau plasma levels

The free amino acids in the plasma were measured using pre-column derivatization with phenyl isothiocyanate (Sigma P1034). The chromatography was developed with a mobile phase gradient of acetonitrile, water based buffer solution and the basic sodium acetate and water, at 42°C (flow 1 mL). Separation was performed on C18 reverse phase column 3.9×300 mm (Pico-tag for free amino acid analysis, Waters, Ireland) and detected at 254 nm. A volume of 50 μL of internal standard solution (methionine sulphone—S0065 Synth-r) was added to 50 μL of each plasma sample. The supernatant was filtered through molecular filter (Sartorius Stedim Biotech, Goettingen, Germany) with a cut-off of 3 kDa and centrifuged at 15,000g for 45 min. The volume of 40 μL of each sample was derivatized and suspended in 500 μL diluent. Twenty microliters were injected into the liquid chromatograph (HPLC system/SCL-10AVP, Shimadzu Scientific Instruments, Columbia, MD, USA). The results were analyzed by the software Class VP version 5:43 (Shimadzu). An amino acid standard solution was derivatized and analyzed together with the samples (cat. A6407 and A6282, respectively; Sigma, Aldrich, St Louis, MO, USA).

Intraperitoneal glucose (ipGTT), insulin (ipITT) and glucagon (ipGluTT) tolerance tests

For ipGTT, 8 h-fasted mice received an ip injection of 2 g/kg BW glucose. Blood samples from the tip of the tail were collected before (time 0) and at 15, 30, 60, 120 and 180 min after glucose administration for glycemia measurement using a glucose analyzer (Accu-Chek Performa,

Roche Diagnostic®, Switzerland). For the ipITT, fed ob and obT mice received an ip injection of 10 U/kg BW human insulin; however for the C and CT groups, 2 U/kg BW human insulin were administered (Humulin® R, Lilly's, São Paulo, SP, BRA). Blood samples were also collected before insulin injection (time 0) and 10, 15, 30 and 60 min after insulin administration for plasma glucose concentration analysis. For the ipGluTT, blood glucose levels (time 0) were measured in 8 h-fasted mice. Subsequently, all mice groups received via ip an injection of 100 µg/kg BW glucagon, and additional blood samples were collected at 5, 10, 20, 40 and 60 min (Batista et al. 2013).

Islet isolation, static insulin and glucagon secretion

Islets were isolated by collagenase digestion of the pancreas. For insulin and glucagon static secretion experiments, groups of four or fifteen islets, respectively, were incubated for 30 min at 37 °C in Krebs–Ringer bicarbonate (KRB) buffer containing 115 mM NaCl, 5 mM KCl, 10 mM NaHCO₃, 2.56 mM CaCl₂, 1 mM MgCl₂ and 15 mM HEPES, supplemented with 5.6 mM glucose plus 0.3 % BSA (Sigma Chemical, St Louis, MO, USA), pH 7.4 and continuously gassed with 95 % O₂/5 % CO₂. This medium was then replaced with fresh buffer and the islets were incubated for further 1 h with different glucose concentrations or 2.8 mM glucose in combination or not with 10 nM SST for insulin release evaluation. For glucagon secretion assessment, the islets were incubated with 5.6 and 0.5 mM glucose. At the end of the incubation period, the insulin content of the medium was measured using human insulin radiolabeled with ¹²⁵I (Genesis, São Paulo, SP, BRA) by RIA, as previously reported (Ribeiro et al. 2010). Glucagon was measured following the manufacturer's instructions by RIA kit (Merck, Millipore, Darmstadt, Germany). For islet insulin or glucagon content, groups of 4 or 15 islets, respectively, were collected and transferred to tubes containing 1 mL deionized water, and the islet-cells were homogenized using an ultrasonic homogenizer (Brinkmann Instruments, Westbury, NY, USA).

Cytoplasmic Ca²⁺ oscillation measurement by fluorescence or confocal microscopy

For intracellular Ca²⁺ concentration ([Ca²⁺]_i) recordings, islets were incubated in KRB medium containing 5.6 mM glucose at 37 °C for 2 h. During the last hour of incubation, islets were loaded with 5 µM of the Ca²⁺-sensitive dye Fura-2 acetoxymethylester (AM). Afterwards, single islets were placed inside a thermostatically-regulated chamber (37 °C) over poly-L-lysine-treated glass coverslips and perfused with a BSA-free KRB buffer containing 11.1 mM glucose as indicated in the figure legends. Fura-2AM

loaded islets were imaged using an inverted epifluorescence microscope (Nikon Eclipse TE200, Tokyo, Japan). A ratio image was acquired every 3 s with a Cool One camera (Photon Technology International, NJ, USA) using a dual filter wheel equipped with 340, 380 and 10 nm bandpass filters, and a range of neutral density filters (Photon Technology International, NJ, USA). Data were acquired using the Image Master version 5.0 software (Photon Technology International, NJ, USA) (Carneiro et al. 2009).

For Ca²⁺ oscillation recordings in α-cells, isolated islets were incubated with 5 µM Fluo-4 for 1 h at room temperature. Islets were placed in a chamber mounted on the microscope stage and perfused with a KRB medium containing 0.5 or 5.6 mM glucose. Low glucose concentrations induce regular Ca²⁺ oscillations in α-cells that are inhibited by high glucose concentrations (Nadal et al. 1999; Quesada et al. 2006). This typical pattern has been used to identify α-cells within the islet. To analyze these Ca²⁺ oscillations, individual cells identified at the periphery of the freshly isolated islets were monitored using a Zeiss LSM 510 laser confocal microscope (Zeiss, Oberkochen, Germany). The Ca²⁺ probe was excited at 488 nm and emission was collected with a band-pass filter at 505–530 nm from an optical section of 8 µm. Images were collected at 2 s intervals and treated with a low pass filter (Nadal et al. 1999; Quesada et al. 2006).

Measurement of NAD(P)H and mitochondrial membrane potential (Δψ_m)

The autofluorescence for NAD(P)H and the Δψ_m were monitored in freshly isolated islets from all groups of mice, in response to increasing glucose concentrations (0.5–22.2 mM glucose), using an inverted epifluorescence microscope (Axiovert 200; Zeiss, Jena, Germany). Images were acquired with an extended C4742-95 digital camera (Hamamatsu Photonics, Barcelona, Spain) using 10-nm band-pass filters (Omega Optics, Madrid, Spain). NAD(P)H autofluorescence was excited with a 360 nm band-pass filter and emission was filtered at 445 ± 25 nm. An image was acquired every 60 s using ORCA software (Hamamatsu Photonics, Barcelona, Spain). The electrical potential across the inner mitochondrial membrane was measured using rhodamine (Rhod)-123. Islets were pre-loaded with 10 µg/mL Rhod-123 for 20 min and an image was acquired every 30 s using conventional fluorescein filters and ORCA software (Hamamatsu Photonics, Barcelona, Spain) (Pertusa et al. 2002).

Pancreas morphometry and immunohistochemistry

For morphometric analyses, pancreases from all groups of mice were removed, weighed, fixed for 16 h in Bouin's

Table 1 Primer sequences for the islet-associated transcription factors and genes involved in islet-cell functions

	Forward (5'–3')	Reverse (5'–3')
Insulin	AGCAGGAAGGTTATTGTTTC	ACATGGGTGTGTAGAAGAAG
Glucagon	GGCTCCTTCTCTGACGAGATGAGCAC	CTGGCACGAGATGTTGTGAAGATGG
SST	GAGCCCAACCAGACAGAGAA	AAGCTGGCTGCAAGAACTTC
GLUT-2	GGAAGAGGCATCGACTGAGCAG	GCCTTCTCCACAAGCAGCACAG
TRPM5	CAAATCCCTCTGGATGAAATTGATG	CCAGCCAGTTGGCATAGA
SSTR2	CTACATCTTCAACGTCTCTTC	ATTGTGAATTGTCTGCCTTG
MafA	CATCCGACTGAAACAGAAG	ATTTCTCCTTGTACAGGTCC
PAX-6	TTTGAGAGGACCCATTATCC	AACCATACCTGTATTCTTGC
PDX-1	GATGAAATCCACCAAAGCTC	TAAGAATTCCTTCTCCAGCTC
Ngn3	GTCGGGAGAACTAGGATG	AAAAGGTTGTTGTGTCTCTG
NeuroD	AGATCGTCACTATTTCAGAACC	AGAACTGAGACACTCATCTG
MafB	AAATTGACATACACACACC	AAGCCTGTCTTGTCTTCTTC
GAPDH	CCTGCACCACCAACTGCTTAG	GCCCCACGGCCATCACGCCA

SST somatostatin, *GLUT-2* glucose transporter 2, *TRPM5* transient receptor potential melastatin 5, *SSTR2* SST receptor 2, *MafA* V-maf musculoaponeurotic fibrosarcoma oncogene homolog A, *PDX-1* pancreatic and duodenal homeobox factor 1, *Pax-6* paired box 6, *Ngn3* neurogenin 3, *NeuroD* neurogenic differentiation 1 or $\beta 2$, *MafB* V-maf musculoaponeurotic fibrosarcoma oncogene homolog B, *GAPDH* glyceraldehyde 3-phosphate dehydrogenase

solution and routinely embedded in Paraplast® (Sigma Chemical, St Louis, MO, USA). From each block, exhaustive 5 μ m serial sections were obtained (every 20th section) and randomly selected for insulin, glucagon and the SST immunoperoxidase reaction. For immunohistochemistry, the paraplast was removed; the sections were rehydrated and washed with 0.05 M Tris–saline buffer (TBS) pH 7.4, and incubated with TBS containing 0.3 % H₂O₂ for endogenous peroxidase activity blockade and permeabilized for 1 h with TTBS (0.1 % Tween® 20 and 0.5 % fat free milk in TBS). The sections were incubated overnight with a polyclonal guinea pig anti-insulin (1:100; Dako North America, Inc., CA, USA), or rabbit anti-glucagon (1:50; Dako North America, Inc., CA, USA) or goat anti-SST (1:50; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) antibody at 4 °C. Subsequently, the sections were incubated with rabbit anti-guinea-pig IgG, or goat anti-rabbit or rabbit anti-goat conjugated antibody with HRP for 1 h and 30 min (1:1500; Zymed Laboratories, Inc., San Francisco, CA, USA). The positive insulin, glucagon or SST cells were detected with diaminobenzidine (DAB; Sigma Chemical, St Louis, MO, USA) solution (10 % DAB and 0.2 % H₂O₂ in TBS). Finally, the sections were quickly stained with Ehrlich's hematoxylin and mounted for observation by microscopy. All islets present in the sections were covered systematically by capturing images with a digital camera (Nikon FDX-35) coupled to a Nikon Eclipse E800 microscope (Nikon, Tokyo, Japan). The islet, β , α and δ -cell and section areas were analyzed using the free software, Image J (<http://imagej.nih.gov/ij>). The islet, β , α and δ -cell masses were calculated by multiplying the pancreas weight by

the total islet or β , or α or δ -cell area per pancreas section (Ribeiro et al. 2012).

