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ORIGINAL ARTICLE

Taurine supplementation increases K_{ATP} channel protein content, improving Ca^{2+} handling and insulin secretion in islets from malnourished mice fed on a high-fat diet

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Abstract Pancreatic β-cells are highly sensitive to sub-optimal or excess nutrients, as occurs in protein-malnutrition and obesity. Taurine (Tau) improves insulin secretion in response to nutrients and depolarizing agents. Here, we assessed the expression and function of Cav and K_{ATP} channels in islets from malnourished mice fed on a high-fat diet (HFD) and supplemented with Tau. Weaned mice received a normal (C) or a low-protein diet (R) for 6 weeks. Half of each group were fed a HFD for 8 weeks without (CH, RH) or with 5 % Tau since weaning (CHT, RHT). Isolated islets from R mice showed lower insulin release with glucose and depolarizing stimuli. In CH islets, insulin secretion was increased and this was associated with enhanced K_{ATP} inhibition and Cav activity. RH islets secreted less insulin at high K^+ concentration and showed enhanced K_{ATP} activity. Tau supplementation normalized K^+-induced secretion and enhanced glucose-induced Ca^{2+} influx in RHT islets. R islets presented lower Ca^{2+} influx in response to tolbutamide, and higher protein content and activity of the Kir6.2 subunit of the K_{ATP}. Tau increased the protein content of the α1.2 subunit of the Cav channels and the SNARE proteins SNAP-25 and Synt-1 in CHT islets, whereas in RHT, Kir6.2 and Synt-1 proteins were increased. In conclusion, impaired islet function in R islets is related to higher content and activity of the K_{ATP} channels. Tau treatment enhanced RHT islet secretory capacity by improving the protein expression and inhibition of the K_{ATP} channels and enhancing Synt-1 islet content.

Keywords High-fat diet · Insulin secretion · K_{ATP} channels · Protein malnutrition · Taurine supplementation · Voltage-sensitive Ca^{2+} channels

Abbreviations

AUC	Area under curve
BSA	Bovine serum albumin
BW	Body weight
C	Control
Cav	Voltage-sensitive Ca^{2+} channel
$Cav_{\alpha 1.2}$	α1.2 subunit of the Cav
$Cav_{\beta 2}$	β2 subunit of the Cav
$[Ca^{2+}]_i$	Intracellular Ca^{2+} concentration
CH	Control mice submitted to HFD
CHOL	Cholesterol
CHT	CH supplemented with Tau
DZX	Diazoxide
FAs	Fatty acids
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GLUT-2	Glucose transporter 2
HFD	High-fat diet
ipGTT	Intraperitoneal glucose tolerance test
ipITT	Intraperitoneal insulin tolerance test
K_{ATP}	ATP-sensitive K^+
KBB	Krebs–bicarbonate buffer
Kir6.2	Subunit 6.2 of the inward-rectifier K^+ channel
Nif	Nifedipine
PK	Protein kinase

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R	Protein-restricted mice
RH	R submitted to HFD
RHT	RH mice supplemented with Tau
RIA	Radioimmunoassay
SNAP-25	Synaptosomal associated protein of 25 kDa
SNARE	Soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor
Synt-1	Syntaxin 1
SUR1	Sulfonylurea receptor 1
Tau	Taurine
Tolb	Tolbutamide
TG	Triglycerides

Introduction

In pancreatic β-cells, stimulus-secretion coupling is initiated by glucose entry through glucose transporters (GLUT)-2, which is followed by full metabolism of the sugar and ATP generation. An increase in ATP/ADP ratio leads to closure of ATP-sensitive potassium (K_{ATP}) channels, depolarizing the plasma membrane and opening the voltage-sensitive Ca^{2+} (Cav) channels. The Ca^{2+} influx activates the exocytotic machinery, which coordinates insulin granule migration and fusion. The former event is mediated by a family of soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins, which includes syntaxin (Synt)-1 and the synaptosomal associated protein of 25 kDa (SNAP-25) (Boschero and Malaisse 1979; Nagamatsu et al. 1999).

Persistent insulin resistance is a determinant factor for the disruption of glucose homeostasis. This condition is associated with enhanced β-cell activity and, if sustained, may lead to cell death and impaired glycemic control (Cnop et al. 2005). Beta-cell dysfunction may manifest in several different ways, causing alterations that include reductions in insulin secretion in response to fuels and non-nutrient secretagogues, which are associated with alterations in the expression and/or activity of proteins that form the K_{ATP} channels (Tokuyama et al. 1996; Moritz et al. 2001; Soriano et al. 2010), Cav channels (Iwashima et al. 2001; Yang and Berggren 2006; Hoppa et al. 2009; Rorsman et al. 2012) and SNARE proteins (Nagamatsu et al. 1999; Batista et al. 2012; Andersson et al. 2012).

Hales and Barker (1992) proposed the 'thrifty phenotype hypothesis', which postulates that fetal programming could represent an important player in the origins of adult morbidity, such as type 2 diabetes, metabolic syndrome and cardiovascular dysfunction (Hales and Barker 1992; Barker et al. 1993). In addition, we also demonstrated that, after weaning, protein malnutrition may program body metabolic functions, which increases the probability of premature type 2 diabetes onset in mice fed on a HFD (Batista et al. 2013b).

Pancreatic islets from rodents fed on a low-protein diet secrete less insulin in response to nutrients and neurotransmitters (Ferreira et al. 2003; Amaral et al. 2010; da Silva et al. 2012). This effect is associated with reduced expression of genes and proteins that are involved in β-cell glucose sensing, altered production and activity of intracellular messengers as well as reduced exocytosis of insulin granules (Delghingaro-Augusto et al. 2004; Ferreira et al. 2004; Amaral et al. 2010; Batista et al. 2012). This effect is compensated, at least in part, by an increased sensitivity to insulin in peripheral tissues (Reis et al. 1997). In contrast, rodents fed on a HFD develop obesity and insulin resistance (Winzell et al. 2007; Araujo et al. 2007), which increases β-cell mass and insulin secretion (Ribeiro et al. 2012).

Tau (2-aminoethanesulfonic acid) is a semi-essential sulfur-containing amino acid that regulates Ca^{2+} homeostasis and K_{ATP} channel activity in different cell types (Satoh and Sperelakis 1998; Han et al. 2004; Lee et al. 2004). Tau is found at higher concentrations in pancreatic islets (Bustamante et al. 2001; Ribeiro et al. 2010) and regulates insulin secretion in response to nutrients by increasing the mobilization of Ca^{2+} in β-cells (Ribeiro et al. 2009, 2010). In protein-malnourished rodents, Tau restores the secretory capacity of β-cells (Batista et al. 2012, 2013a) and prevents morpho-functional alterations in pancreatic islets from HFD mice (Ribeiro et al. 2012).

We herein attempt to determine the role of Tau in glucose control in malnourished mice fed on a HFD. We previously reported that Tau prevents obesity, hyperphagia and glucose intolerance in control but not in malnourished HFD mice (Batista et al. 2013b). These Tau beneficial actions were associated with improved insulin sensitivity in the liver (Batista et al. 2013b) and hypothalamus (Solon et al. 2012; Camargo et al. 2013). However, the involvement of Tau in pancreatic β-cell function in this nutritional background is still unknown. This study aimed to verify insulin secretion in response to glucose and non-fuel secretagogues, focusing on the role of Cav and K_{ATP} channels, and exocytotic proteins in isolated islets from malnourished mice fed on a HFD and supplemented with Tau. We provide evidence that Tau supplementation ameliorates β-cell function in malnourished HFD mice partly through increased protein content of the Kir6.2 subunit of the K_{ATP} channel and its inhibition in response to fuel and depolarizing stimuli.

Materials and methods

Materials

^{125}I human insulin was purchased from Genesis (São Paulo, SP, BRA) and routine reagents were from Sigma Chemical (St Louis, MO, USA).

