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Journal of Nutritional Biochemistry 22 (2011) 554–559
Journal of Nutritional Biochemistry

Reduced expression of SIRT1 is associated with diminished glucose-induced insulin secretion in islets from calorie-restricted rats☆

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Received 19 November 2009; received in revised form 13 April 2010; accepted 20 April 2010

Abstract

Alterations in food intake such as caloric restriction modulate the expression of SIRT1 and SIRT4 proteins that are involved in pancreatic β-cell function. Here, we search for a possible relationship between insulin secretion and the expression of SIRT1, SIRT4, PKC and PKA in islets from adult rats submitted to CR for 21 days. Rats were fed with an isocaloric diet (CTL) or received 60% (CR) of the food ingested by CTL. The dose–response curve of insulin secretion to glucose was shifted to the right in the CR compared with CTL islets (EC_{50} of 15.1±0.17 and 10.5±0.11 mmol/L glucose). Insulin release by the depolarizing agents arginine and KCl was reduced in CR compared with CTL islets. Total islet insulin content and glucose oxidation were also reduced in CR islets. Leucine-stimulated secretion was similar in both groups, slightly reduced in CR islets stimulated by leucine plus glutamine but higher in CR islets stimulated by ketoisocaproate (KIC). Insulin secretion was also higher in CR islets stimulated by carbachol, compared with CTL islets. No differences in the rise of cytosolic Ca^{2+} concentrations stimulated by either glucose or KCl were observed between groups of islets. Finally, SIRT1, but not SIRT4, protein expression was lower in CR compared with CTL islets, whereas no differences in the expression of PKC and PKA proteins were observed. In conclusion, the lower insulin secretion in islets from CR rats was, at least in part, due to an imbalance between the expression of SIRT1 and SIRT4.

Keywords: Insulin secretion; SIRT1; SIRT4; Caloric restriction

1. Introduction

Inappropriate diets and food intake contribute to the appearance of metabolic diseases.

During the past 70 years a series of studies involving caloric restriction (CR) were undertaken showing that this kind of intervention modulates various molecular and biochemical pathways preventing several diseases such as hypertension, obesity and diabetes [1–3].

CR, a reduction of food intake without undernutrition, also increases longevity in several multicellular organisms [4,5]. A food restriction of 20–40% retards physio-pathological alterations associated with oldness, increasing by 50% the longevity in rodents [6,7].

In mammals submitted to CR for short periods of time a rapid adaptation in fat mobilization from adipose tissue was observed. This implies a profound modification in gene expression as well as in the interaction between enzymes, hormones and other effectors involved in the adaptation of the organisms for the utilization of stored fuels [8,9]. In rodents, CR reduces glycemia and insulinemia preventing glycation that hampers several biological functions. These effects are attributed to regulatory proteins that simultaneously reduce the insulin secretion and increase the insulin sensitivity of tissues [10–13].

The proteins SIRT1 and SIRT4 are involved in pancreatic β-cell function and both are modulated by food modifications, especially in CR situations [14–21]. SIRT1 is a nuclear protein that deacetylates substrates such as histones, NF-kB, p53, FOXO, PPARγ and PGC-α, regulating processes involved in cellular survival. In addition, SIRT1 contributes to the maintenance of glucose homeostasis [14]. SIRT1 acts as a potentiator of insulin secretion in response to glucose and KCl in β cells. In these cells, SIRT1 reduces the expression of UCP-2 and increases ATP

☆ This work was partly supported by the following Brazilian foundations: Conselho Nacional de Pesquisa (CNPq), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Progroma dePós Graduação em Ciências Biomédicas - UNIARARAS, and Instituto Nacional de Obesidade e Diabetes (INOD).

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doi:10.1016/j.jnutbio.2010.04.010

production, as observed in BESTO transgenic mice (β-cell specific Sirt1-overexpressing) [15]. SIRT4, highly expressed in the β cells, is a mitochondrial enzyme that uses NAD to ADP-ribosylate and reduces glutamate dehydrogenase (GDH) activity. Corroborating this observation, it was demonstrated that the loss of SIRT4 in insulinoma cell line activates GDH, increasing amino acid-stimulated insulin secretion [16]. It is well known that dietary intervention such as low-protein diet also modifies the expression of PKA and PKC, reducing glucose-induced insulin secretion in pancreatic islets [22]. These proteins are important for the mechanism of insulin secretion, and the effect of CR on their levels was not yet investigated.