Quantitative real-time PCR

Total RNA from all groups of islets was isolated using the RNeasy Plus Mini kit (Qiagen, Hilden, Germany) and the RNA concentration was measured in a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). cDNA was synthesized from 500 ng RNA using the high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA). Quantitative PCR reactions were performed using the CFX96 Real Time System (Bio-Rad, Hercules, CA, USA). Reactions were carried out in a final volume of 10 μ L, containing 200 nM primers, 1 μ L cDNA and iQ™ SYBR® Green supermix (Applied Biosystems, Foster City, CA, USA). Samples were subjected to the following thermal cycler conditions: 10 min at 95 °C, 45 cycles (10 s at 95 °C, 7 s at 60 °C, 15 s at 72 °C) and melting curve from 65 to 95 °C with a slope of 0.1 °C/s. Primer sequences used for mice genes are provided in Table 1. The resulting values were analyzed by CFX Manager version 1.6 software (Bio-Rad, Hercules, CA, USA). The relative mRNA levels were determined by the $2^{-\Delta\Delta C_t}$ method and normalized by the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene.

Statistical analysis

Results are presented as mean \pm SEM for the number of independent experiments (n) indicated. The area under

Table 2 Obesity parameters in 90 day-old male C, CT, ob and obT mice

	C	CT	ob	obT
BW (g)	29 ± 1 ^a	28 ± 1 ^a	50 ± 1 ^b	47 ± 1 ^b
Lee index	312 ± 5 ^a	323 ± 7 ^a	373 ± 4 ^b	365 ± 5 ^b
Retroperitoneal fat pad (mg/g BW)	1.9 ± 0.3 ^a	2.3 ± 0.3 ^a	14.3 ± 2.4 ^b	11.9 ± 2.7 ^b
Perigonadal fat pad (mg/g BW)	6.8 ± 0.7 ^a	8.2 ± 1.0 ^a	52.3 ± 5.7 ^b	55.3 ± 5.0 ^b

Data are mean values ± SEM ($n = 8-16$). Different letters represent significant differences (Two-way ANOVA followed by Holm-Sidak post test, $P \leq 0.05$)

the curve (AUC) was calculated by trapezoidal integration using GraphPad Prism[®] version 5.00 for Windows (San Diego, CA, USA). Data were analyzed by two-way analysis of variance (ANOVA), followed by the Holm-Sidak post test using SigmaStat version 3.5 for Windows (Jandel Scientific Software Inc., San Jose, CA, USA). The level of significance was set at $P \leq 0.05$.

Results

Obesity evaluation and general nutritional features

Final BW and Lee index were 73 and 19 % higher in 90-day-old male ob mice, compared with C mice

($P < 0.0001$; Table 2). In addition, ob mice displayed a substantial fat accumulation with approximately 7-fold higher retroperitoneal and perigonadal fat stores, compared with the C group ($P < 0.0001$; Table 2). Tau-supplemented ob mice presented BW, Lee index, retroperitoneal and perigonadal fat stores similar to that observed in ob mice (Table 2).

In addition, ob mice were hyperglycemic and hyperinsulinemic under fasting and fed conditions, compared with C mice ($P < 0.01$, $P < 0.002$; Table 3). The ob group presented an increase of 69 % in plasma CHOL levels ($P < 0.001$), but reduced plasma NEFA concentrations in the fasting state, in comparison with the C mice ($P < 0.05$; Table 3). In obT mice, blood glucose levels, in the fed state, and insulinemia in both the fed and fasted states, were normalized (Table 3). Whereas, an increase in plasma CHOL levels under fed conditions and higher fasting NEFA plasma concentrations were observed in obT mice, compared with the ob group ($P < 0.01$, $P < 0.03$; Table 3). Under fasting conditions, no alterations in plasma glucagon levels were observed between groups (Table 3). Tau plasma levels were increased by 112 and 107 % in fasted obT and CT mice, compared with their respective controls ($P < 0.05$; Table 3).

Glucose homeostasis

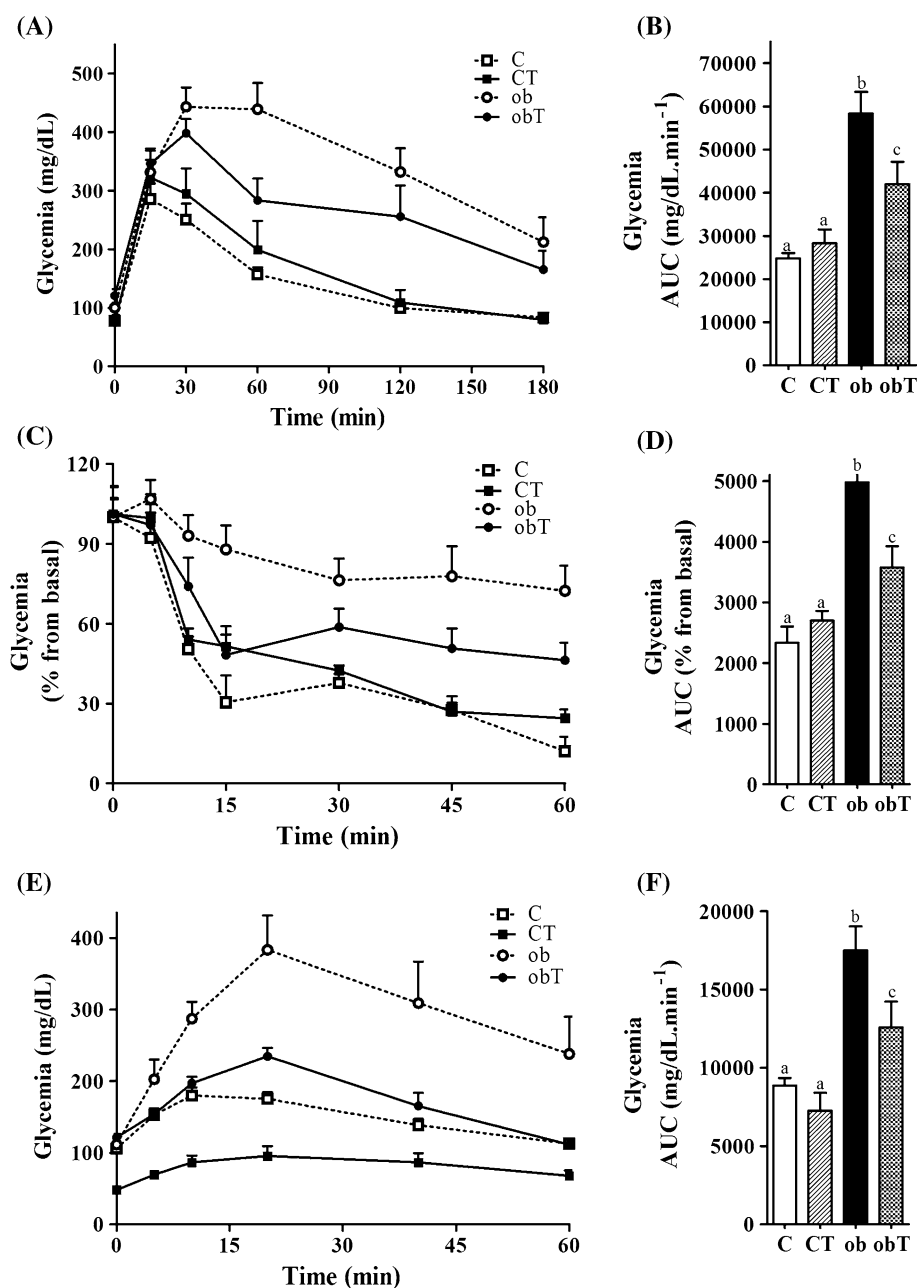
At 90 days of age, mice of all groups were submitted to an ipGTT. After glucose administration, blood glucose reached

Table 3 Plasma biochemical parameters in 12 h-fasted and fed 90-day-old male C, CT, ob and obT mice

	C	CT	ob	obT
Glycemia (mg/dL)				
Fasted	74 ± 4 ^a	96 ± 6 ^a	105 ± 9 ^b	119 ± 9 ^b
Fed	134 ± 12 ^a	134 ± 4 ^a	174 ± 7 ^b	127 ± 7 ^a
Insulin (ng/mL)				
Fasted	0.21 ± 0.1 ^a	0.18 ± 0.1 ^a	5.57 ± 0.9 ^b	2.42 ± 0.7 ^a
Fed	1.10 ± 0.1 ^a	0.56 ± 0.1 ^a	49.26 ± 14.9 ^b	3.92 ± 1.2 ^a
CHOL (mg/dL)				
Fasted	135 ± 15 ^a	167 ± 13 ^a	228 ± 13 ^b	241 ± 19 ^b
Fed	151 ± 15 ^a	155 ± 20 ^a	200 ± 28 ^a	307 ± 30 ^b
TG (mg/dL)				
Fasted	116 ± 24	87 ± 19	142 ± 28	111 ± 24
Fed	159 ± 13	155 ± 13	126 ± 35	140 ± 44
NEFA (mmol/L)				
Fasted	1.48 ± 0.2 ^{a,b}	1.11 ± 0.1 ^b	1.06 ± 0.1 ^c	1.41 ± 0.2 ^{a,c}
Fed	0.66 ± 0.1 ^a	1.04 ± 0.2 ^{a,b}	0.87 ± 0.1 ^a	1.40 ± 0.2 ^b
Tau (mmol/L)				
Fasted	1.3 ± 0.2 ^a	2.7 ± 0.2 ^b	1.3 ± 0.3 ^a	2.8 ± 0.5 ^b
Glucagon (pg/mL)				
Fasted	55 ± 10	71 ± 6	63 ± 8	76 ± 10

Data are mean values ± SEM ($n = 8-16$). Different letters represent significant differences (Two-way ANOVA followed by Holm-Sidak post test, $P \leq 0.05$)

Fig. 1 Tau supplementation ameliorates glucose homeostasis in ob mice. Changes in blood glucose during the ipGTT (a), ipITT (c) and ipGluTT (e). Total plasma glucose concentrations during ipGTT (b), ipITT (d) and ipGluTT (f) expressed by the AUC. Data are mean \pm SEM ($n = 5$ –8 mice). Different letters over the bars represent significant differences (Two-way ANOVA followed by Holm-Sidak post test, $P \leq 0.05$)



maximal values at 15 min in C and CT, and at 30 min in ob, and obT mice (Fig. 1a). In the ob group, hyperglycemia initiated at 30 min and persisted until 120 min ($P < 0.001$; Fig. 1a). Tau supplementation decreased glycemia at 60 min in obT, compared with ob mice ($P < 0.005$). Total plasma glucose levels (AUC), during ipGTT, were 1.8-fold higher in ob than in C mice ($P < 0.0001$; Fig. 1b). Tau supplementation improved glucose tolerance in obT, with a reduction of 36 % in the total glycemia, compared with ob mice ($P < 0.0001$; Fig. 1b).