Animals and groups

All procedures involving animals were approved by the ethics committee at UNICAMP (license number: 2234-1). Male C57Bl/6 mice were obtained from the breeding colony at UNICAMP and were maintained at 22 ± 1 °C, on a 12-h light–dark cycle, with free access to food and water. Weaned 30-day old mice were randomly distributed into the following groups: mice that received a normal protein diet (14 % protein) without (Control group: C) or with 5 % Tau in their drinking water; or mice that were fed on a protein-restricted diet (6 % protein) without (Restricted group: R) or with Tau supplementation. After 6 weeks, C and R groups were subdivided and were fed either a normal or HFD (35 % fat) for 8 weeks (CH and RH). All Tau-treated mice were kept on the supplementation protocol and were fed a HFD for 8 weeks (CHT and RHT). Tau supplementation did not alter water consumption and its intake was estimated in 5.8 g/kg for CHT and 6.3 g/kg for RHT mice. All experimental procedures listed below were developed at the end of the diet and Tau treatment (14 weeks). Diet compositions were previously described (Batista et al. 2013b).

General nutritional parameters

At the end of the experimental period (14 weeks), the final body weight and nasoanal length were measured in all groups for the calculation of the Lee Index [from the ratio of body weight $(g)^{1/3}$/Nasoanal length (cm) × 1,000], which is used as a predictor of obesity in rodents (Bernardis and Patterson 1968). In addition, fasted and fed mice were euthanized in a CO_2 chamber, which was followed by decapitation. Their blood was collected in heparinized tubes (5,000 IU diluted 1:1,000), centrifuged at 10,600*g* and the obtained plasma was stored at −20 °C until use (Batista et al. 2013b). Plasma glucose was measured using a glucose analyzer (Accu-Chek® Performa, Roche Diagnostic, San Francisco, USA), and insulin was measured by radioimmunoassay (RIA) as previously described (Ribeiro et al. 2010). Triglycerides (TG) and total cholesterol (CHOL) were measured according to the manufacturer's instructions (Roche Diagnostics, Germany).

Intraperitoneal glucose (ipGTT) and insulin tolerance test (ipITT)

For the ipGTT, blood glucose and insulin levels (time 0) were measured (as previously described) in mice submitted to an overnight fast. A glucose load of 2 g/kg body weight was then administered by ip injection, and additional blood samples were collected at 15, 30, 60, 90, 120 and 180 min to measure the levels of plasma glucose. For the ipITT, fed mice were injected with 1.5 U/kg body weight of human insulin (Biohulin ®R, Biobrás, M. Claros, MG, BRA). Blood samples were collected before insulin injection and at the times 10, 15, 30, 45 and 60 min for glucose analysis.

Islet isolation and static insulin secretion

Islets were isolated by collagenase digestion of the pancreas. For static incubations, groups of 4 islets from each mouse group were first incubated for 30 min at 37 °C in Krebs–bicarbonate buffer (KBB) with the following composition: 115 mmol/L NaCl, 5 mmol/L KCl, 2.56 mmol/L $CaCl_2$, 1 mmol/L $MgCl_2$, 10 mmol/L $NaHCO_3$, 15 mmol/L HEPES, supplemented with 5.6 mmol/L glucose, 0.3 % of BSA, and equilibrated with a mixture of 95 % O_2/5 % CO_2 to give pH 7.4. This medium was then replaced with fresh buffer, and the islets were incubated for 1 h with 2.8 and 11.1 mmol/L glucose. The medium also contained 30 mmol/L K^+, 20 mmol/L L-arginine (Arg), 0.5 mmol/L tolbutamide (Tolb), 0.25 mmol/L diazoxide (DZX) and 10 µmol/L nifedipine (Nif) when necessary. When 30 mmol/L K^+ was used, NaCl in KBB was withdrawn from the incubation medium and the same amount of KCl was added. At the end of the incubation period, the insulin content of the medium was measured by RIA.

Cytoplasmatic Ca^{2+} Oscillations

Fresh pancreatic islets were incubated with fura-2 acetoxymethyl ester (5 µmol/L) for 1 h at 37 °C in KBB buffer that contained 5.6 mmol/L glucose, 0.3 % BSA and pH 7.4. The islets were then washed with the same medium and were placed in a chamber that was thermostatically regulated at 37 °C on the stage of an inverted microscope (Nikon UK, Kingston, UK). Islets were then perifused with albumin-free KBB that was continuously gassed with 95 % O_2/5 % CO_2, pH 7.4, which contained 2.8 mmol/L glucose with or without 30 mmol/L K^+ or 0.5 mmol/L Tolb. In another series of experiments, islets were perifused with KBB that contained increasing glucose concentrations (2.8 and 11.1 mmol/L) with or without 250 µmol/L DZX, as indicated in the figures. A ratio image was acquired every 5 s with an ORCA-100 CCD camera (Hammamatsu Photonics Iberica, Barcelona, Spain), in conjunction with a

Lambda-10-CS dual filter wheel (Sutter Instrument Company, CA, USA), which was equipped with 340 and 380 nm, 10 nm bandpass filters, and a range of neutral density filters (Omega opticals, Stanmore, UK). Data were obtained using ImageMaster3 software (Photon Technology International, NJ, USA) (Carneiro et al. 2009).

Islet insulin and DNA content

Groups of 4 islets were collected and transferred to 1.5 mL tubes. Deionized water (1 mL) was added to the samples, which was followed by the sonication of the pancreatic cells (3 times, 10 s pulses); the islet insulin content was then measured by RIA. DNA was measured using fluorimetric probes and a standard curve of known DNA concentrations (Ribeiro et al. 2009).

Western blotting

Isolated islets from C, CH, CHT, R, RH and RHT mice were solubilized in homogenization buffer that contained the following: 100 mmol/L Tris pH 7.5, 10 mmol/L sodium pyrophosphate, 100 mmol/L sodium fluoride, 10 mmol/L EDTA, 10 mmol/L sodium vanadate, 2 mmol/L PMSF and 1 % Triton-X 100. The islets were disrupted using a Polytron PT 1200 C homogenizer (Brinkmann Instruments, NY, USA), employing three 10 s pulses. The extracts were then centrifuged at 12,600*g* at 4 °C for 5 min to remove insoluble materials. The protein concentration in the supernatants was assayed using the Bradford dye method (Bradford 1976) with BSA as a standard curve and the Bradford reagent (Bio-Agency Lab., São Paulo, SP, BRA). For SDS gel electrophoresis and Western blot analysis, the samples were treated with a Laemmli sample buffer containing dithiothreitol. After heating to 95 °C for 5 min, the proteins were separated by electrophoresis (50 µg protein/lane, 10 % gels). After electrophoresis, proteins were transferred to nitrocellulose membranes. The nitrocellulose filters were treated overnight with a blocking buffer (5 % non-fat dried milk, 10 mmol/L Tris, 150 mmol/L NaCl, and 0.02 % Tween 20) and were subsequently incubated with a polyclonal antibody against α1.2 (1:1,000, ACC-013; Alomone, Jerusalem, Israel) and β2 (1:1,000, ACC-105; Alomone, Jerusalem, Israel) subunits of the Cav, SUR1 (1:1,000, sc-25683) and Kir6.2 (1:500, AB-5495, Millipore, MA, USA) subunits of the K_{ATP} channel, SNAP-25 (1:1,000; S-5187, Sigma Chemical St Louis, MO, USA) and Synt-1 (1:1,000, sc-12736). All "sc" primary antibodies that were used were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). The visualization of specific protein bands was performed by incubating the membranes with appropriate secondary antibodies (1:10,000, Invitrogen, São Paulo, SP, BRA), followed by exposure to an ImageQuant LAS 4000 Mini (GE® Healthcare Bio-Sciences, Uppsala, Sweden), which detects the chemiluminescence in the nitrocellulose membranes. The band intensities were quantified with the ImageQuant TL 7.0 Software (GE® Healthcare Bio-Sciences, Uppsala, Sweden). After assaying the target proteins, Western blotting was repeated using a rabbit polyclonal antibody to the glyceraldehyde 3-phosphate dehydrogenase protein (GAPDH; 1:1,000; cat. no. sc-25778, Santa Cruz Biotechnologies, CA, USA) as an internal control.

Statistical analysis

The results are presented as mean ± SEM for the number of determinations (n) indicated. Statistical analyses were performed using an one-way analysis of variance (ANOVA) followed by Duncan's multiple range test ($P < 0.05$) or Student's *t* test with the Statistica 5.0 software (Statsoft, Tulsa, OK, USA).