In the present study, we try to establish a relationship between insulin secretion with the expression of SIRT1, SIRT4, PKC and PKA in islets from adult rats submitted to CR for 21 days. We noticed that reduced insulin secretion stimulated by glucose and depolarizing agents, observed in islets from CR rats, coincides with a reduced expression of SIRT1 and of glucose oxidation. However, the imbalance between SIRT1 and SIRT4 did not interfere with the insulin secretion stimulated by leucine and its derivative ketoisocaproate (KIC), and by the potentiator of secretion, CCh.

2. Material and methods

2.1. Animals

Male Wistar rats (8 weeks old) from the University of Araras Animal Breeding Center were kept at 22±1°C with a 12-h light/dark cycle. The rats were separated randomly into two groups and kept in individual cages. The control rats (CTL) were fed with an isocaloric commercial diet *ad libitum*, whereas those from the restrict group (CR) received 60% of the food ingested by CTL. During the experimental period, the rats had free access to water. All the experiments were approved by the Ethics Commission on Animal Experiments (CEEA), University of Araras, SP, Brazil.

2.2. Chemicals and antibodies

Bovine serum albumin (BSA, fraction V), Ficoll, RPMI-1640 medium, collagenase type V, KIC (alpha-ketoisocaproate), carbachol, *tris*-[hydroxymethyl]amino-methane (Tris), phenylmethylsulfonylfluoride (PMSF), dithiothreitol (DTT), triton X-100, Tween 20 and glycerol were purchased from Sigma (St. Louis, MO, USA). The reagents and apparatus for sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting were from Bio-Rad (Richmond, CA, USA). Aprotinin was from Bayer (São Paulo, SP, Brazil). Nitrocellulose membrane (Hybond ECL, 0.45 μm) and an ECL chemiluminescence–Western blotting analysis system were from Amersham (Buckinghamshire, UK). Anti-SIRT1 (C14H4) was from Cell Signaling, anti-SIRT4 (EB 06288) was from Everest Biotech, anti-β-actin and anti-antibodies PKC and PKA were from Santa Cruz Technology (Santa Cruz, CA, USA). Dr. Leclercq-Meyer (Faculty of Medicine, Brussels Free University, Brussels, Belgium) kindly provided the antiserum against insulin. Standard insulin was from Novo-Nordisk (Copenhagen, Denmark). Dextran T70 was from Pharmacia (Uppsala, Sweden). Arginine, leucine, KCl and glucose were from Fluka Biochemik (Switzerland). ^{125}I-insulin (human) was from New England Nuclear Co. (Boston, MA, USA).

2.3. Biochemical analysis

Blood samples were obtained from the tail tip of the rats and allowed to clot; the sera were subsequently stored at −20°C for biochemical analyses. Serum glucose, serum albumin, cholesterol and triglyceride levels were measured according to the manufacturer's instructions (Laborlab, Guarulhos, SP, Brazil). Insulin was measured by RIA [23], and hepatic and muscle glycogen as described elsewhere [24].

2.4. Oral glucose tolerance test

An oral glucose tolerance test (oGTT) was performed in CTL and CR rats. After a 15-h fast, glucose (200 g/L) was administered orogastrically through a catheter at a dose of 2 g/kg body weight. Blood samples were obtained from the cut tip of the tail at 0 and 15, 30, 60, 120 and 180 min after glucose administration for serum glucose determination. The glucose response during the oGTT was calculated by estimating the total area under the curves, using the trapezoidal method [25].

2.5. Insulin content and secretion in isolated islets

Fed rats were euthanized by decapitation and the pancreas was removed. Islets were isolated by handpicking after collagenase digestion of the pancreas, following a technique previously described [26]. Groups of five islets were first incubated for 30 min at 37°C in Krebs-bicarbonate solution containing 5.6 mmol/L glucose and equilibrated with 95% O_2/5% CO_2 (pH 7.4). The solution was then replaced with fresh Krebs-bicarbonate buffer and the islets were further incubated for 1 h in the presence of increasing concentrations of glucose (2.8–27.7 mmol/L) or 2.8 mmol/L glucose plus 40 mmol/L KCl, 10 mmol/L arginine, 10 mmol/L leucine, 10 mmol/L KIC, 10 mmol/L leucine plus 5 mmol/L glutamine, and 10 μmol/L carbachol. The incubation medium contained (in millimoles per liter) 115 NaCl, 5 KCl, 10 $NaHCO_3$, 2.56 $CaCl_2$, 1 $MgCl_2$, 15 Hepes and BSA 0.3% (w/v). The insulin contained in each sample was measured as indicated above using rat insulin as the standard. The glucose concentration producing a response that was 50% of the maximum (EC_{50}) was calculated as the mean negative logarithm (pD2). The islet insulin content was measured as reported [27].