During the ipITT, ob mice displayed enhanced blood glucose compared with C mice (Fig. 1c), indicating an impaired insulin action, as confirmed by an increase of

124 % in the total glycemia in ob compared with the C mice, during the test ($P < 0.0001$; Fig. 1d). Tau treatment improved the insulin action in obT mice, decreasing the AUC of the ipITT by 27 %, in comparison with ob mice ($P < 0.001$; Fig. 1d).

Hepatic glucose production was also assessed by an ipGluTT. Fasted ob mice showed higher glycemia after glucagon administration (Fig. 1e), exhibiting an exacerbation in hepatic glucose mobilization, since the total glycemia during the test was 97 % higher in ob than in C mice ($P < 0.001$; Fig. 1f). A reduction of 39 % in hepatic glucose production was observed in obT mice, when compared with ob mice ($P < 0.001$; Fig. 1f).

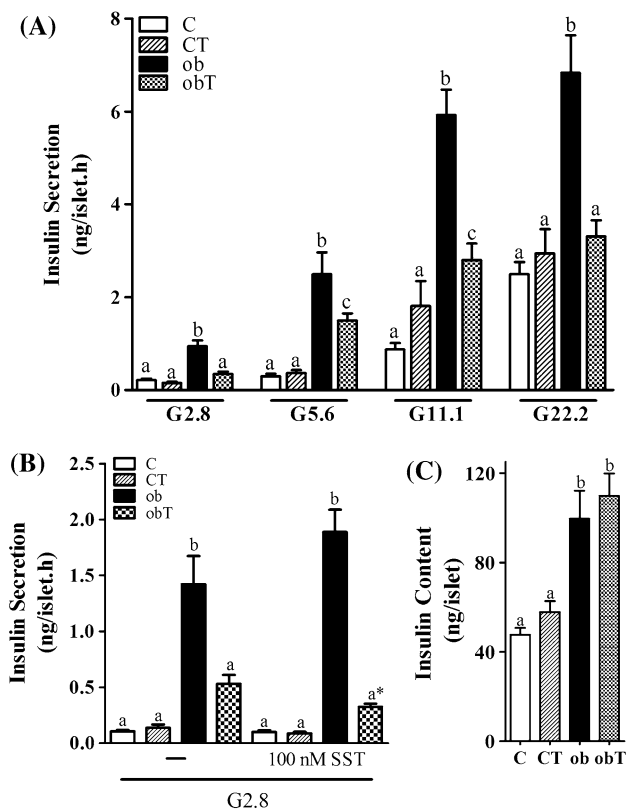


Fig. 2 Tau supplementation prevented insulin hypersecretion in ob islets, enhancing β -cell responsiveness to SST. Static insulin secretion in response to increasing glucose (G) concentrations (a), or at 2.8 mM glucose with or without 100 nM SST (b), and total islet insulin content (c) in isolated islets from 90-day-old male C, CT, ob and obT mice. Groups of 4 islets of similar sizes were incubated for 1 h under different stimuli, as indicated in the figure. Each bar represents mean \pm SEM ($n = 12\text{--}24$). Different letters over the bars represent significant differences (Two-way ANOVA followed by Holm-Sidak post test, $P < 0.05$). *Indicates a significant difference compared with obT islets incubated at 2.8 mM glucose ($P \leq 0.05$)

Insulin secretion, Ca^{2+} handling and glucose metabolism in β -cells

To evaluate β -cell function, isolated islets from mice of all groups were exposed to increasing glucose concentrations (Fig. 2a). Islets from ob mice secreted higher amounts of insulin at all glucose concentrations, compared with C islets ($P < 0.001$). Tau supplementation normalized insulin secretion in obT at 2.8 and 22.2 mM glucose, and significantly reduced secretion at 5.6 and 11.1 mM glucose, which was 40 and 53 % lower, respectively, than those observed for ob islets ($P < 0.001$ and $P < 0.0001$, respectively; Fig. 2a). In addition, when ob islets were incubated with 2.8 mM glucose and 100 nM SST, no alterations in insulin secretion were observed (Fig. 2b). In contrast, obT islets presented a 39 % reduction in insulin release in response to 100 nM SST ($P < 0.03$; Fig. 2b). However, the

higher content of insulin in the ob islets (130 % higher than C islets; $P < 0.0001$) was not altered by Tau treatment. It is, therefore, plausible that the lower insulin secretion in obT is due to a better responsiveness to SST in these islets.

Figure 3a–f shows cytoplasmic Ca^{2+} oscillations, in response to 11.1 mM glucose, in islets isolated from all groups of mice. The switch from 2.8 to 11.1 mM glucose produced a typical pattern of glucose-induced cytoplasmic Ca^{2+} influx, which was characterized by an initial small decrease, followed by an abrupt and sustained increase, and subsequent oscillations that varied among the groups (Fig. 3a, b, d and e). Islets from ob mice presented a higher Ca^{2+} influx, induced by 11.1 mM glucose, as shown by the higher AUC and amplitude of cytoplasmic Ca^{2+} , when compared with C islets ($P < 0.0001$; Fig. 3e, f); in contrast, ob islets did not present Ca^{2+} oscillations in response to glucose (Fig. 3c). Tau treatment decreased the total Ca^{2+} influx, compared with the ob group ($P < 0.0001$; Fig. 3e), and normalized the amplitude of $[\text{Ca}^{2+}]_i$ in obT islets, without restoring the typical oscillatory Ca^{2+} flux (Fig. 3f).

Mitochondrial ATP synthesis is dependent upon $\Delta\psi_m$ (Newsholme et al. 2007). We explored this parameter by monitoring Rhod123 fluorescence in islets from all groups (Scaduto and Grotyohann 1999). Increases in the concentrations of glucose leads to a proportional fall in the intensity of Rhod123 fluorescence (Fig. 3g), which reflects the mitochondrial hyperpolarization, attributable to matrix proton extrusion (Scaduto and Grotyohann 1999). Tau treatment did not alter $\Delta\psi_m$, under basal and stimulatory glucose concentrations, in both C and ob islets (AUC for C $302,879 \pm 14,751$, CT $315,233 \pm 34,231$, ob $274,600 \pm 12,208$ and obT $322,829 \pm 18,916$ F480 min^{-1}).

We also measured NAD(P)H production to verify whether an alteration in glucose metabolism contributes to the increased islet glucose responsiveness in the ob group. At lower levels of glucose (0.5–5.6 mM), NAD(P)H values were higher in ob islets (Fig. 3h). Stimulatory glucose concentrations (11.1–22.2 mM), raised NAD(P)H autofluorescence in islets from all groups, but NAD(P)H was higher in ob, compared with C islets ($P < 0.02$; Fig. 3i). NAD(P)H values were similar for the ob and obT islets (Fig. 3h, i).

Glucagon secretion and Ca^{2+} handling in α -cells

It is known that dysfunction of the α -cells also contributes to the disruption of glucose homeostasis in T2D (Walker et al. 2011). Therefore, we investigated α -cell function in islets of all groups (Fig. 4). Ob islets secreted high amounts of glucagon at a low glucose concentration (0.5 mM) ($P < 0.005$). At 5.6 mM glucose, glucagon secretion tends to be lower in ob, compared with C and CT islets. At this glucose concentration, ob secreted significantly less glucagon than obT ($P < 0.02$; Fig. 4a); however,