Results

Mice features

Body weight (BW) and nasoanal length were lower in R compared with C mice ($P < 0.0002$ and $P < 0.002$; Table 1). The HFD intake increased BW and fat depots in the RH and CH groups ($P < 0.001$; Table 1), without altering the Lee index (Table 1). Tau supplementation prevented obesity development only in CHT mice, as judged by a similar BW and retroperitoneal fat pads to C group (Table 1). Mice fed on a low-protein diet developed malnutrition, as indicated by a reduction in the total plasma protein levels in R compared with C mice ($P < 0.003$; Table 2). No further alterations were observed in the plasma parameters of R mice (Table 2). The CH and RH groups displayed an increase in total fed CHOL plasma levels compared with their respective controls ($P < 0.0001$). Only CH mice displayed hyperinsulinemia in fed and fasted conditions ($P < 0.007$ and $P = 0.05$; Table 2). Tau supplementation lowered plasma CHOL in fasted CHT mice compared with CH ($P < 0.04$). Tau also normalized insulinemia in the fasting state and partially decreased insulinemia in the fed state in CHT mice (Table 2).

Glucose homeostasis

At the end of the experimental period, all mice groups were submitted to an ipGTT and ipITT. In the ipGTT, after the glucose load, plasma glucose concentrations reached

Table 1 Final body weight (BW) and obesity parameters in C, CH, CHT, R, RH, RHT mice

	C	CH	CHT	R	RH	RHT
BW (g)	27 ± 1a	36 ± 1b	29 ± 1a	19 ± 1c	32 ± 1d	30 ± 1d
Nasoanal length (cm)	9.2 ± 0.1a	9.2 ± 0.1a	9.6 ± 0.2a	8.0 ± 0.3b	9.1 ± 0.2a	8.8 ± 0.3a
Lee index	326 ± 3	357 ± 7	317 ± 2	328 ± 11	345 ± 11	345 ± 10
Retroperitoneal fat pad (mg)	125 ± 13a	445 ± 40b	242 ± 54a	65 ± 15a	356 ± 71b	269 ± 50b
Perigonadal fat pad (mg)	376 ± 37a	1,559 ± 76b	1,209 ± 88c	304 ± 35a	1,062 ± 137c,d	856 ± 138d

The data are the mean ± SEM of 7–15 mice of each group. *Different letters* indicate significant differences, $P < 0.05$

Table 2 Blood glucose, plasma insulin, cholesterol (CHOL), triglycerides (TG) albumin, and total proteins levels in fasted and fed C, CH, CHT, R, RH and RHT mice

	C	CH	CHT	R	RH	RHT
Glycemia (mg/dL)						
Fasted	84 ± 6	87 ± 5	87 ± 4	76 ± 6	82 ± 5	92 ± 4
Fed	138 ± 3	149 ± 4	149 ± 3	123 ± 3	155 ± 6	147 ± 2
Insulin (ng/mL)						
Fasted	0.35 ± 0.05a	1.11 ± 0.28b	0.37 ± 0.11a, b	0.13 ± 0.04a	0.60 ± 0.21a, b	0.26 ± 0.07a
Fed	0.82 ± 0.13a	1.87 ± 0.57b	1.68 ± 0.23a, b	0.34 ± 0.05a	1.49 ± 0.11a	1.77 ± 0.28a
CHOL (mg/dL)						
Fasted	138 ± 9a	223 ± 15b	178 ± 16c	145 ± 7a	192 ± 9b,c	188 ± 9b,c
Fed	134 ± 13a	212 ± 18b	262 ± 13b	148 ± 5a	238 ± 17b	258 ± 27b
TG (md/dL)						
Fasted	84 ± 8	107 ± 8	113 ± 8	90 ± 10	106 ± 9	117 ± 9
Fed	201 ± 21a, b	230 ± 29b	270 ± 22b	131 ± 5a	244 ± 41b	262 ± 23b
Albumin (mg/dL)						
Fasted	3.7 ± 0.1	3.6 ± 0.1	3.5 ± 0.1	3.8 ± 0.1	3.6 ± 0.1	3.4 ± 0.1
Total proteins (mg/dL)						
Fasted	5.8 ± 0.3a	5.4 ± 0.1a	5.8 ± 0.2a	4.7 ± 0.3b	5.9 ± 0.2a	5.0 ± 0.3a

The data are the mean ± SEM of 7–18 mice of each group. Different letters indicate significant differences, $P < 0.05$

maximal levels at 15 min in C, CHT and R groups and at 30 min in CH, RH and RHT mice (Fig. 1a, b). HFD treatment modified glucose homeostasis in RH and CH mice, since CH mice presented higher glycemia at 30, 60 and 90 min of the test when compared with C ($P < 0.05$, $P < 0.01$ and $P < 0.05$, respectively; Fig. 1a), and in the RH group higher plasma glucose levels were observed at all times of the ipGTT than in the R mice ($P < 0.05$; Fig. 1b). RHT mice also presented higher glucose levels at 60 and 90 min ($P < 0.05$; Fig. 1b), and the CHT group presented increased glycemia at 90 min of the ipGTT ($P < 0.05$; Fig. 1a). The total glycemia during ipGTT, expressed by the area under the curve (AUC), was lower in R compared with C mice ($P < 0.04$; Fig. 1c). RH and CH groups displayed glucose intolerance, as judged by the higher AUC compared with the C group ($P < 0.003$ and $P < 0.0001$; Fig. 1c). Tau supplementation normalized the glucose tolerance only in CHT mice (Fig. 1a, c). During the ipITT, a decrease in glycemia was observed after 10 min of the insulin administration in all groups (Fig. 1d, e). HFD treatment lowered the insulin action in RH and CH mice, since in RH, glycemia was higher than in R mice at all times of the test ($P < 0.05$; Fig. 1d), and in the CH group a higher plasma glucose was observed at 10 and 30 min of the ipITT in comparison with C ($P < 0.0005$ and $P < 0.05$; Fig. 1c). Tau supplementation did not prevent the reduction in insulin action in RHT, since higher glycemia was observed at all times in this group ($P < 0.05$; Fig. 1d). The R group presented a lower AUC when compared with C mice ($P < 0.02$), indicating a rapid decrease in plasma glucose levels after insulin administration and, therefore, increased insulin sensitivity (Fig. 1e, f). However, CH and RH displayed a higher AUC compared with the C group, suggesting a lower insulin action ($P < 0.005$ and $P < 0.0007$; Fig. 1f). Tau treatment did not alter the insulin sensitivity in CHT and RHT groups (Fig. 1d, f).