2.6. Glucose oxidation

Groups of 25 islets were incubated for 2 h at 37°C in Krebs-Ringer buffer (KRB) supplemented with 11.1 mmol/L glucose with trace amounts of D-[U-^{14}C]glucose (20 μCi/ml) for $^{14}CO_2$ formation. Islet glucose metabolism was stopped with HCl (1N) with consequent cell cleavage. $^{14}CO_2$ released was absorbed by NaOH (1 mol/L) for 1 h at 4°C, obtaining $NaH^{14}CO_3$. Scintillation fluid was added and radioactivity was counted in a liquid scintillation counter.

2.7. Measurements of intracellular free Ca^{2+} concentration

Freshly isolated islets were incubated in RPMI 1640 medium supplemented with 5% fetal calf serum, 11.1 mmol/L glucose, 100 UI of penicillin/ml, 100 μg of streptomycin/ml at 37°C in 95% O_2/5% CO_2 for 4–6 h. Thereafter, islets were transferred to plates containing a KRB (millimoles per liter concentrations were 115 NaCl, 5 KCl, 24 $NaHCO_3$, 1 $MgCl_2$, 2.6 $CaCl_2$, 25 Hepes; pH 7.4) with 5 mmol/L glucose and 5 μmol/L fura2/AM for 1 h [25]. Islets were then transferred to a thermostatically regulated open chamber (37°C), placed on the stage of an inverted microscope (Nikon UK, Kingston, UK) and perfused with KRB at a flow rate of 1.5 ml/min. A ratio image was acquired approximately every 5 s with an ORCA-100 CCD camera (HamLamatsu Photonics Ibérica, Barcelona, Spain), in conjunction with a Lambda-10-CS dual-filter wheel (Sutter Instrument Company, California, USA), 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 the ImageMaster5 software (Photon Technology International, New Jersey, USA).

2.8. Protein analysis by Western Blotting

A pool of at least 300 clean islets from each experimental group were transferred to an Eppendorf and homogenized, by sonication 15 s, in 200 μL of solubilization buffer (10 ml/L Triton-X 100, 100 mmol/L Tris[hydroxymethyl]amino-methane (Tris) pH 7.4, 10 mmol/L sodium pyrophosphate, 100 mmol/L sodium fluoride, 10 mmol/L ethylendiaminetetracetic acid (EDTA), 10 mmol/L sodium vanadate, 2 mmol/L PMSF and 0.1 mg/ml aprotinin at 4°C). The samples (total islets extracts) were treated with Laemmli buffer containing 100 mmol/L DTT, heated in a boiling water bath for 4 min and subjected to 8% SDS-PAGE in a Bio-Rad minigel apparatus (Mini-Protean, Bio-Rad). The prestained molecular mass standards used were myosin (205 kDa), beta-galactosidase (116 kDa), BSA (80 kDa) and ovalbumin (49.5 kDa). Electrotransfer of proteins from the gel to the nitrocellulose membranes was performed for 90 min at 120 V (constant) in a Bio-Rad miniature transfer apparatus (Mini-Protean) with 0.02% SDS and β-mercaptoethanol added to the transfer buffer to enhance the elution of high-molecular-mass proteins. The nonspecific protein binding to the nitrocellulose was reduced by preincubating the filter for 2 h at 22°C in blocking buffer (5% nonfat dry milk, 10 mmol/L Tris, 150 mmol/L NaCl and 0.02% Tween 20). The nitrocellulose blots were incubated for 4 h at 22°C with antibodies against the PKA, PKC, SIRT1 and SIRT4 diluted in blocking buffer (3% nonfat dry milk, 10 mmol/L Tris, 150 mmol/L NaCl and 0.02% Tween 20), followed by washing for 30 min in blocking buffer without milk. Proteins were detected using the ECL kit chemiluminescence reagent, and the autoradiography using preflashed Kodak XAR film (Eastman Kodak, Rochester, NY, USA) with a Cronex Lightning Plus intensifying screen (DuPont, Wilmington, DE) for 5–30 min. Images of the developed autoradiographs were scanned (Hewlett Packard Scanjet 5P) and band intensities were quantitated by optical densitometry (Scion Image Corporation) of the developed autoradiographs that were used at exposures in the linear range.