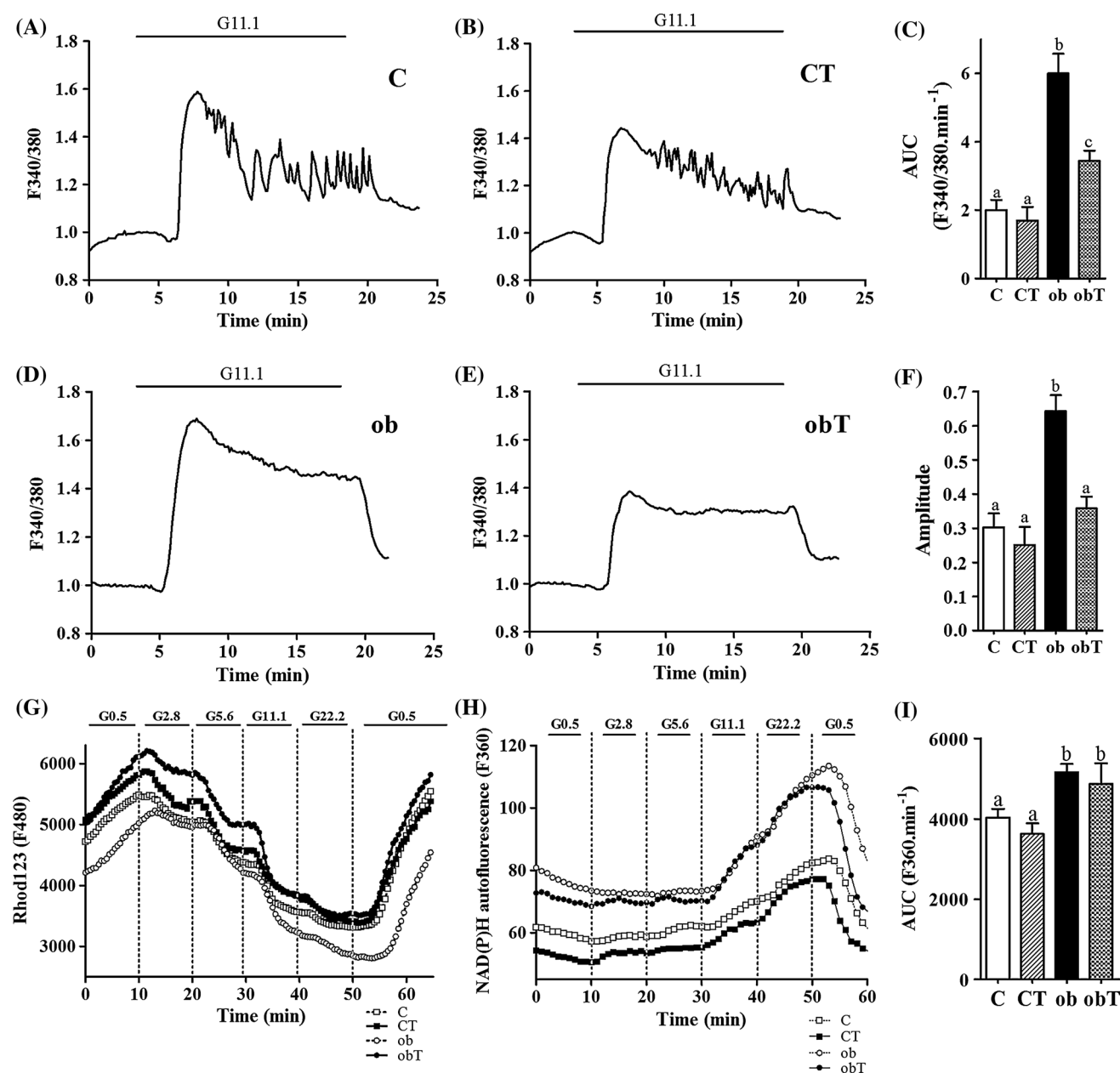


Fig. 3 Tau partially decreases glucose-induced Ca^{2+} influx, but does not alter NAD(P)H production and $\Delta\Psi_m$ in ob islets. Representative curves of 11.1 mM glucose-induced intracellular Ca^{2+} oscillations in islets from C (a), CT (b), ob (d) and obT (e). The AUC (c) and amplitude (f) of $[\text{Ca}^{2+}]_i$ in response to 11.1 mM glucose. The experiments were performed in a perfusion system in a medium that contained 2.8 or 11.1 mM glucose (G2.8 and G11.1, respectively). Changes

in $\Delta\Psi_m$ (g) and NAD(P)H fluorescence (h) in response to increasing glucose concentrations (as indicated by horizontal bars) in islets from C, CT, ob and obT mice. Total NAD(P)H production, expressed as AUC (i) in C, CT, ob and obT islets. Different letters over the bars indicate significant differences (Two-way ANOVA followed by Holm-Sidak post test, $P \leq 0.05$)

at 0.5 mM glucose, obT secreted significantly less glucagon than ob ($P < 0.001$), and similar glucagon concentrations to those observed for C and CT islets. No differences in the total glucagon content were observed in islets from all groups (Fig. 4b). Augmented glucagon release, at 0.5 mM glucose, was accompanied by higher Ca^{2+} oscillations in ob, compared with C islets ($P < 0.0001$; Fig. 5a, d,

respectively). Although there was no difference in glucagon secretion, an increase in Ca^{2+} oscillations was also observed in CT, compared with C, and in obT compared with C and ob islets ($P < 0.0001$; Fig. 5a, c). The numbers of Ca^{2+} oscillations in α -cells, at 5.6 mM glucose, were 23 % lower in obT (Fig. 5e), compared with ob islets ($P < 0.01$; Fig. 5d, f).

Pancreatic islet morphometry

Table 4 shows a morphometric analysis of the pancreases from mice of all groups. The pancreas weight did not differ between groups. Histological analysis revealed that pancreatic islets from ob were hypertrophic with increased β -cell and α -cell areas, compared with the C islets ($P < 0.0001$; Table 4). In addition, islet, β and α -cell masses were higher

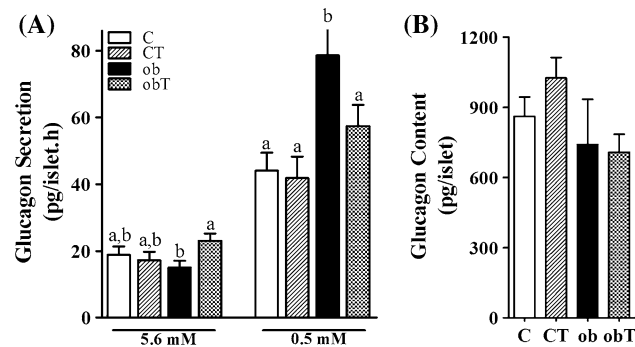


Fig. 4 Tau supplementation prevents α -cell hyperfunction in ob islets. Glucagon secretion (a) and total glucagon content (b) in islets from 90-day-old male C, CT, ob and obT mice. Groups of 15 islets of similar sizes were incubated for 1 h at 5.6 or 0.5 mM glucose (G), as indicated in the figure. Data are mean \pm SEM ($n = 16$ –25). Different letters over the bars represent significant differences (Two-way ANOVA followed by Holm-Sidak post test, $P \leq 0.05$)

in the ob pancreas ($P < 0.001$; Fig. 6). Tau-supplemented ob mice demonstrated reductions of 61 and 52 % in islet and β -cell areas, respectively, compared with ob ($P < 0.0001$); however, Tau supplementation did not alter the α -cell area, which remained similar to that of ob mice and higher than that of C islets ($P < 0.05$; Table 4). In terms of mass, Tau supplementation reduced obT islet and β -cell masses by 47 and 53 %, respectively, compared with those of ob mice ($P < 0.0001$; Fig. 6m, n). In obT islets, the α -cell mass was higher than those of C and CT, and similar to that of ob ($P < 0.0001$; Fig. 6o). Additionally, Tau treatment increased the δ -cell area in CT and δ -cell mass in the obT pancreas ($P < 0.04$; Table 4; Fig. 6p).

Gene expression

Insulin mRNA expression was enhanced in ob islets, compared with C islets ($P < 0.001$; Fig. 7a), whereas glucagon, SST, GLUT-2, transient receptor potential melastatin 5 (TRPM5), SST receptor (SSTR) 2 genes and MafB (V-maf musculoaponeurotic fibrosarcoma oncogene homolog B) were downregulated in ob, compared with C islets ($P < 0.05$; Fig. 7a–c). In contrast, ob islets presented upregulation of paired box (Pax)-6, pancreatic and duodenal homeobox factor (PDX)-1 and neurogenin (Ngn)-3 mRNAs ($P < 0.0001$; Fig. 7c). The expression of insulin, SST, Pax-6, and PDX-1 genes was not modified in obT,

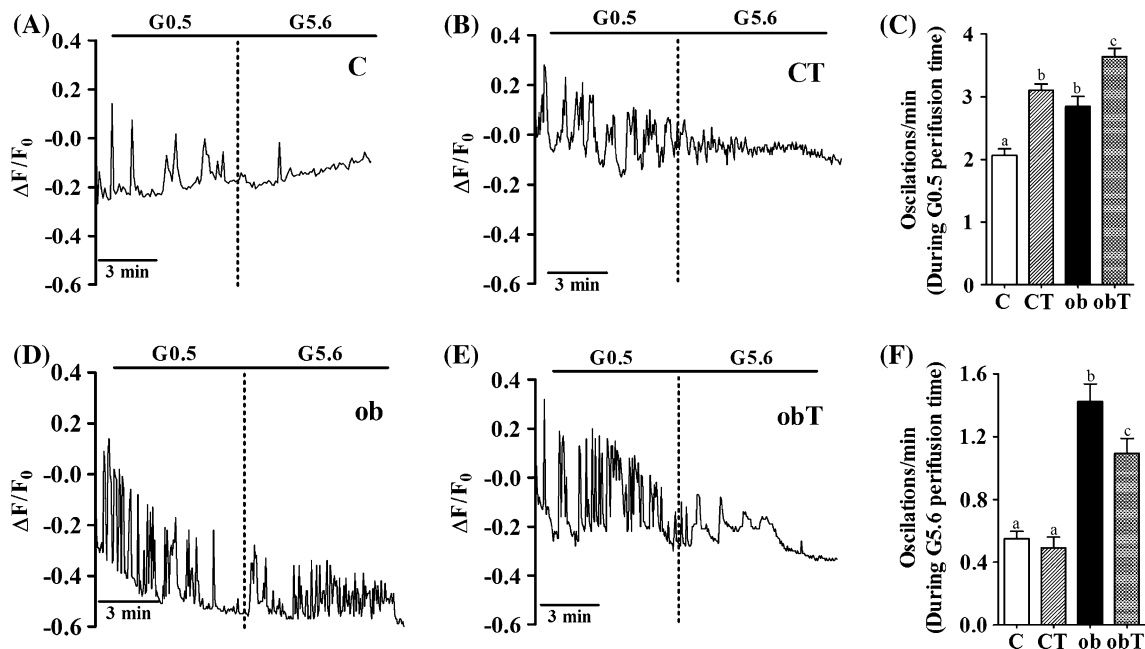


Fig. 5 Tau supplementation enhances cytoplasmic Ca^{2+} oscillations in α -cells from ob mice. Representative curves of Ca^{2+} oscillations in α -cells from C (a), CT (b), ob (d) and obT (e) islets. Individual Ca^{2+} signals were measured in 8- μm optical sections from intact islets using confocal microscopy and fluorescent dye Fluo-4. The fre-

quency of $[\text{Ca}^{2+}]_i$ in response to 0.5 (c) and 5.6 (f) mM glucose. Data are mean \pm SEM ($n = 23$ –61 α -cells from 5 different mice). Different letters over the bars represent significant differences (Two-way ANOVA followed by Holm-Sidak post test, $P \leq 0.05$)

Table 4 Morphometric analysis of pancreases from 90-day-old male C, CT, ob and obT mice

	C	CT	ob	obT
Pancreas weight (mg)	232 ± 7	212 ± 17	254 ± 30	227 ± 8
Islet area (μm ²)	4191 ± 302 ^a	5172 ± 293 ^a	28,919 ± 1549 ^b	17,955 ± 1155 ^c
β-Cell area (μm ²)	2733 ± 277 ^a	3425 ± 276 ^a	16,022 ± 1153 ^b	7599 ± 824 ^c
α-Cell area (μm ²)	1143 ± 106 ^a	1528 ± 136 ^a	3610 ± 457 ^b	2720 ± 310 ^b
δ-Cell area (μm ²)	886 ± 99 ^a	1220 ± 189 ^b	805 ± 74 ^a	781 ± 70 ^a
Number of islets per section	42 ± 5 ^a	60 ± 6 ^a	85 ± 9 ^b	116 ± 18 ^c
Number of analyzed islets	672	1073	1282	815

For parameter calculations, see “Materials and methods”. Different letters represent significant differences (Two-way ANOVA followed by Holm-Sidak post test, $P \leq 0.05$)

compared with islets from the ob group (Fig. 7a, c). But, glucagon, GLUT-2 and TRPM5 gene expressions were restored in obT islets (Fig. 7a, b). In addition, obT islets expressed higher MafA (V-maf musculoaponeurotic fibrosarcoma oncogene homolog A), Ngn3 and NeuroD (Neurogenic differentiation 1, also called β2) mRNAs ($P < 0.05$; Fig. 7c). Tau supplementation also enhanced the expressions of SST, Pax-6, Ngn3, NeuroD and MafB genes in the CT islets ($P < 0.05$; Fig. 7a, c).