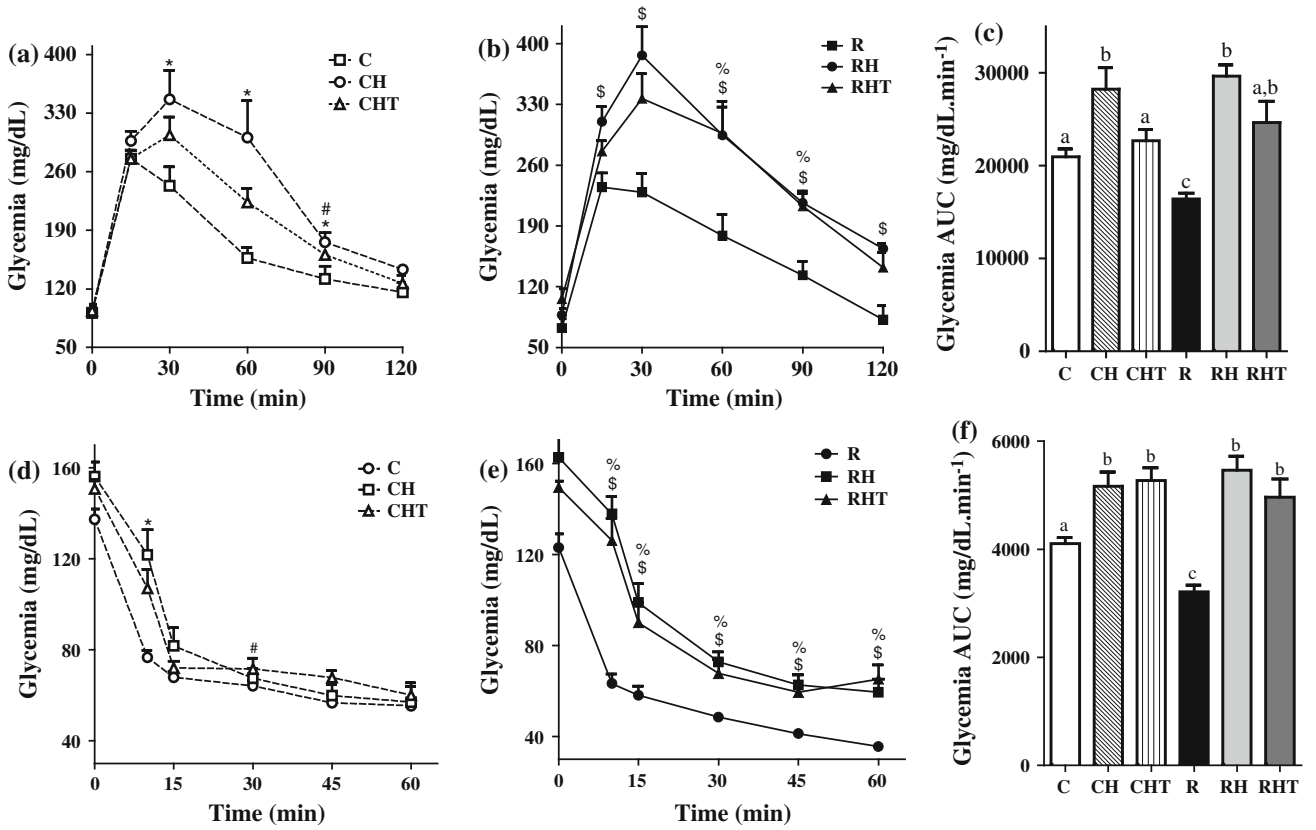

Fig. 1 Changes in plasma glucose concentrations during the ipGTT and ipITT in C, CH and CHT (**a**, **d**), and in R, RH and RHT mice (**b**, **e**). Total plasma glucose concentration, expressed as the area under the curve (AUC), during the ipGTT (**c**; integration area after glucose injection = 0–180 min) and ipITT (**f**; integration area after insulin administration = 0–60 min). Data are mean ± SEM obtained from 5 to 8 mice. *CH is different from C; #CHT is different from C; $RH different from R; and %RHT is different from R group. *Different letters* over the *bars* represent significant differences, $P < 0.05$

Glucose-induced insulin secretion

Figure 2 shows the insulin secretion induced by sub- and stimulatory glucose concentrations. At basal glucose levels, insulin secretion was similar between groups. Islets from R mice secreted less insulin in the presence of 11.1 mmol/L glucose compared with the C group ($P < 0.006$). CH islets displayed a higher secretion of insulin compared with C ($P < 0.03$); however, insulin secretion by RH was similar to that of C islets (Fig. 2). Insulin secretion of islets isolated from CHT and RHT mice was similar to secretion from the C group at 11.1 mmol/L glucose. The insulin secreted by RHT was also similar to that displayed by CH (Fig. 2).

Insulin secretion in response to K^+, L-arginine and tolbutamide

Figure 3 shows the insulin secretion induced by depolarizing agents at basal glucose concentrations. Isolated islets from R mice released less insulin in response to 2.8 mmol/L glucose plus 30 mmol/L K^+ (Fig. 3a), 20 mmol/L Arg (Fig. 3b) and 0.5 mmol/L Tolb (Fig. 3c), when compared with C islets ($P < 0.0001$, $P < 0.0001$ and $P < 0.05$). RH islets displayed a lower insulin secretion in the presence of 2.8 mmol/L glucose plus K^+ compared with C group ($P < 0.01$; Fig. 3a). However, CH islets did not show any alteration in the release of insulin with depolarization stimuli in relation to C (Fig. 3). Tau supplementation restored the insulin secretion in RHT islets to the same levels of the C at 2.8 mmol/L glucose plus 30 mmol/L K^+ (Fig. 3a) and enhanced the hormone release in the presence of 0.5 mmol/L Tolb ($P < 0.01$; Fig. 3c). In contrast, CHT islets presented a similar insulin secretion to C with depolarizing stimuli (Fig. 3).

In the presence of 250 µmol/L DZX, a K_{ATP} channel opener (Gribble and Reimann 2002), R and RH islets did not show a significant inhibition of insulin release, whereas in the C, CH, CHT and RHT groups a significant inhibitory effect was promoted by DZX (Fig. 4a). The Δ of DZX inhibition (G11.1–DZX) was lower in R compared with C islets (0.13 ± 0.01 vs 0.42 ± 0.02 ng/mL, respectively;

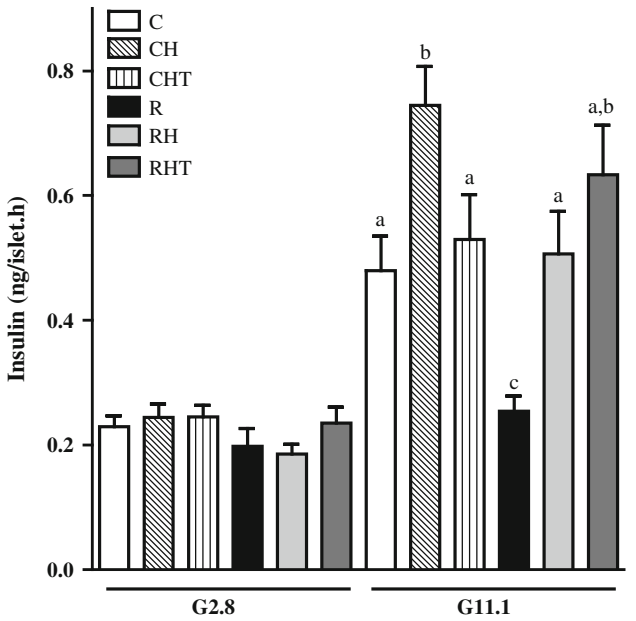

Fig. 2 Glucose-induced insulin secretion in islets from C, CH, CHT, R, RH and RHT mice. Groups of 4 islets were incubated for 1 h with 2.8 or 11.1 mmol/L glucose (G2.8 and G11.1, respectively). The data are mean ± SEM of 12–23 groups of islets. *Different letters* over the *bars* represent significant differences between the groups in the same glucose concentration evaluated, $P < 0.05$

$P < 0.0001$). RH, CH and RHT islets presented a higher Δ of DZX inhibition, when compared with C (RH 0.47 ± 0.04, CH 0.63 ± 0.04 and RHT 0.53 ± 0.027 ng/mL, $P < 0.03$). However, the inhibitory effects of DZX in CHT islets were similar to C (0.42 ± 0.020 ng/mL).

Finally, we assessed the glucose-induced insulin secretion in the presence of the dihydropyridine nifedipine (Nif), a L-type Ca^{2+} channel blocker (Yang and Berggren 2006). At 10 μmol/L Nif, R islets did not show an efficient inhibition upon insulin secretion (Fig. 4b). However, a discrete reduction in insulin release in R islets was observed when the Δ (G11.1–Nif) was evaluated (0.15 ± 0.01 ng/mL) in comparison with C (0.43 ± 0.03 ng/mL; $P < 0.0001$). In CH, RH and RHT islets a higher inhibition was observed upon the release of hormone (Δ = CH 0.57 ± 0.03, RH 0.51 ± 0.05 and RHT 0.54 ± 0.03 ng/mL, $P < 0.002$, $P < 0.05$ and $P < 0.01$). Finally, CHT islets displayed a lower inhibition of insulin release (0.35 ± 0.02 ng/mL) compared with C ($P < 0.04$).