2.9. Statistical analysis

The results are presented as the mean±S.E.M. for the number of rats or islets (n) indicated. When comparing the CTL and CR groups, a nonpaired t test was used. These data were log transformed to correct for variance heterogeneity or nonnormality and then analyzed by two-way ANOVA, followed by the Tukey–Kramer test to calculate individual differences between groups and among glucose concentrations, and to verify the interactions between these factors.

Table 1
Serum levels of fasting glucose, albumin, cholesterol, triglycerides, insulin and liver glycogen, and muscle and adipose tissue weight of control (CTL) and of rats maintained on caloric restriction (CR) for 21 days

	CTL	CR	*n*
Glucose (mg/dl)	90±5.04	92±6.07	6
Albumin (g/dl)	8.66±0.66	9.64±0.56	9
Cholesterol (mg/dl)	77.4±3	60±1.11 *	6
Triglyceride (mg/dl)	152.4±17	101±5.13 *	6
Insulin (ng/ml)	0.65±0.03	0.36±0.05 *	6
Hepatic glycogen (g/100 g tissue)	3.34±0.33	2.45±0.24	12
Muscle glycogen (g/100 g tissue)	0.44±0.02	0.45±0,03	12
Adipose tissue (g)	2.43±0.15	1.81±0.08 *	12

Values are expressed as mean±S.E.
* *P*<.05 *vs.* CTL.

3. Results

3.1. Animal features

Table 1 shows that plasma glucose and albumin levels were similar between groups. Plasma insulin, cholesterol and triglyceride levels of CR were significantly lower than those of CTL rats (*P*<.05). Muscular glycogen content was similar in both groups, whereas a marginal reduction in the hepatic glycogen of CR compared with CTL rats was noticed (NS). Epididymal adipose tissue was lower in CR compared with CTL rats (*P*<.05). Weight gain during the 21 days of restriction was lower in CR than in CTL rats reaching 222±7 and 272±7 g body weight (*P*<.05) (Fig. 1). Fig. 2 shows that the oGTT result was similar between groups with the area under the curves reaching 84.6±11 and 87.8±9 cm^2 for CR and CTL, respectively (NS).

3.2. Insulin content and secretion in isolated islets

The total insulin content in islets from CR was reduced compared with CTL rats (73.82±6.7 and 113.83±17.5 ng/islet, *n*=14, *P*<.05). The insulin secretion in isolated islets, stimulated by increasing concentrations of glucose, followed a sigmoidal curve in both CR and CTL islets (Fig. 3). However, the dose–response curve was shifted to the right in the CR, compared with CTL islets with an EC_{50} of 15.1±0.17 and 10.5±0.11 mmol/L glucose, respectively (*P*<.05). The insulin secretion stimulated by agents that depolarize the β-cell membrane such as arginine and high concentrations of KCl was also lower in CR compared with CTL islets (Fig. 4A). At 10 mmol/L arginine, the insulin secretion was 1.4±0.12 and 2.01±0.1 ng/islet (*P*<.05), and

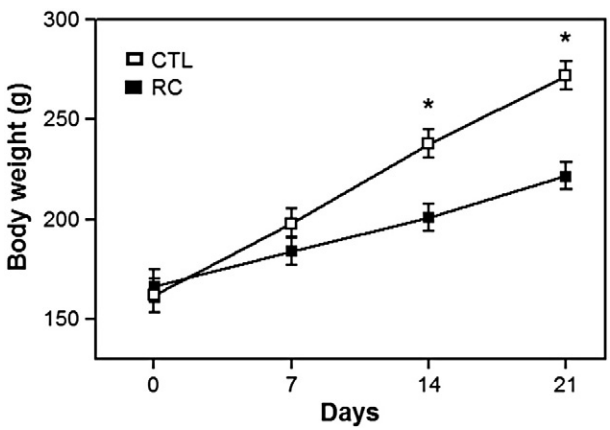

Fig. 1. Weight gain of CTL and CR rats. Values are expressed as mean±S.E. *Significantly different *vs.* CR; *P*<.05; *n*=10.