Discussion

Obesity predisposes to several chronic diseases such as T2D (Kahn et al. 2006). In this disease insulin resistance is the main factor, but pancreatic islet dysfunction also contributes to the disruption of body glucose homeostasis (Unger and Orci 2010; Walker et al. 2011; Cerf 2013). Therefore, to prevent or treat T2D, strategies for the regulation of the endocrine pancreatic cell populations must be developed to maintain adequate β, α and δ-cell physiology and its paracrine interactions to preserve normal body glucose control.

Here, although Tau supplementation did not prevent obesity in ob mice, as previously reported in diet or glutamate-induced obesity, as well as in other type of genetic obesity (Tsuboyama-Kasaoka et al. 2006; Nardelli et al. 2011; Batista et al. 2013); better glucose tolerance, insulin action and lower glucagon-induced hepatic glucose output were observed in the obT group (Fig. 1). Previous reports showed that Tau improves glucose control in pre- and diabetic conditions (Tsuboyama-Kasaoka et al. 2006; Kim et al. 2012; Batista et al. 2013; Vettorazzi et al. 2014), and this effect may be due to the interaction of the amino acid with the insulin receptor (Maturo and Kulakowski 1988; Carneiro et al. 2009), enhancing Akt activation in different insulin target tissues (Baek et al. 2012; Das et al. 2012; Ribeiro et al. 2012; Batista et al. 2013).

We also show here that Tau can ameliorate glucose homeostasis by regulating islet-cell morphology and

function. Pancreatic islets from adult ob mice displayed an altered β-cell threshold for glucose-induced insulin release which contributes to the severe hyperinsulinemia in these rodents (Chen et al. 1993). These alterations are concomitant with abnormal cytoplasmic Ca^{2+} oscillations, probably due to the lower expression of the TRPM5 cation channel (Colsoul et al. 2014). The ob pancreas also presents morphological alterations with severe islet hypertrophy (Tomita et al. 1992). Several of these features were found in our study, and we also observed higher NAD(P)H production (Fig. 3h), despite a reduced expression of GLUT-2 mRNA, which is indicative of an early dysfunction of glucose utilization in β-cells (Fig. 7b). As such, decreased expression of the GLUT-2 gene may occur in T2D and is associated with reduced β-cell responsiveness to glucose (Johnson et al. 1990; Ohneda et al. 1993).

Tau supplementation prevented insulin hypersecretion and hyperinsulinemia in obT mice, decreasing islet Ca^{2+} influx and normalizing the expression of the GLUT-2 and TRPM5 channel genes. The TRPM5 is a monovalent cation-permeable channel expressed in pancreatic β-cells that is activated by $[\text{Ca}^{2+}]_i$ and causes membrane depolarization by Na^+ influx (Uchida and Tominaga 2011). The lack of TRPM5 in β-cells leads to glucose intolerance due to reduced insulin secretion in mice (Colsoul et al. 2010). Unfortunately, although we did not observe any restoration of Ca^{2+} oscillations in response to glucose in the obT islets (Fig. 3e), the normalization of GLUT-2 and TRPM5 transcripts may delay the onset of β-cell dysfunction in the obT group.

Tau supplementation may prevent alterations or restore endocrine pancreatic mass in malnutrition (Boujendar et al. 2002), obesity (Ribeiro et al. 2012), type 1 diabetes (Arany et al. 2004) and T2D (Lee et al. 2011). In these pre- and diabetic conditions, Tau seems to protect pancreatic islets against oxidative stress and cytokines (Boujendar et al. 2002; Arany et al. 2004; Lee et al. 2011). Our study also revealed that Tau prevents islet hypertrophy (Table 4), and the increase in islet and β-cell masses in the obT pancreas

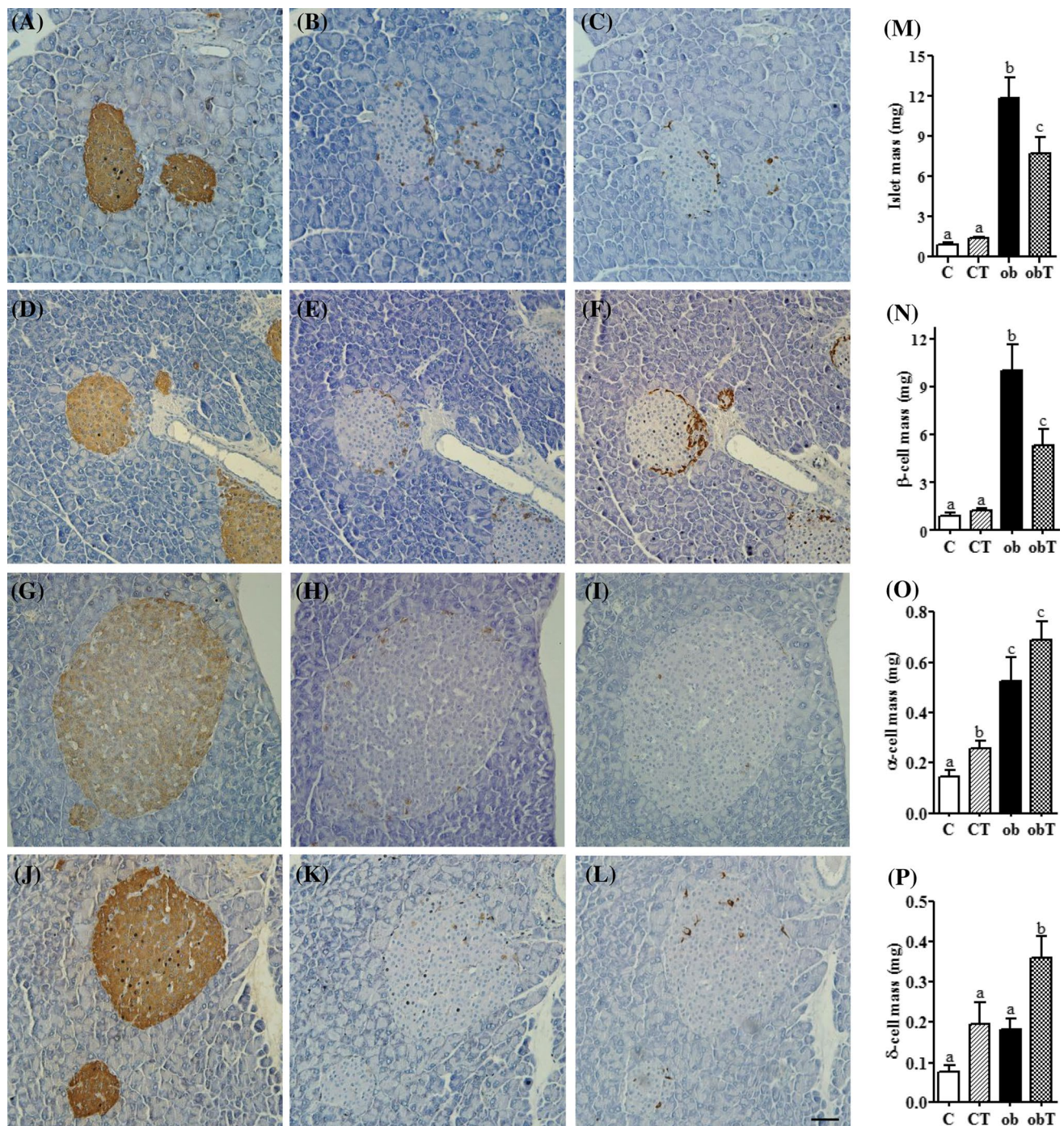


Fig. 6 Tau supplementation regulates endocrine pancreatic mass content in ob mice. *Panels* show paraffin-embedded pancreas sections (5 μ m thick) from the C (a, b, c), CT (d, e, f), ob (g, h, i) and obT (j, k, l) groups, which were immunolabelled for insulin (a, d, g, j), glucagon (b, e, h, k) or SST (c, f, i, l). Bar 50 μ m. Mean \pm SEM of islet (m), β -cell (n), α -cell (o) and δ -cell (p) masses of the endo-