Islet Ca^{2+} handling

The total intracellular Ca^{2+} influx in response to 30 mmol/L K^+ was similar between all groups of islets (Fig. 5d). In response to 0.5 mmol/L Tolb, the Ca^{2+} influx by R was lower than C islets ($P < 0.04$; Fig. 6d). In this condition Ca^{2+} influx did not differ in CH or RH mice supplemented

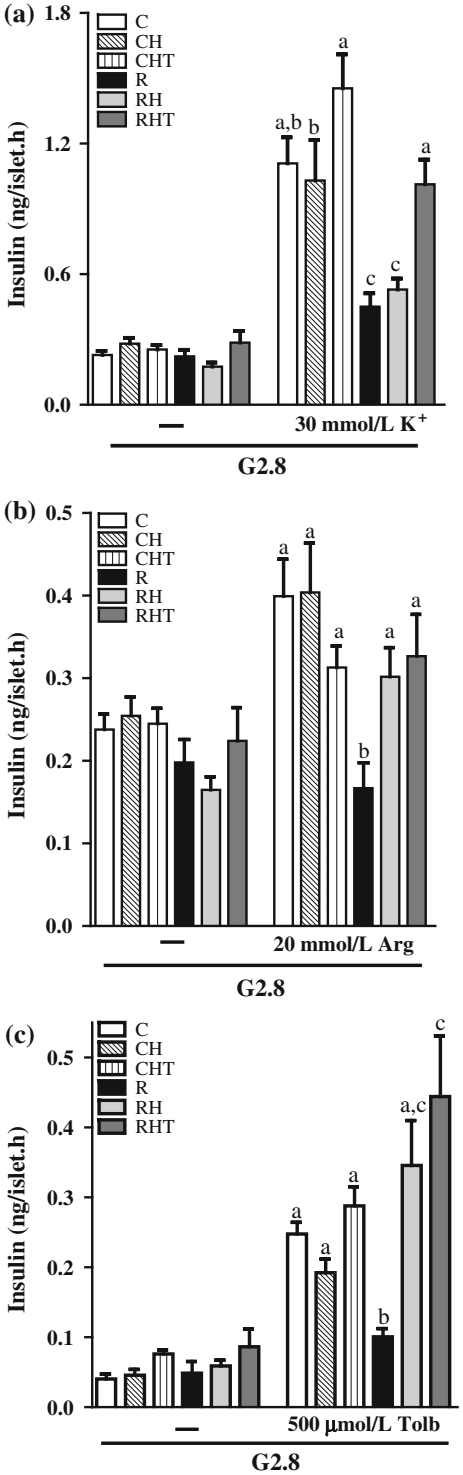

Fig. 3 Insulin secretion induced by 30 mmol/L K^+ (**a**), 10 mmol/L Arg (**b**) or 0.5 mmol/L Tolb (**c**) in isolated islets from C, CH, CHT, R, RH and RHT mice. Groups of 4 islets of similar sizes were incubated for 1 h at 2.8 mmol/L glucose (G2.8) with or without depolarizing agents (as indicated by the *horizontal lines*). The data are mean ± SEM of 13–24 groups of islets. *Different letters* over the *bars* represent significant differences between the groups in the same stimuli, $P < 0.05$

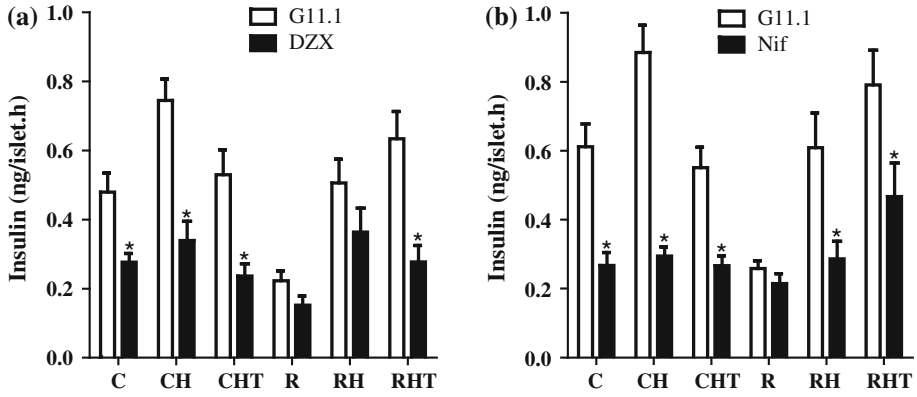

Fig. 4 Insulin secretion in response to 11.1 mmol/L glucose (G11.1) with or without 250 µmol/L diazoxide (DZX) (**a**) or 10 µmol/L nifedipine (Nif) (**b**) in isolated islets from C, CH, CHT, R, RH and RHT mice. The data are mean ± SEM (n = 9-15 groups of islets). *$P < 0.05$ vs the same group in the G11.1 condition (Student's *t* test)

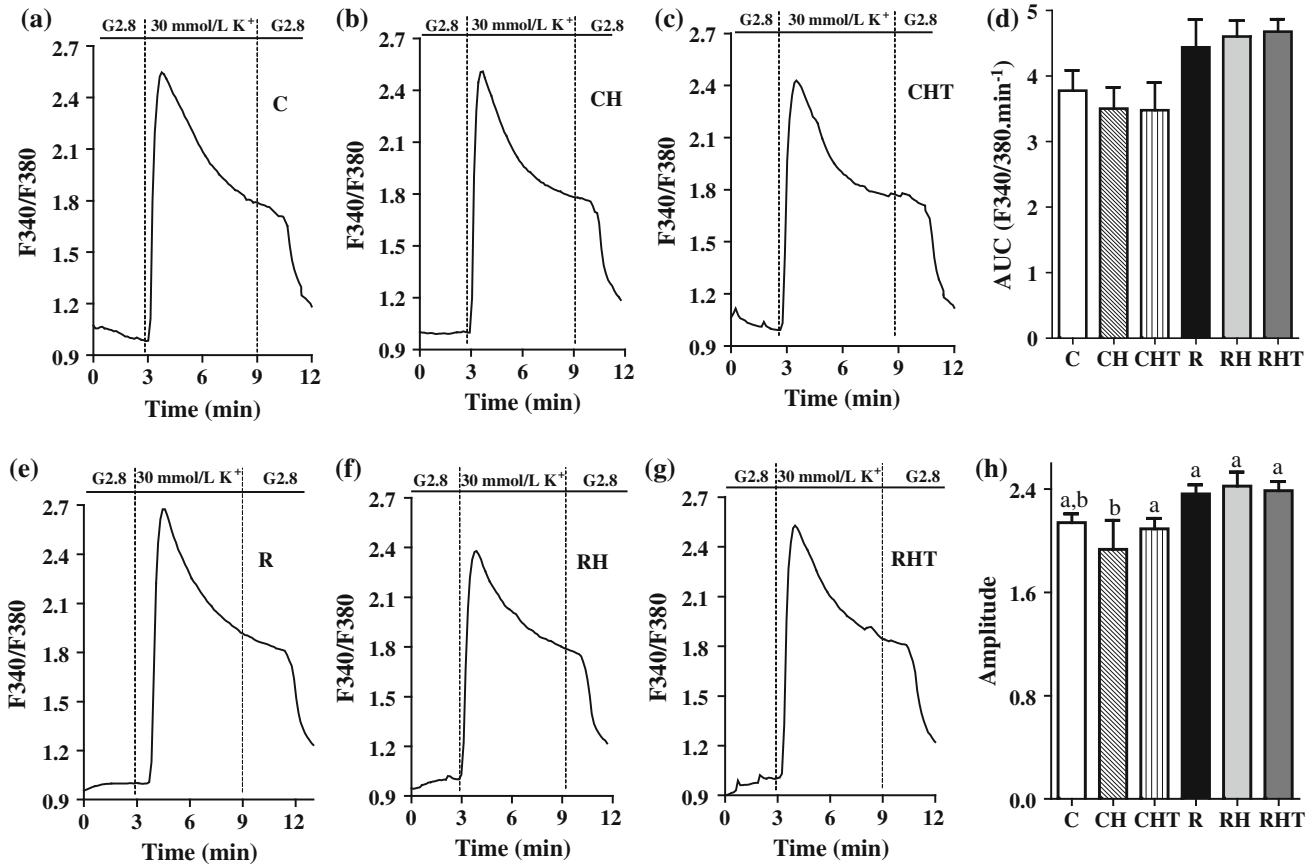

Fig. 5 Representative curves of the 30 mmol/L K^+-induced Ca^{2+} influx in isolated islets from C (**a**), CH (**b**), CHT (**c**), R (**e**), RH (**f**) and RHT (**g**) mice. **d** The AUC (during K^+ stimulus = 3–9 min) and **h** the amplitude of the $[Ca^{2+}]_i$ in response to 30 mmol/L K^+. The experiments were performed in a perifusion system in the presence of 2.8 mmol/L glucose (G2.8) with or without 30 mmol/L K^+ (horizontal lines). The values are the ratio of F340/F380 registered for each group. The data are mean ± SEM obtained from 4 to 6 independent experiments. *Different letters* over the *bars* indicate significant differences ($P < 0.05$)

or not with Tau (Fig. 6). The switch from 2.8 to 11.1 mmol/L produced a typical pattern of glucose-induced cytoplasmatic Ca^{2+} influx, which was characterized by an initial decrease followed by an abrupt and sustained increase, and subsequent oscillations in islets of all groups (Fig. 7a–f). These characteristics varied among groups and are presented in Fig. 7g–i. The glucose-induced Ca^{2+} influx did not differ between C and R mice with or without the HFD treatment (Fig. 7g). Tau supplementation enhanced the total intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in RHT islets compared with C ($P < 0.04$; Fig. 7g). However, the frequency of the cytoplasmatic