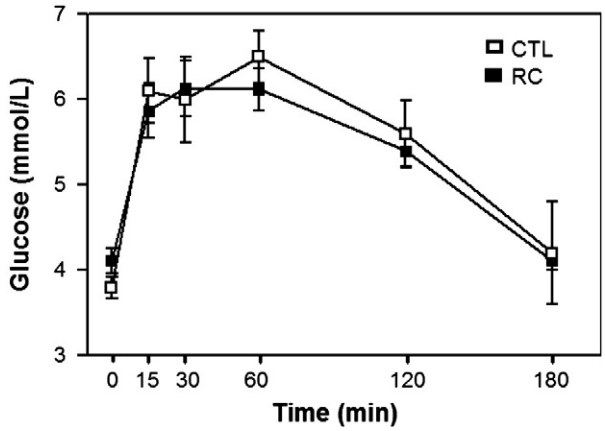

Fig. 2. Oral glucose tolerance test in CTL and CR rats, fasted for 14 h. At Time 0, the rats received a glucose overload of 2 g/kg body weight by gavage. Samples for glucose determination were collected from the tip of the tail at 0, 15, 30, 60, 120 and 180 min. Values are expressed as mean±SE; *n*=8.

at 40 mmol/L KCl was 0.33±0.045 and 1.13±0.1 ng/islet per hour (*P*<.05), respectively, in CR and CTL islets. The insulin secretion stimulated by other fuels such as leucine, KIC and the combination of leucine and glutamine was also analyzed (Fig. 4B). At 10 mmol/L leucine, the insulin secretion was similar between groups reaching 9.54±0.77 and 9.82±0.92 ng/islet per hour in CR and CTL islets, respectively (NS). However, in the presence of KIC the insulin secretion was higher in CR than in CTL islets (8.5±0.71 and 3.9 ±0.73 ng/islet per hour; *P*<.05). In the presence of 10 mmol/L leucine and 5 mmol/L glutamine, insulin secretion was lower in CR than in CTL islets (6.51±0.42 and 8.07±0.38 ng/islet per hour, respectively; *P*<.05). Finally, the insulin secretion stimulated by the cholinergic agent Cch was higher in CR compared with CTL islets (4.9±0.27 and 3.57±0.46, respectively; *P*<.05).

3.3. Glucose oxidation

D-[U-^{14}C]glucose conversion to $^{14}CO_2$ (glucose oxidation) was lower in islets from CR, compared with CTL rats (17.33±1.0 *vs.* 24.5±0.5 pmol/islet per 2 h, respectively, *n*=12, *P*<.05).

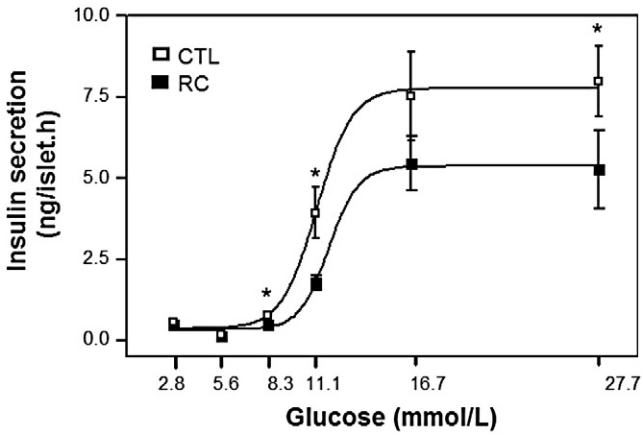

Fig. 3. Dose–response curves to increasing concentrations of glucose (2.8–27.7 mmol/L) in CTL (open squares) and CR (closed squares) islets. Groups of four islets were incubated in Krebs bicarbonate buffer containing 5.6 mmol/L glucose for 30 min. Then, the medium was replaced by fresh medium and the islets were further incubated at different glucose concentrations for 1 h. Values are expressed as mean±S.E. *Significantly different *vs.* CR; *P*<.05; *n*=10.