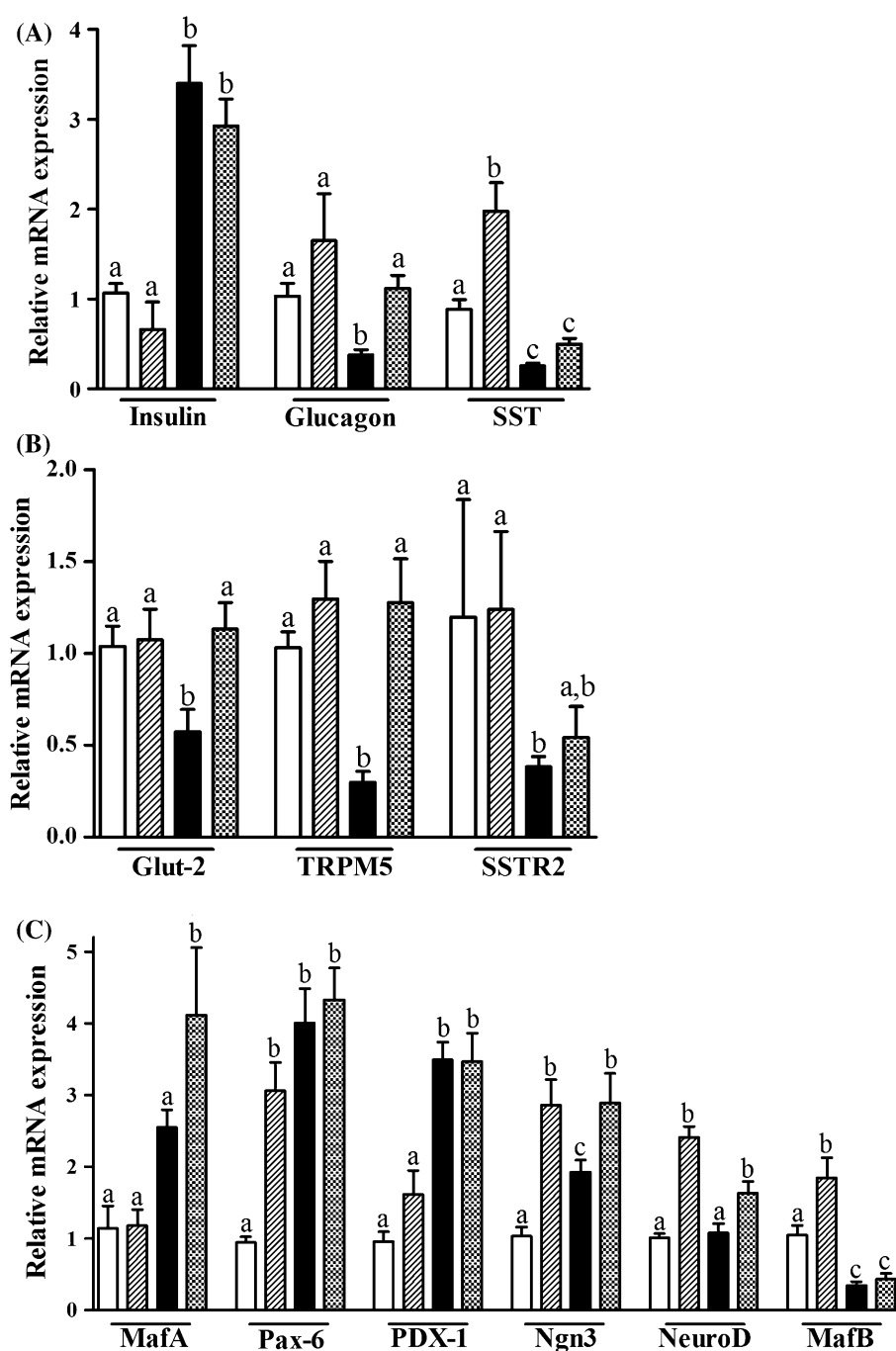
crine pancreas from C, CT, ob and obT mice. Each endocrine pancreatic mass was calculated by multiplying the pancreas weight by the total islet, β , α or δ -cell area per pancreas section. Data are obtained from at least 12 pancreas sections analyzed. Different letters over the bars represent significant differences (Two-way ANOVA followed by Holm-Sidak post test, $P \leq 0.05$)

(Fig. 6), but enhances δ -cell mass (Fig. 6p), and β -cell responsiveness to SST (Fig. 2b). Tau also increased δ -cell area and SST mRNA content in CT islets (Table 4; Fig. 7c). These findings support the idea that Tau enhances the SST

paracrine action in islets from ob and C supplemented mice.

In mice α and β -cells, SST is known to decrease hormone secretion mainly through SSTR2 and SSTR5,

Fig. 7 Tau regulates endocrine pancreatic genes in ob islets. Relative insulin, glucagon, SST, GLUT-2, TRPM5, SSTR2, MafA, Pax-6, PDX-1, Ngn3, NeuroD and MafB mRNA expressions in islets from 90-day-old C, CT, ob and obT mice. Data are mean \pm SEM ($n = 6$ –12). Different letters over the bars represent significant differences between the groups for the same gene evaluated (Two-way ANOVA followed by Holm-Sidak post test, $P \leq 0.05$)



respectively (Strowski et al. 2000). However, in human islets, SSTR2 predominates in α and β -cells (Kailey et al. 2012). In human β -cells, SST causes hyperpolarization through the activation of G protein-gated inwardly rectifying K^+ channels. In addition, the SSTR2 hormone inhibits Ca^{2+} influx through voltage-gated P/Q-type Ca^{2+} channels, and directly inhibits Ca^{2+} -dependent exocytosis in α and β -cells (Gromada et al. 2001a, b; Kailey et al. 2012). Insulin and glucagon hypersecretion, and impaired glucose-induced suppression of glucagon release have

been reported in SST knockout mice (Hauge-Evans et al. 2009). Decreased SST secretion may be a compensatory mechanism by which the body attempts to overcome insulin resistance in T2D. Accordingly, we observed that ob islets did not respond to SST (Fig. 2b) and presented lower expression of SST and SSTR2 genes (Fig. 7c, f), without any alteration in δ -cell pancreatic mass (Fig. 6). These results indicate that islet hypersecretion in ob islets is associated with a lower paracrine inhibitory action of SST upon β and α -cells.

In α -cells, low concentrations of glucose induce electrical activity, Ca^{2+} oscillations and glucagon secretion, but all these events are inhibited when glucose levels are raised (Quesada et al. 2008). However, in T2D, impaired α -cell responsiveness to glucose and glucagon hypersecretion may occur, aggravating the disruption of body glucose control (Unger and Orci 2010; Walker et al. 2011). Here, in response to low glucose, α -cells from ob mice hypersecreted glucagon, likely due to a higher frequency of cytoplasmic Ca^{2+} oscillations (Figs. 4a, 5d). Furthermore, an impaired α -cell electrical activity was evidenced in ob islets, since under basal glucose concentrations, α -cells also exhibited enhanced Ca^{2+} oscillations (Fig. 5d).

In pancreatic islet-cells, Tau co-localized with glucagon and SST positive cells (Bustamante et al. 2001), however, the effects of Tau on δ -cells of pancreatic islets have not been previously reported. It has been shown, though, that Tau induces SST release from median eminence extracts (Aguila and McCann 1985), indicating that increased Tau concentrations in the islet milieu may regulate δ -cell secretion and mass, preventing alterations in glucagon secretion in obT (Figs. 4a, 7b).

Several transcription factors are involved in pancreas development and islet-cell differentiation and function. The PDX-1 and MafA transcription factors regulate insulin gene expression (Kaneto et al. 2009). Exposure of β -cells to high glucose or fatty acid decreased DNA binding activities of these transcription factors (Sharma et al. 1995; Hagman et al. 2005; Kaneto et al. 2009). In addition, MafA knockout mice become diabetic due to a lower insulin secretion, in association with reductions in insulin, PDX-1, NeuroD1, and GLUT-2 transcripts (Zhang et al. 2005). In db/db mice, islet nuclear MafA expression was markedly decreased with age and was not detected during senescence (Matsuoka et al. 2010). Here, increased PDX-1 gene expression in ob islets may account for the enhanced insulin mRNA content and β -cell mass, as previously demonstrated in PDX-1 haploinsufficient mice (Johnson et al. 2003). However, it is possible that a non-compensatory increase in MafA mRNA in ob islets impairs GLUT-2 expression. As such, the increase in MafA transcripts induced by Tau supplementation contributes to maintaining normal GLUT-2 mRNA expression in obT islets and, together with an enhancement in NeuroD gene expression (Fig. 7c), maintains the normal β -cell sensitivity to glucose and insulin release (Gu et al. 2010).

Paired box 6 is a transcription factor necessary to control the precise expression of glucagon, insulin, SST and PP hormones (Sander et al. 1997). In the β -cell, Pax-6 directly regulates the expression of prohormone convertase (PC) 1/3, a serine protease involved in proinsulin processing (Wen et al. 2009), and also controls the transcription of the insulin, PDX-1, MafA and GLUT-2 genes (Gosmain

et al. 2012). Furthermore, Pax-6 is crucial for glucagon biosynthesis through the direct and indirect controls of the proglucagon and PC2 genes, respectively. Pax-6 controls the expression and activation of the promoter regions of the MafB, cMaf, and NeuroD1 genes (Gosmain et al. 2010). The inactivation of Pax-6 in pancreatic islets from 6-month-old mice reduced the number of insulin, glucagon, SST, PDX-1, GLUT-2 and PC 1/3 positive cells (Hart et al. 2013). In addition, MafB is a transcript factor found in adult α -cells and regulated glucagon gene expression (Arner et al. 2006). In this way, decreased glucagon mRNA content in ob islets (Fig. 7a) is associated with lower MafB mRNA (Fig. 7c). Our data suggest that increased α -cell mass and SST mRNA in CT islets is partly due to high Pax-6 expression in this group (Figs. 6o, 7a, c).

Additional islet-associated transcription factors were modulated by Tau, preventing this alteration in the obT group (Fig. 7). Ngn3 is a transcript factor that can suppress exocrine fate and promote endocrine fate during both embryogenesis and adulthood in differentiated acinar cells (Rukstalis and Habener 2009). Ngn3 binds directly to the promoters of the β -cell transcription factors NeuroD and Pax-4, promoting differentiation of progenitor cells in the β -cell lineage (Huang et al. 2000).

It is also supposed that the exocrine cells of the pancreas can undergo transdifferentiation into the endocrine lineage, since co-expression of Ngn3, PDX-1 and MafA in the mouse pancreas transforms exocrine cells into β -cells (Zhou et al. 2008). In addition, these transcript factors also regulate the endocrine fate of other islet-cells, since in adult mice, Ngn3 overexpression promotes δ -specification from acinar pancreatic cells, whereas the combination of Ngn3 and MafA genes induce α -cell lineage. Taken together, while Ngn3 regulation is complex and depending of several transcriptional and post-transcriptional regulators (Rukstalis and Habener 2009), our results indicate that Tau supplementation enhances δ -cell area in the CT and mass in obT, increasing Ngn3 mRNA expression (Fig. 7c).

Collectively, our results indicate that Tau supplementation ameliorates glucose homeostasis in ob mice displaying a central action in the regulation of endocrine pancreatic cells. The prevention of the β -cell hypersecretion in the obT group was associated with a better β -cell responsiveness to SST, attenuated islet Ca^{2+} handling and β -cell mass in the pancreas. Tau maintained normal gene expression of GLUT-2, in association with an increase in the transcription of MafA gene. Tau also prevented α -cell hypersecretion in obT islets, an effect that was associated with an improved α -cell oscillatory Ca^{2+} profile in response to low and high glucose concentrations. Enhanced δ -cell mass in the obT pancreas is partly due to the higher Ngn3 mRNA promoted by Tau. Additionally, SST and the δ -cell area were higher in islets

from CT mice, which could be due at least in part to higher Pax-6 and Ngn3 gene expressions. These data suggest that Tau may prevent glucagon and insulin compensatory hypersecretion in obesity and T2D, by maintenance of at least SST paracrine interactions in the endocrine pancreas.

Acknowledgments This study forms part of a PhD Thesis (JC Santos-Silva) and was supported by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 2009/54153-7), Conselho Nacional para o Desenvolvimento Científico e Tecnológico (CNPq 238836/2012-6) and Instituto Nacional de Obesidade e Diabetes (CNPq/FAPESP), Ministerio de Economía y competitividad BFU2011-28358 and Generalitat Valenciana PROMETEO/2011/080. The authors also thank Marise Carneossi, Salomé Ramon and Maria Luisa Navarro for excellent technical assistance and Nicola Conran for editing English.