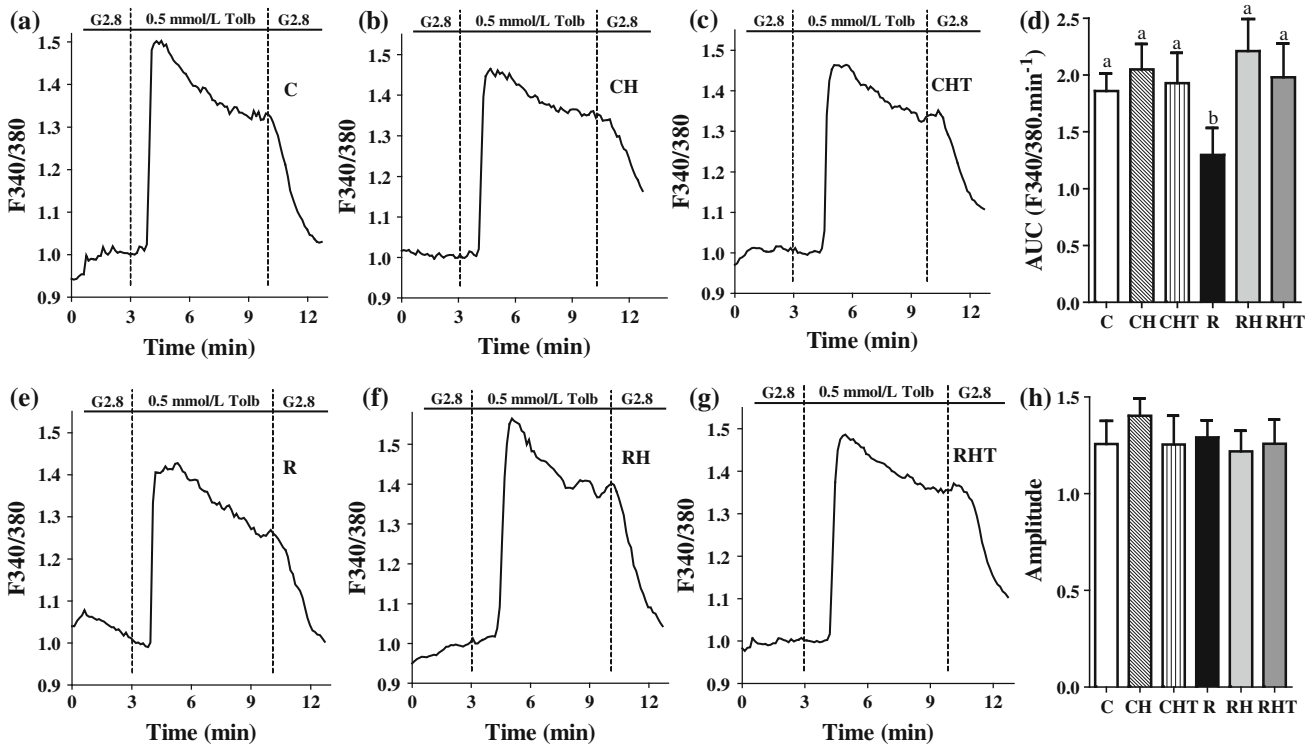

Fig. 6 Ca^{2+} influx induced by 0.5 mmol/L tolbutamide (Tolb) in islets from C (**a**), CH (**b**), CHT (**c**), R (**e**), RH (**f**) and RHT (**g**) mice. AUC (during Tolb stimulus = 3-10 min) (**d**) and amplitude (**h**) of the Ca^{2+} influx in response to 500 μmol/L Tolb. The values are the ratio of F340/F380 that was registered for each group. The data are mean ± SEM from 7-8 independent experiments. Different letters indicate significant differences ($P < 0.05$)

Ca^{2+} oscillations was higher in R and CHT than in C islets ($P < 0.01$ and $P < 0.05$, respectively; Fig. 7i). The amplitude of Ca^{2+} influx did not differ between groups (Fig. 7h). When 250 μmol/L DZX was added to the perfusion medium, a similar decrement in cytoplasmatic Ca^{2+} was observed between R and C islets (Fig. 7a, d, j). A lower decrement in $[Ca^{2+}]$i was observed in the CH group ($P < 0.005$; Fig. 7j), whereas DZX induced a marked reduction of $[Ca^{2+}]_i$ in RHT, compared with C islets ($P < 0.02$; Fig. 7j).

Islet insulin and DNA content

Islets from R mice displayed a 49 % decrease in the content of insulin when compared with C islets (25 ± 5 vs 51 ± 5 ng/islet, respectively; $P < 0.05$). Insulin storage did not differ between CH (78 ± 10 ng/islet), RH (70 ± 10 ng/islet), CHT (61 ± 5 ng/islet) and RHT (70 ± 11 ng/islet) islets in comparison with C. The total DNA islet content was not modified by protein malnutrition (R 19 ± 2 vs C 21 ± 2 ng/islet), HFD feeding (RH 15 ± 3 vs CH 26 ± 4 ng/islet) or Tau treatment (RHT 18 ± 3 vs CHT 19 ± 3 ng/islet).

Expression of islet proteins involved in insulin secretion

R islets displayed increased protein levels of the Kir6.2 subunit of the K_{ATP} channel compared with C ($P < 0.03$, Fig. 8a), whereas no alteration in the protein content of SUR1 was observed in R islets (Fig. 8b). The HFD intake increased the levels of the SUR1 protein only in RH islets ($P < 0.003$; Fig. 8b). Tau supplementation had different effects on the protein expression in islets of the RHT and CHT groups. In CHT islets, Tau increased the protein content of the Cav channel α1.2 subunit (Fig. 8c), Synt-1 (Fig. 8e) and SNAP-25 (Fig. 8f; $P < 0.003$, $P < 0.01$ and $P < 0.009$, respectively). In the RHT group, Tau treatment increased the protein levels of Kir6.2 (Fig. 8a) and Synt-1 (Fig. 8e), compared with the C group ($P < 0.007$ and $P < 0.008$).

Discussion

The present study confirms previous observations from our research group regarding the development of obesity and glucose intolerance in malnourished mice fed on a HFD.