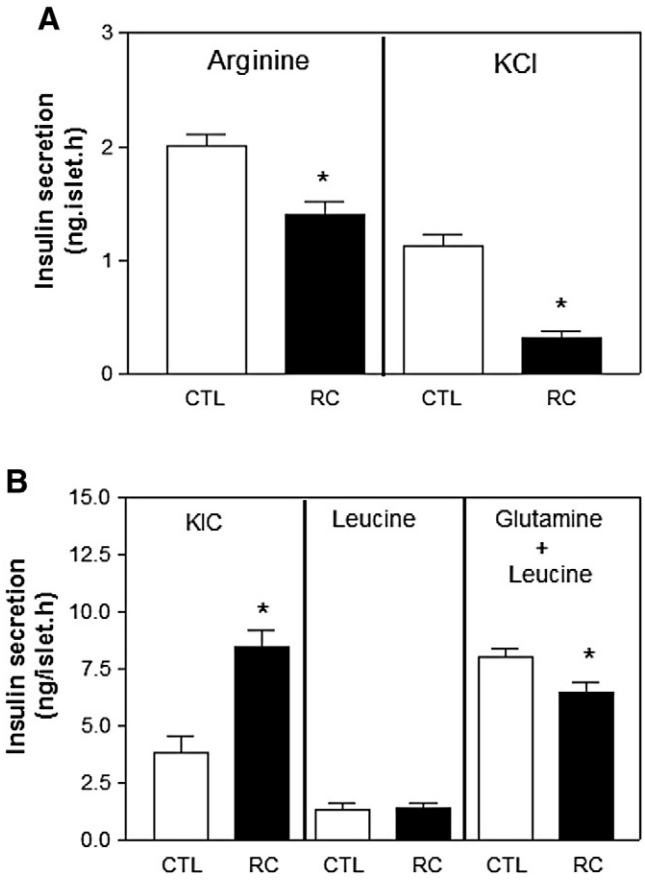

Fig. 4. Insulin secretion stimulated by 2.8 mmol/L glucose plus arginine (10 mmol/L; A), KCl (40 mmol/L; A), leucine (10 mmol/L; B) with or without glutamine (5 mmol/L; B) and KIC (10 mmol/L; B) in CTL (white columns) and CR islets (black columns). Groups of four islets were incubated in Krebs bicarbonate buffer containing 5.6 mmol/L glucose for 30 min. Then, the medium was replaced by fresh medium and the islets further incubated in the presence of the stimulators indicated above for 1 h. Values are means ±S.E. *Significantly different *vs.* CR; $P<.05$; $n=12$ groups of islets.

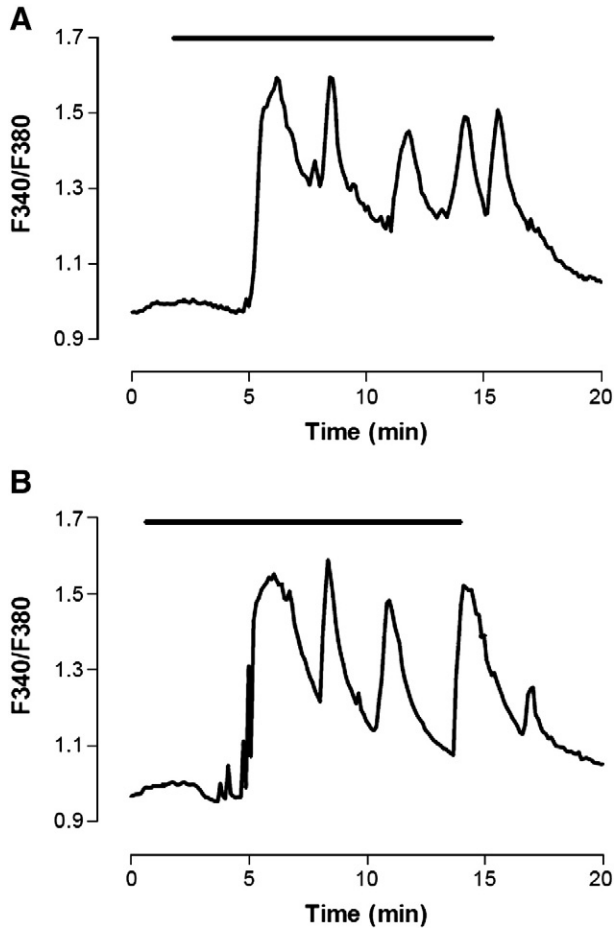

Fig. 5. Representative curves of changes in intracellular Ca^{2+} concentrations in response to 11.1 mmol/L glucose (horizontal bars) in islets isolated from CTL (A) and CR (B) rats; $n=6–10$ islets from three different rats.