Conflict of interest All contributing authors declare no conflicts of interest.

References

- Aguila MC, McCann SM (1985) Stimulation of somatostatin release from median eminence tissue incubated in vitro by taurine and related amino acids. *Endocrinology* 116(3):1158–1162. doi:[10.1210/endo-116-3-1158](https://doi.org/10.1210/endo-116-3-1158)
- Arany E, Strutt B, Romanus P, Remacle C, Reusens B, Hill DJ (2004) Taurine supplement in early life altered islet morphology, decreased insulinitis and delayed the onset of diabetes in non-obese diabetic mice. *Diabetologia* 47(10):1831–1837. doi:[10.1007/s00125-004-1535-z](https://doi.org/10.1007/s00125-004-1535-z)
- Artner I, Le Lay J, Hang Y, Elghazi L, Schisler JC, Henderson E, Sosa-Pineda B, Stein R (2006) MafB: an activator of the glucagon gene expressed in developing islet alpha- and beta-cells. *Diabetes* 55(2):297–304
- Baek YY, Cho DH, Choe J, Lee H, Jeoung D, Ha KS, Won MH, Kwon YG, Kim YM (2012) Extracellular taurine induces angiogenesis by activating ERK-, Akt-, and FAK-dependent signal pathways. *Eur J Pharmacol* 674(2–3):188–199. doi:[10.1016/j.ejphar.2011.11.022](https://doi.org/10.1016/j.ejphar.2011.11.022)
- Batista TM, Ribeiro RA, da Silva PM, Camargo RL, Lollo PC, Boschero AC, Carneiro EM (2013) Taurine supplementation improves liver glucose control in normal protein and malnourished mice fed a high-fat diet. *Mol Nutr Food Res* 57(3):423–434. doi:[10.1002/mnfr.201200345](https://doi.org/10.1002/mnfr.201200345)
- Bernardis LL, Patterson BD (1968) Correlation between ‘Lee index’ and carcass fat content in weanling and adult female rats with hypothalamic lesions. *J Endocrinol* 40(4):527–528
- Boujendar S, Reusens B, Merezak S, Ahn MT, Arany E, Hill D, Remacle C (2002) Taurine supplementation to a low protein diet during foetal and early postnatal life restores a normal proliferation and apoptosis of rat pancreatic islets. *Diabetologia* 45(6):856–866. doi:[10.1007/s00125-002-0833-6](https://doi.org/10.1007/s00125-002-0833-6)
- Bustamante J, Lobo MV, Alonso FJ, Mukala NT, Gine E, Solis JM, Tamarit-Rodríguez J, Martín Del Río R (2001) An osmotic-sensitive taurine pool is localized in rat pancreatic islet cells containing glucagon and somatostatin. *Am J Physiol Endocrinol Metab* 281(6):E1275–E1285
- Cabrera O, Berman DM, Kenyon NS, Ricordi C, Berggren PO, Caicedo A (2006) The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. *Proc Natl Acad Sci USA* 103(7):2334–2339. doi:[10.1073/pnas.0510790103](https://doi.org/10.1073/pnas.0510790103)
- Carneiro EM, Latorraca MQ, Araujo E, Beltra M, Oliveras MJ, Navarro M, Berna G, Bedoya FJ, Velloso LA, Soria B, Martín F (2009) Taurine supplementation modulates glucose homeostasis and islet function. *J Nutr Biochem* 20(7):503–511. doi:[10.1016/j.jnutbio.2008.05.008](https://doi.org/10.1016/j.jnutbio.2008.05.008)
- Cerf ME (2013) Beta cell dysfunction and insulin resistance. *Front Endocrinol* 4:37. doi:[10.3389/fendo.2013.00037](https://doi.org/10.3389/fendo.2013.00037)
- Chen NG, Tassava TM, Romsos DR (1993) Threshold for glucose-stimulated insulin secretion in pancreatic islets of genetically obese (ob/ob) mice is abnormally low. *J Nutr* 123(9):1567–1574
- Colsoyl B, Jacobs G, Philippaert K, Owsianik G, Segal A, Nilius B, Voets T, Schuit F, Vennekens R (2014) Insulin downregulates the expression of the Ca²⁺-activated nonselective cation channel TRPM5 in pancreatic islets from leptin-deficient mouse models. *Pflug Arch* 466(3):611–621. doi:[10.1007/s00424-013-1389-7](https://doi.org/10.1007/s00424-013-1389-7)
- Colsoyl B, Schraenen A, Lemaire K, Quintens R, Van Lommel L, Segal A, Owsianik G, Talavera K, Voets T, Margolskee RF, Kokrashvili Z, Gilon P, Nilius B, Schuit FC, Vennekens R (2010) Loss of high-frequency glucose-induced Ca²⁺ oscillations in pancreatic islets correlates with impaired glucose tolerance in Trpm5^{−/−} mice. *Proc Natl Acad Sci USA* 107(11):5208–5213. doi:[10.1073/pnas.0913107107](https://doi.org/10.1073/pnas.0913107107)
- Das J, Vasan V, Sil PC (2012) Taurine exerts hypoglycemic effect in alloxan-induced diabetic rats, improves insulin-mediated glucose transport signaling pathway in heart and ameliorates cardiac oxidative stress and apoptosis. *Toxicol Appl Pharmacol* 258(2):296–308. doi:[10.1016/j.taap.2011.11.009](https://doi.org/10.1016/j.taap.2011.11.009)
- Gosmain Y, Katz LS, Masson MH, Cheyssac C, Poisson C, Philippe J (2012) Pax6 is crucial for beta-cell function, insulin biosynthesis, and glucose-induced insulin secretion. *Mol Endocrinol* 26(4):696–709. doi:[10.1210/me.2011-1256](https://doi.org/10.1210/me.2011-1256)
- Gosmain Y, Marthinet E, Cheyssac C, Guerardel A, Mamin A, Katz LS, Bouzakri K, Philippe J (2010) Pax6 controls the expression of critical genes involved in pancreatic alpha cell differentiation and function. *J Biol Chem* 285(43):33381–33393. doi:[10.1074/jbc.M110.147215](https://doi.org/10.1074/jbc.M110.147215)
- Gromada J, Hoy M, Buschard K, Salehi A, Rorsman P (2001a) Somatostatin inhibits exocytosis in rat pancreatic alpha-cells by Gi(2)-dependent activation of calcineurin and depriming of secretory granules. *J Physiol* 535(Pt 2):519–532
- Gromada J, Hoy M, Olsen HL, Gotfredsen CF, Buschard K, Rorsman P, Bokvist K (2001b) Gi2 proteins couple somatostatin receptors to low-conductance K⁺ channels in rat pancreatic alpha-cells. *Pflug Arch* 442(1):19–26
- Gu C, Stein GH, Pan N, Goebbels S, Hornberg H, Nave KA, Herrera P, White P, Kaestner KH, Sussel L, Lee JE (2010) Pancreatic beta cells require NeuroD to achieve and maintain functional maturity. *Cell Metab* 11(4):298–310. doi:[10.1016/j.cmet.2010.03.006](https://doi.org/10.1016/j.cmet.2010.03.006)
- Hagman DK, Hays LB, Parazzoli SD, Poitout V (2005) Palmitate inhibits insulin gene expression by altering PDX-1 nuclear localization and reducing MafA expression in isolated rat islets of Langerhans. *J Biol Chem* 280(37):32413–32418. doi:[10.1074/jbc.M506000200](https://doi.org/10.1074/jbc.M506000200)
- Hart AW, Mella S, Mendrychowski J, van Heyningen V, Kleinjan DA (2013) The developmental regulator Pax6 is essential for maintenance of islet cell function in the adult mouse pancreas. *PLoS One* 8(1):e54173. doi:[10.1371/journal.pone.0054173](https://doi.org/10.1371/journal.pone.0054173)
- Hauge-Evans AC, Anderson RL, Persaud SJ, Jones PM (2012) Delta cell secretory responses to insulin secretagogues are not mediated indirectly by insulin. *Diabetologia* 55(7):1995–2004. doi:[10.1007/s00125-012-2546-9](https://doi.org/10.1007/s00125-012-2546-9)
- Hauge-Evans AC, King AJ, Carmignac D, Richardson CC, Robinson IC, Low MJ, Christie MR, Persaud SJ, Jones PM (2009) Somatostatin secreted by islet delta-cells fulfills multiple roles as a paracrine regulator of islet function. *Diabetes* 58(2):403–411. doi:[10.2337/db08-0792](https://doi.org/10.2337/db08-0792)