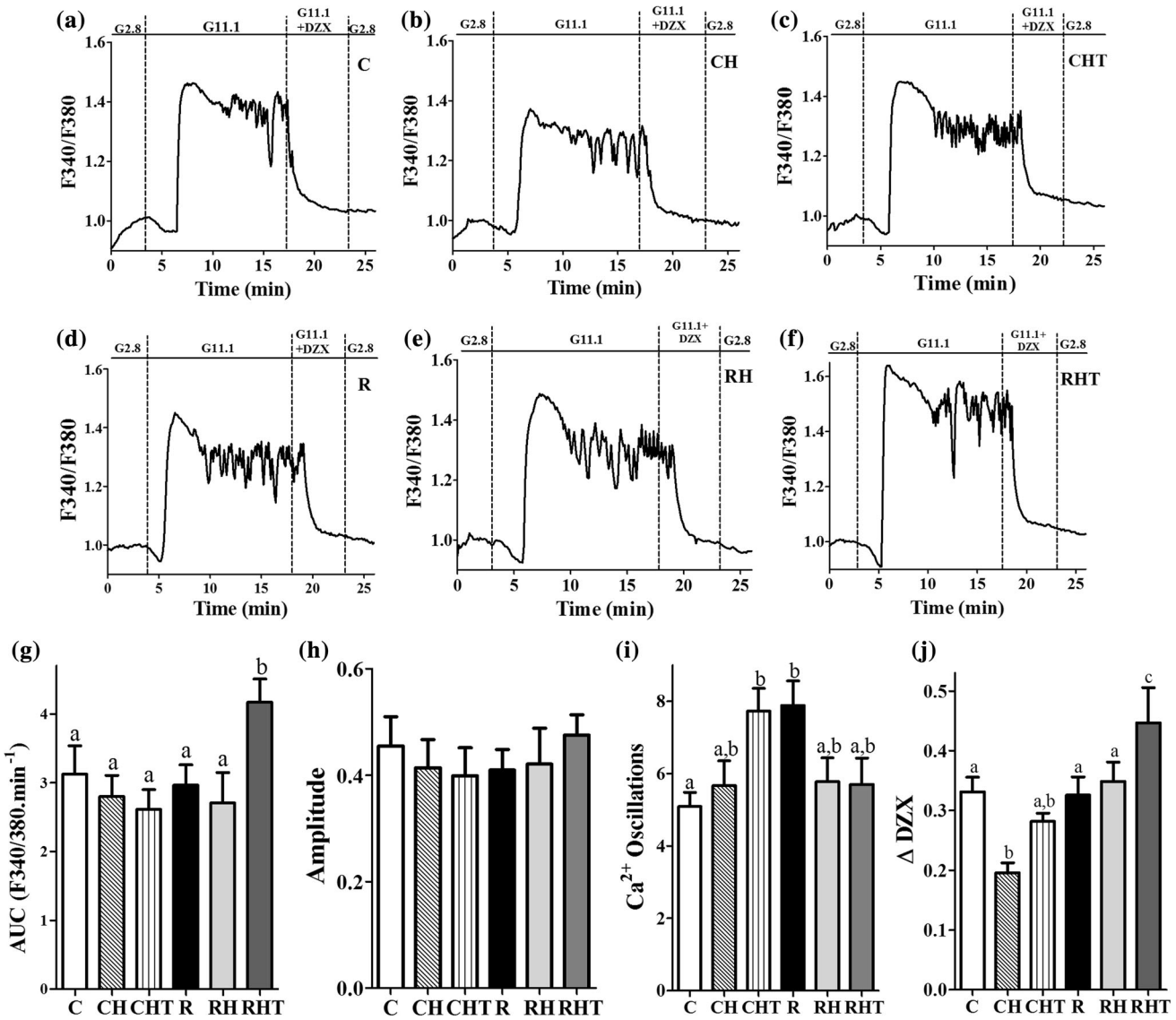

Fig. 7 Representative curves of 11.1 mmol/L glucose with or without 250 μmol/L DZX-induced intracellular Ca^{2+} oscillations in islets from C (**a**), CH (**b**), CHT (**c**), R (**d**), RH (**e**) and RHT (**f**) mice. The AUC (during G11.1 condition = 3–18 min) (**g**), amplitude (**h**) and frequency of Ca^{2+} oscillations (**i**) of the $[Ca^{2+}]_i$ in response to 11.1 mmol/L glucose. The experiments were performed in a perifusion system in a medium that contained 2.8 and 11.1 mmol/L glucose (G2.8 and G11.1, respectively) with or without DZX. The amplitude was calculated by subtracting the highest fluorescence ratio obtained at G11.1 from G2.8 fluorescence values immediately before changing glucose concentration in the perifusium. **j** Potency of DZX inhibition (Δ DZX) calculated by subtraction of the highest value of $[Ca^{2+}]_i$ at G11.1, immediately before DZX perifusion time, from the lowest $[Ca^{2+}]_i$ registered during DZX perifusium. Values are the ratio of F340/F380 registered for each group. The data are mean ± SEM that were obtained from 8 to 10 independent experiments. *Different letters* over the *bars* represent significant differences ($P < 0.05$)

Tau supplementation promoted beneficial effects on BW, adiposity, plasma lipid profile and whole-body glucose control, which were hindered by dietary protein restriction, as previously reported (Batista et al. 2013b; Camargo et al. 2013). Herein we extend these observations to a comprehensive analysis of β-cell function in the same mouse model.

Previous studies on nutritional programming have identified intrauterine protein malnutrition as a risk factor for the development of obesity, insulin resistance and type 2 diabetes in humans and rodents (Hales and Barker 1992; Bol et al. 2009). Here, post-weaned low-protein mice developed glucose intolerance and insulin resistance after feeding on a HFD (Fig. 1). However, the magnitude of this effect was the same observed in CH mice. Tau treatment only improved glucose control in CHT mice, since the RHT group was persistently glucose intolerant (Fig. 1). These differences may be related to developmental and epigenetic

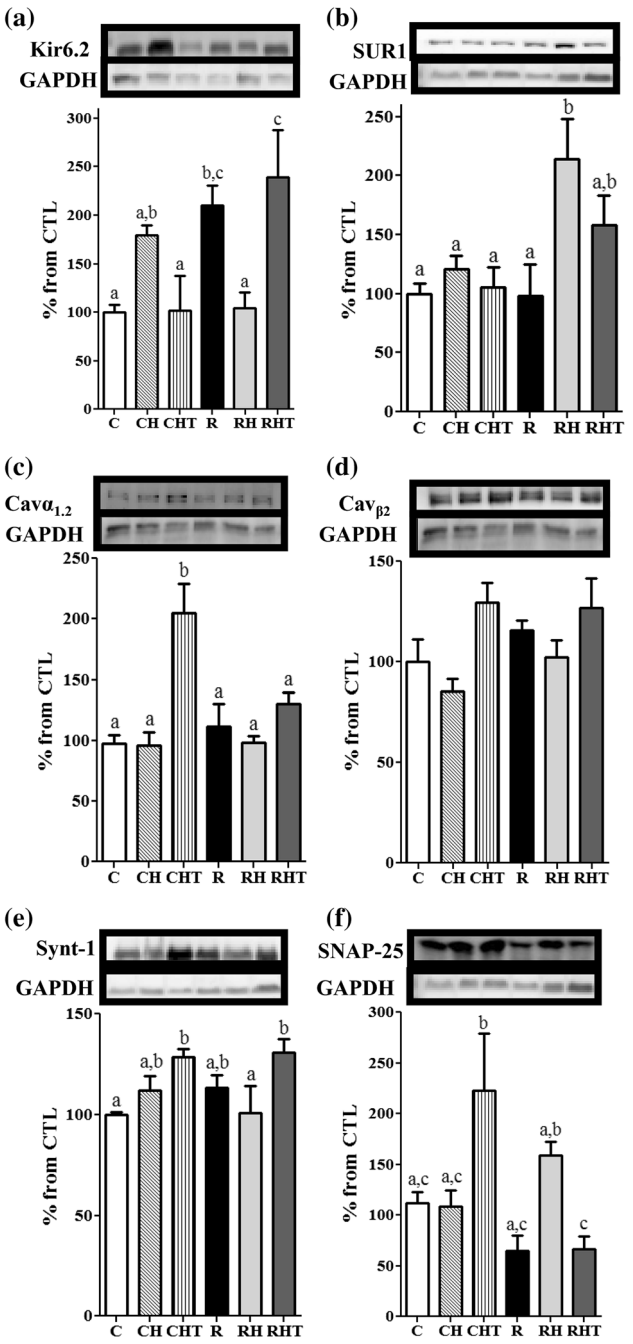

Fig. 8 Protein expression in isolated islets from C, CH, CHT, R, RH and RHT mice. Protein extracts were processed for the Western blot detection of Kir 6.2 (**a**), SUR1 (**b**), Cav α1.2 (**c**), Cav β2 (**d**), Synt-1 (**e**), SNAP-25 (**f**) and GAPDH (internal control). The data are mean ± SEM of the optical densitometric values ($n = 3$–5). *Different letters* over the *bars* represent significant differences ($P < 0.05$)

mechanisms activated in malnourished β-cells, which impair their function in adulthood leading to a worse glucose control (Remacle et al. 2007; Sandovici et al. 2011).

Our data support the notion that a low-protein background leads to differential effects of both HFD and Tau upon insulin secretion. Compensatory insulin secretion to compensate peripheral hormone resistance was impaired in RH islets and this was linked to increased SUR1 protein content (Fig. 8b), which may impair K_{ATP} channel inhibition (Fig. 4a) and β-cell depolarization. Tau supplementation normalized insulin secretion in CHT (Fig. 2) and increased islet exocytotic proteins (Fig. 8). In the RHT group, insulin release was improved by supplementation due to increased protein expression and inhibition of the Kir6.2 subunit of the K_{ATP} channels (Figs. 4a, 8a).