3.4. Glucose- and KCl-induced cytoplasmic Ca^{2+} alterations

We investigated the calcium handling by islets challenged either by 11.1 mmol/L glucose or by 40 mmol/L KCl. After increasing the glucose concentrations from 2.8 to 11.1 mmol/L, cytosolic Ca^{2+} was significantly enhanced, reaching maximal values after 6–7 min in both groups of islets. Ca^{2+} oscillations were also noticed during the plateau phase (8–17 min). The Ca^{2+} returned closed to basal values when glucose concentrations were reduced to 2.8 mmol/L glucose (Fig. 5A and B). As judged by the analysis of AUC, no differences in the calcium increase were observed between the two groups of islets (CTL 1.076±0.193 and CR 0.893±0.144). KCl also significantly increased the cytosolic Ca^{2+} concentrations in both CR and CTL islets, and no differences were observed in the AUC between those islets (not shown).

3.5. Protein expression

Western blotting analysis showed a reduction in the expression of SIRT1 but not of SIRT4 in CR compared with CTL islets (2383±817 and 8210±1560 arbitrary units; $P<.05$) (Fig. 6A). No differences in the expression of PKC and PKA proteins were observed between groups (Fig. 6B).

4. Discussion

Low body weight, reduced periepididymal fat pad, hypocholesterolemia, hypotriglyceridemia and hypoinsulinemia associated with normal glycemia, albuminemia, and hepatic and muscle glycogen contents (Fig. 1 and Table 1) are features usually found in animals submitted to caloric restriction, indicating the adequacy of the animal model used in this work [9]. In addition, the normoglycemia associated with hypoinsulinemia (Table 1) as well as the lack of differences between oGTT in CTL and CR rats (Fig. 2) indicates that, although lower, the insulin secreted in CR rats is sufficient to keep normoglycemia.

The hypoinsulinemia of CR rats (Table 1) may result from a reduced insulin secretion to glucose in these rats. This assumption is based on the observation that in *ex vivo* islets the dose–response curve of insulin secretion to increasing concentrations of glucose is shifted to the right in CR compared with CTL, indicating a lower sensitivity to glucose in the former (Fig. 3). This lower secretion may be attributed, at least in part, to lower insulin content and/or reduced glucose oxidation in those islets. This lower sensitivity to glucose coincides with an enhancement of the peripheral insulin sensitivity in rats maintained for 21 days at a diet with 60% calorie reduction, as reported early [28–31]. This increased sensitivity is justified by a concomitant augmentation in muscle expression of GLUT 4 [28] accompanied by an increase in the IR tyrosine phosphorylation, IRS-1 expression [29] and AKT-2 phosphorylation in this tissue [30,31]. In