- Hellman B (2009) Pulsatility of insulin release—a clinically important phenomenon. *Upsala J Med Sci* 114(4):193–205. doi:[10.3109/03009730903366075](https://doi.org/10.3109/03009730903366075)
- Hellman B, Salehi A, Gylfe E, Dansk H, Grapengiesser E (2009) Glucose generates coincident insulin and somatostatin pulses and antisynchronous glucagon pulses from human pancreatic islets. *Endocrinology* 150(12):5334–5340. doi:[10.1210/en.2009-0600](https://doi.org/10.1210/en.2009-0600)
- Huang HP, Liu M, El-Hodiri HM, Chu K, Jamrich M, Tsai MJ (2000) Regulation of the pancreatic islet-specific gene BETA2 (neuroD) by neurogenin 3. *Mol Cell Biol* 20(9):3292–3307
- Johnson JD, Ahmed NT, Luciani DS, Han Z, Tran H, Fujita J, Mislener S, Edlund H, Polonsky KS (2003) Increased islet apoptosis in Pdx1[±] mice. *J Clin Invest* 111(8):1147–1160. doi:[10.1172/JCI16537](https://doi.org/10.1172/JCI16537)
- Johnson JH, Ogawa A, Chen L, Orci L, Newgard CB, Alam T, Unger RH (1990) Underexpression of beta cell high Km glucose transporters in noninsulin-dependent diabetes. *Science* 250(4980):546–549
- Kahn SE, Hull RL, Utzschneider KM (2006) Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* 444(7121):840–846. doi:[10.1038/nature05482](https://doi.org/10.1038/nature05482)
- Kailey B, van de Bunt M, Cheley S, Johnson PR, MacDonald PE, Gloyd AL, Rorsman P, Braun M (2012) SSTR2 is the functionally dominant somatostatin receptor in human pancreatic beta- and alpha-cells. *Am J Physiol Endocrinol Metab* 303(9):E1107–E1116. doi:[10.1152/ajpendo.00207.2012](https://doi.org/10.1152/ajpendo.00207.2012)
- Kaneto H, Matsuoka TA, Kawashima S, Yamamoto K, Kato K, Miyatsuka T, Katakami N, Matsuhisa M (2009) Role of MafA in pancreatic beta-cells. *Adv Drug Deliv Rev* 61(7–8):489–496. doi:[10.1016/j.addr.2008.12.015](https://doi.org/10.1016/j.addr.2008.12.015)
- Kim KS, da Oh H, Kim JY, Lee BG, You JS, Chang KJ, Chung HJ, Yoo MC, Yang HI, Kang JH, Hwang YC, Ahn KJ, Chung HY, Jeong IK (2012) Taurine ameliorates hyperglycemia and dyslipidemia by reducing insulin resistance and leptin level in Otsuka Long-Evans Tokushima fatty (OLETF) rats with long-term diabetes. *Exp Mol Med* 44(11):665–673. doi:[10.3858/emmm.2012.44.11.075](https://doi.org/10.3858/emmm.2012.44.11.075)
- Lee E, Ryu GR, Ko SH, Ahn YB, Yoon KH, Ha H, Song KH (2011) Antioxidant treatment may protect pancreatic beta cells through the attenuation of islet fibrosis in an animal model of type 2 diabetes. *Biochem Biophys Res Commun* 414(2):397–402. doi:[10.1016/j.bbrc.2011.09.087](https://doi.org/10.1016/j.bbrc.2011.09.087)
- Lindstrom P (2010) beta-cell function in obese-hyperglycemic mice [ob/ob Mice]. *Adv Exp Med Biol* 654:463–477. doi:[10.1007/978-90-481-3271-3_20](https://doi.org/10.1007/978-90-481-3271-3_20)
- Matsuoka TA, Kaneto H, Miyatsuka T, Yamamoto T, Yamamoto K, Kato K, Shimomura I, Stein R, Matsuhisa M (2010) Regulation of MafA expression in pancreatic beta-cells in db/db mice with diabetes. *Diabetes* 59(7):1709–1720. doi:[10.2337/db08-0693](https://doi.org/10.2337/db08-0693)
- Maturo J, Kulakowski EC (1988) Taurine binding to the purified insulin receptor. *Biochem Pharmacol* 37(19):3755–3760
- Nadal A, Quesada I, Soria B (1999) Homologous and heterologous asynchronicity between identified alpha-, beta- and delta-cells within intact islets of Langerhans in the mouse. *J Physiol* 517(Pt 1):85–93
- Nardelli TR, Ribeiro RA, Balbo SL, Vanzela EC, Carneiro EM, Boschero AC, Bonfleur ML (2011) Taurine prevents fat deposition and ameliorates plasma lipid profile in monosodium glutamate-obese rats. *Amino Acids* 41(4):901–908. doi:[10.1007/s00726-010-0789-7](https://doi.org/10.1007/s00726-010-0789-7)
- Newsholme P, Haber EP, Hirabara SM, Rebelato EL, Procopio J, Morgan D, Oliveira-Emilio HC, Carpinelli AR, Curi R (2007) Diabetes associated cell stress and dysfunction: role of mitochondrial and non-mitochondrial ROS production and activity. *J Physiol* 583(Pt 1):9–24. doi:[10.1113/jphysiol.2007.135871](https://doi.org/10.1113/jphysiol.2007.135871)
- Ohneda M, Johnson JH, Inman LR, Chen L, Suzuki K, Goto Y, Alam T, Ravazzola M, Orci L, Unger RH (1993) GLUT2 expression and function in beta-cells of GK rats with NIDDM. Dissociation between reductions in glucose transport and glucose-stimulated insulin secretion. *Diabetes* 42(7):1065–1072
- Pertusa JA, Nesher R, Kaiser N, Cerasi E, Henquin JC, Jonas JC (2002) Increased glucose sensitivity of stimulus-secretion coupling in islets from *Psammomys obesus* after diet induction of diabetes. *Diabetes* 51(8):2552–2560
- Quesada I, Todorova MG, Alonso-Magdalena P, Beltra M, Carneiro EM, Martin F, Nadal A, Soria B (2006) Glucose induces opposite intracellular Ca²⁺ concentration oscillatory patterns in identified alpha- and beta-cells within intact human islets of Langerhans. *Diabetes* 55(9):2463–2469. doi:[10.2337/db06-0272](https://doi.org/10.2337/db06-0272)
- Quesada I, Tuduri E, Ripoll C, Nadal A (2008) Physiology of the pancreatic alpha-cell and glucagon secretion: role in glucose homeostasis and diabetes. *J Endocrinol* 199(1):5–19. doi:[10.1677/JOE-08-0290](https://doi.org/10.1677/JOE-08-0290)
- Ribeiro RA, Bonfleur ML, Amaral AG, Vanzela EC, Rocco SA, Boschero AC, Carneiro EM (2009) Taurine supplementation enhances nutrient-induced insulin secretion in pancreatic mice islets. *Diabetes Metab Res Rev* 25(4):370–379. doi:[10.1002/dmrr.959](https://doi.org/10.1002/dmrr.959)
- Ribeiro RA, Santos-Silva JC, Vettorazzi JF, Cotrim BB, Molioli DD, Boschero AC, Carneiro EM (2012) Taurine supplementation prevents morpho-physiological alterations in high-fat diet mice pancreatic beta-cells. *Amino Acids* 43(4):1791–1801. doi:[10.1007/s00726-012-1263-5](https://doi.org/10.1007/s00726-012-1263-5)
- Ribeiro RA, Vanzela EC, Oliveira CA, Bonfleur ML, Boschero AC, Carneiro EM (2010) Taurine supplementation: involvement of cholinergic/phospholipase C and protein kinase A pathways in potentiation of insulin secretion and Ca²⁺ handling in mouse pancreatic islets. *Br J Nutr* 104(8):1148–1155. doi:[10.1017/S0007114510001820](https://doi.org/10.1017/S0007114510001820)
- Ripps H, Shen W (2012) Review: taurine: a “very essential” amino acid. *Mol Vis* 18:2673–2686
- Rukstalis JM, Habener JF (2009) Neurogenin3: a master regulator of pancreatic islet differentiation and regeneration. *Islets* 1(3):177–184. doi:[10.4161/isl.1.3.9877](https://doi.org/10.4161/isl.1.3.9877)
- Sander M, Neubuser A, Kalamaras J, Ee HC, Martin GR, German MS (1997) Genetic analysis reveals that PAX6 is required for normal transcription of pancreatic hormone genes and islet development. *Genes Dev* 11(13):1662–1673
- Scaduto RC Jr, Grotjohann LW (1999) Measurement of mitochondrial membrane potential using fluorescent rhodamine derivatives. *Biophys J* 76(1 Pt 1):469–477. doi:[10.1016/S0006-3495\(99\)77214-0](https://doi.org/10.1016/S0006-3495(99)77214-0)
- Sharma A, Olson LK, Robertson RP, Stein R (1995) The reduction of insulin gene transcription in HIT-T15 beta cells chronically exposed to high glucose concentration is associated with the loss of RIPE3b1 and STF-1 transcription factor expression. *Mol Endocrinol* 9(9):1127–1134. doi:[10.1210/mend.9.9.7491105](https://doi.org/10.1210/mend.9.9.7491105)
- Strowski MZ, Parmar RM, Blake AD, Schaeffer JM (2000) Somatostatin inhibits insulin and glucagon secretion via two receptors subtypes: an in vitro study of pancreatic islets from somatostatin receptor 2 knockout mice. *Endocrinology* 141(1):111–117. doi:[10.1210/endo.141.1.7263](https://doi.org/10.1210/endo.141.1.7263)
- Tomita T, Doull V, Pollock HG, Krizsan D (1992) Pancreatic islets of obese hyperglycemic mice (ob/ob). *Pancreas* 7(3):367–375
- Tsuboyama-Kasaoka N, Shozawa C, Sano K, Kamei Y, Kasaoka S, Hosokawa Y, Ezaki O (2006) Taurine (2-aminoethanesulfonic acid) deficiency creates a vicious circle promoting obesity. *Endocrinology* 147(7):3276–3284. doi:[10.1210/en.2005-1007](https://doi.org/10.1210/en.2005-1007)
- Uchida K, Tominaga M (2011) The role of thermosensitive TRP (transient receptor potential) channels in insulin secretion. *Endocr J* 58(12):1021–1028

- Unger RH, Orci L (2010) Paracrinology of islets and the paracrinopathy of diabetes. *Proc Natl Acad Sci USA* 107(37):16009–16012. doi:[10.1073/pnas.1006639107](https://doi.org/10.1073/pnas.1006639107)
- Vettorazzi JF, Ribeiro RA, Santos-Silva JC, Borck PC, Batista TM, Nardelli TR, Boschero AC, Carneiro EM (2014) Taurine supplementation increases K channel protein content, improving Ca handling and insulin secretion in islets from malnourished mice fed on a high-fat diet. *Amino Acids*. doi:[10.1007/s00726-014-1763-6](https://doi.org/10.1007/s00726-014-1763-6)
- Walker JN, Ramracheya R, Zhang Q, Johnson PR, Braun M, Rorsman P (2011) Regulation of glucagon secretion by glucose: paracrine, intrinsic or both? *Diabetes Obes Metab* 13(Suppl 1):95–105. doi:[10.1111/j.1463-1326.2011.01450.x](https://doi.org/10.1111/j.1463-1326.2011.01450.x)
- Wen JH, Chen YY, Song SJ, Ding J, Gao Y, Hu QK, Feng RP, Liu YZ, Ren GC, Zhang CY, Hong TP, Gao X, Li LS (2009) Paired box 6 (PAX6) regulates glucose metabolism via proinsulin processing mediated by prohormone convertase 1/3 (PC1/3). *Diabetologia* 52(3):504–513. doi:[10.1007/s00125-008-1210-x](https://doi.org/10.1007/s00125-008-1210-x)
- Zhang C, Moriguchi T, Kajihara M, Esaki R, Harada A, Shimohata H, Oishi H, Hamada M, Morito N, Hasegawa K, Kudo T, Engel JD, Yamamoto M, Takahashi S (2005) MafA is a key regulator of glucose-stimulated insulin secretion. *Mol Cell Biol* 25(12):4969–4976. doi:[10.1128/MCB.25.12.4969-4976.2005](https://doi.org/10.1128/MCB.25.12.4969-4976.2005)
- Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA (2008) In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. *Nature* 455(7213):627–632. doi:[10.1038/nature07314](https://doi.org/10.1038/nature07314)