Protein malnutrition was reported to lower the expression of several β-cell-related genes and proteins such as GLUT-2, protein kinase (PK)-A, PKC and muscarinic type 3 receptor (Delghingaro-Augusto et al. 2004; Ferreira et al. 2004; da Silva et al. 2012; Batista et al. 2012). These alterations impair insulin secretion in response to glucose and non-fuel secretagogues in malnourished rodents (Ferreira et al. 2004; da Silva et al. 2012; Batista et al. 2012). In accordance, we observed that R islets secreted less insulin in response to stimulatory glucose concentrations (Fig. 2). Increased activity of the K_{ATP} channel was observed in R islets and contributes to a lower membrane potential at resting and to impaired β-cell electrical activity in the presence of glucose (Soriano et al. 2010). Here, we also demonstrated that R islets presented enhanced K_{ATP} channel activity upon a glucose stimulus (Fig. 4a) and this effect was associated with increased protein expression of the Kir6.2 subunit (Fig. 8a).

Increased expression of Kir6.2 hampers β-cells function. Clonal INS-1 β-cells incubated with low glucose displayed an increased amount of the Kir6.2 protein and channel activity (Smith et al. 2006). In ghrelin-treated rodent islets, a reduction was observed in glucose-induced insulin secretion, an effect associated with enhanced Kir6.2 mRNA and protein levels (Peng et al. 2012). Accordingly, we demonstrated that R islets secrete less insulin in part due to lower K_{ATP} inhibition that, together with reduced islet insulin content, results in decreased fuel-induced secretion. This notion is also supported by decreased insulin secretion and Ca^{2+} influx in response to 2.8 mmol/L glucose plus Tolb (Figs. 3c, 6), confirming a disruption in the activity of the K_{ATP} channels in R islets.

In normal protein HFD mice, insulin release at 11.1 mmol/L glucose was enhanced with no alteration in response to other depolarizing stimuli (Fig. 3). These effects may be associated with an increased inhibition of K_{ATP} and higher activity of Cav channels upon glucose stimulus, as judged by the increased inhibition upon hormone release in the presence of DZX or Nif (Fig. 4). Mouse β-cells acutely exposed to palmitate in vitro, showed increased activity of the L-type Ca^{2+} channels in response to glucose and depolarizing agents (Olofsson et al. 2004). In response to 0.5 mmol/L octanoate,

pancreatic islets displayed acutely reduced K_{ATP} channel activity, which improved the depolarization of β-cells. However, chronic exposure to fatty acids (FAs) leads to impaired electrical activity and insulin release (Best et al. 2011). A 72 h incubation with 1 mmol/L palmitate impaired electrical activity and Ca^{2+} handling and hampered insulin release by more than 50 % (Hoppa et al. 2009). Long-chain acyl-CoAs were shown to interact and activate the K_{ATP} channel, which reduces the secretion of insulin (Branstrom et al. 2004). Therefore, we suggest that in a systemic whole-body setting endowed with a lipid removal system such as circulating lipoproteins, the HFD works in a similar fashion to that observed when islets where acutely exposed to FAs in vitro (Best et al. 2011; Hoppa et al. 2009), which enhanced insulin secretion to compensate peripheral resistance observed in CH mice.

In RH islets, the protein–malnutrition background led to differential effects of the HFD on islet function, as judged by loss of glucose-induced hypersecretion and lower insulin release at high K^+ (Figs. 2, 3). In addition, the islet content of SUR1 protein was higher in RH than in C and R, which may account for the partial increase of insulin release in response to Tolb (Fig. 3). The inhibitory effect of DZX on the secretion of insulin (Fig. 4a) was similar to that observed in R, whereas the Nif-induced inhibition of insulin secretion was reestablished (Fig. 4b). These data suggest that the malnutrition period may impair the function of K_{ATP} channels that, in turn, increase β-cell susceptibility to damages that are produced by dietary FAs in RH islets. This impairment hampers the closure of the K_{ATP} channel and the compensatory hypersecretion that is typically observed in insulin-resistant rodents and humans, which is an effect that may contribute to the early onset of type 2 diabetes.

Tau enhances the nutrient-induced secretion of insulin by regulating the handling of cytoplasmatic Ca^{2+} and the expression of a series of proteins in β-cells (Ribeiro et al. 2009, 2010; Batista et al. 2012). We found that Tau supplementation normalized the glucose-induced insulin secretion in islets from CHT mice (Fig. 2), whereas it increased insulin release in response to K^+ and Tolb in RHT islets (Figs. 3a, c, 7j). These effects in the RHT group may be linked to the increased expression of the K_{ATP} protein (Fig. 8a) and to its inhibition (Figs. 4a, 7j). In addition, the activity of Cav channels was improved, as judged by the increased effect of Nif on the inhibition of insulin secretion (Fig. 4b) and by the enhancement of $[Ca^{2+}]_i$ in response to glucose (Fig. 7g).

Tau closed the K_{ATP} channels in a dose-dependent manner in skeletal muscle cells (Tricarico et al. 2000) and decreased the opening time of the K_{ATP} channel in mouse β-cells, which favors a sustained depolarization of the plasma membrane (Navarro 2009). This effect may be related to the enhanced sensitivity of K_{ATP} to sulfonylureas, which is promoted by Tau (Park et al. 2004). Supporting this assumption, Tau was found to interact with the benzamide site of SUR in *Xenopus laevis* oocytes (Lim et al. 2004). Indirectly, our data with DZX and Nif indicate that Tau potentiated the inhibition of the K_{ATP} channels in RHT islets upon glucose and depolarizing stimuli, which increased the probability of the opening of Cav channels and favored a better Ca^{2+} influx (Fig. 7g). These effects, together with the higher content of the Synt-1 protein in RHT islets (Fig. 8e), contribute to ameliorating the coupling of stimulus-secretion in RHT β-cells.

Here, we demonstrate for the first time that Tau supplementation enhanced the protein content of the Cav channel α1.2 subunit (Fig. 8c), Synt-1 (Fig. 8e) and SNAP-25 (Fig. 8f) in CHT islets. However, Nif inhibited insulin secretion to a lesser extent (Fig. 4b), and DZX induced a similar reduction in the release of insulin in CHT and C islets (Fig. 4a). These effects support the notion that Tau preserves the physiological activity of the K_{ATP} in CHT islets.

Despite the reduced effect of Nif in CHT islets, Tau enhanced the protein content of Cav channels without altering the total $[Ca^{2+}]_i$ in response to glucose and depolarizing stimuli (Figs. 5d, 6d, 7g). However, Ca^{2+} oscillations were increased in CHT islets indicating a possible alteration in the activity of the Cav channel (Fig. 7i). This effect is in accordance with previous reports that Tau enhanced Ca^{2+} oscillations in rodent β-cells (Park et al. 2004; Carneiro et al. 2009) and that the amino acid may act as a $[Ca^{2+}]_i$ buffering molecule, increasing the Ca^{2+} current at low $[Ca^{2+}]_i$, and inhibiting it at high $[Ca^{2+}]_i$ (Satoh and Sperelakis 1998).

In conclusion, our study demonstrated that R islets secreted less insulin due to the enhanced expression and activity of the Kir6.2 subunit of the K_{ATP} channel, which impairs the depolarization of β-cells in response to glucose and other depolarizing agents. The HFD treatment in R mice increased the expression of the islet SUR1 protein, which may also affect the depolarization of the β-cell membrane. In the CH group, despite a lack of changes in the protein content of the CH islets, the enhancement of insulin secretion occurred due to a better inhibition of K_{ATP} activity and an increase in Cav activity to compensate insulin resistance. Tau supplementation improved β-cell function in RHT islets by enhancing the protein content of the K_{ATP} channel and their inhibition in response to depolarization, together with the increased expression of the Synt-1 protein. A beneficial effect of Tau was also noted in the CHT group, which was represented by an increase in the islet protein content of the Cav channel α1.2 subunit, Synt-1 and SNAP-25 and the normalization of the

insulin release. Thus, Tau is a potent regulator of β-cell function and its specific actions may depend on the nutritional protein and lipid status.

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

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