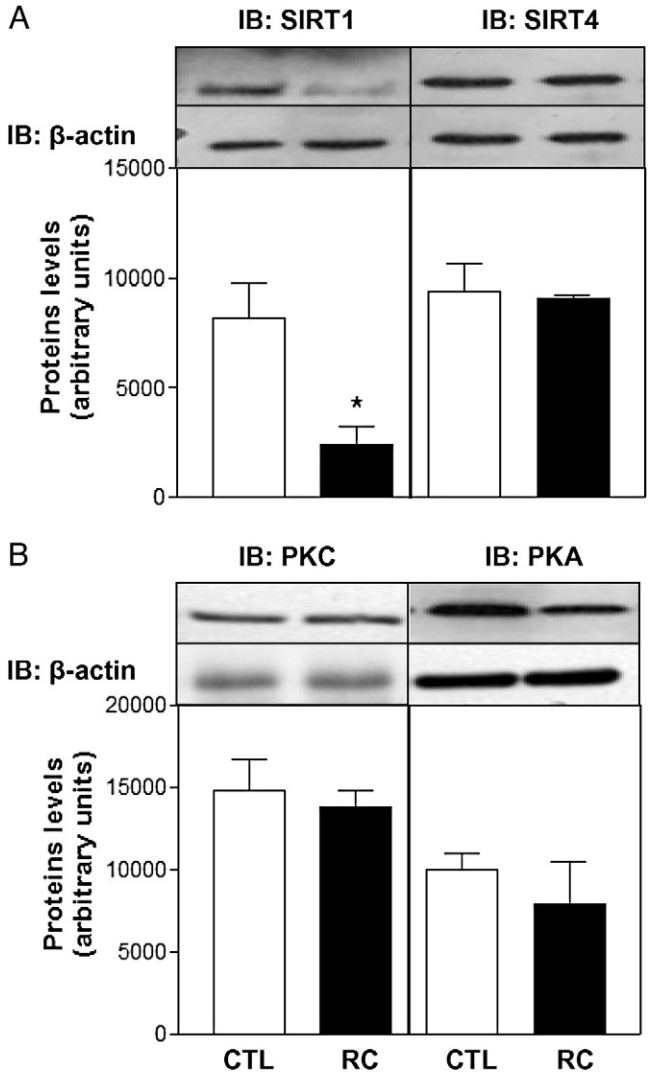

Fig. 6. Representative immunoblotting showing SIRT1, SIRT4 (6A), PKC and PKA (6B) expressions in islets from CTL and CR rats. Loading control was done with anti-β-actin antibody. Values are expressed as mean±S.E. (n=5). *Significantly different *vs.* CR, P<.05.

addition, rats also showed increased plasma levels of adiponectin and a reduction of IL-6, triglycerides and cholesterol, which reinforces the higher peripheral sensitivity to insulin in these animal models [32].

On the contrary, the insulin secretion induced by leucine was similar between islets from CR and CTL islets. The secretion was even higher in CR islets stimulated by KIC, a leucine product [33], compared with CTL islets, indicating that the reduced total insulin content was not the only thing responsible for the lower secretion induced by glucose in CR islets. Interestingly, the insulin secretion stimulated by the combination of leucine plus glutamine was slightly reduced in CR than in CTL islets, indicating a lower allosteric action of GDH in the former islets (Fig. 4B). These results, together with those observed for KIC, support the idea that the similar insulin secretion observed in response to leucine alone could be due to a better transamination of this amino acid, counteracting the lower allosteric effect. Arginine-stimulated secretion in CR was lower than in CTL islets. A similar response was observed when the islets were challenged with 40 mmol/L KCl (Fig. 4A). Glucose, arginine and high concentrations of KCl stimulate insulin release, inducing membrane cell depolarization [34]. Thus, the next series of experiments were performed to test the hypothesis that the lower insulin secretion induced by glucose or by depolarizing agents was due to a defect on Ca^{2+} handling by the islets. However, the ability of glucose and of depolarizing KCl concentrations to increase cytosolic Ca^{2+} levels and oscillations was similar in both CR and CTL islets. These results clearly indicate that the lower insulin secretion in response to glucose, arginine and KCl cannot be attributed to defects on Ca^{2+} mobilization by the CR islets.

In contrast to that observed with glucose and depolarizing agents, Cch-induced secretion was higher in CR than in CTL islets. As it is known, Cch potentiates secretion, increasing the production of IP3 and DAG [35]. Although we have not addressed this issue in this work, we are tempted to speculated that, due to the calorie restriction, it is possible that in the CR rats there is an exacerbation of the cholinergic systems making the islets more sensitive to Ach and consequently to Cch. However, this speculation was not supported by alterations in PKC expression, maybe due to the short period of food restriction.

The reduction of glucose-induced secretion in islets from CR rats parallels a concomitant reduction in the expression of SIRT1 protein that positively regulates secretion. These results agree with the lower insulin secretion observed in insulinoma cells where the SIRT1 mRNA was repressed [15] and in islets from transgenic SIRT1 knockout mice [36]. On the contrary, transgenic mice overexpressing SIRT1 in β-cells secreted more insulin in response to glucose administration [37]. CR did not alter the expression of SIRT4 in our animal model. This protein is highly expressed in β-cells and acts as an inhibitor of secretion as judged by the lower insulin release in knockout mice and in insulinoma β-cell line with reduced SIRT4 expression [16]. Thus, our data concerning the expression of SIRT1 and SIRT4 indicate that the reduced insulin secretion in islets from CR rats may be a consequence of an imbalance between both proteins [16,20]. Interestingly, SIRT4 is important for the control of GDH activity. Thus, at the beginning of the CR period GDH is more active in accelerating glutamate metabolism and, by doing so, allows β-cells to the obtain energy from other fuels [17,18]. We do not know yet how the imbalance between SIRT1 and 4 interferes with GDH activity. However, based on KIC results a possible increase in the transamination of leucine occurs in CR rats, which could explain the normal insulin secretion when the islets were challenged by this amino acid.

In conclusion, in CR rats the lower insulin secretion induced by glucose is compensated by an increase in insulin sensitivity maintaining the normoglycemia. The lower insulin secretion in the islets is not due to alterations in Ca^{2+} handling but to an imbalance between the expression of SIRT1 and SIRT4.

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