Endurance training stimulates growth and survival pathways and the redox balance in rat pancreatic islets

Vivian C. Calegari,1 Julia L. Abrantes,1 Leonardo R. Silveira,2 Flavia M. Paula,1 José Maria Costa Jr.,1 Alex Rafacho,1,3 Lício A. Velloso,4 Everardo M. Carneiro,1 Jose R. Bosqueiro,5 Antonio C. Boschero,1 and Claudio C. Zoppi1

1Department of Anatomy, Cellular Biology and Physiology and Biophysics, Institute of Biology and 4Laboratory of Cell Signaling, State University of Campinas (UNICAMP), Campinas; 2School of Physical Education and Sports, Faculty of Medicine, Department of Biochemistry and Immunology, University of Sao Paulo (USP), Ribeirão Preto, Sao Paulo; 3Department of Physiological Sciences, Center of Biological Sciences, Federal University of Santa Catarina (UFSC), Florianópolis, Santa Catarina; and 5Department of Physical Education, School of Science, Sao Paulo State University, UNESP, Bauru, Sao Paulo, Brazil

Submitted 14 March 2011; accepted in final form 14 December 2011

Calegari VC, Abrantes JL, Silveira LR, Paula FM, Costa JM Jr, Rafacho A, Velloso LA, Carneiro EM, Bosqueiro JR, Boschero AC, Zoppi CC. Endurance training stimulates growth and survival pathways and the redox balance in rat pancreatic islets. J Appl Physiol 112: 711–718, 2012. First published December 15, 2011; doi:10.1152/japplphysiol.00318.2011.—Endurance training has been shown to increase pancreatic β-cell function and mass. However, whether exercise modulates β-cell growth and survival pathways signaling is not completely understood. This study investigated the effects of exercise on growth and apoptotic markers in rat pancreatic islets. Male Wistar rats were randomly assigned to 8-wk endurance training or to a sedentary control group. After that, pancreatic islets were isolated; gene expression and the total content and phosphorylation of several proteins related to growth and apoptotic pathways as well as the main antioxidant enzymes were determined by real-time polymerase chain reaction and Western blot analysis, respectively. Reactive oxygen species (ROS) production was measured by fluorescence. Endurance training increased the time to reach fatigue by 50%. Endurance training resulted in increased protein phosphorylation content of AKT (75%), AKT substrate (AS160; 100%), mTOR (60%), p70s6k (90%), and ERK1/2 (50%), compared with islets from control group. Catalase protein content was 50% higher, whereas ROS production was 49 and 77% lower in islets from trained rats under basal and stimulating glucose conditions, respectively. Bax and cleaved caspase-3 protein contents were reduced by 25 and 50% in islets from trained rats, respectively. In conclusion, these results demonstrate that endurance training favors β-cell growth and survival. However, whether exercise modulates β-cell growth and survival pathways signaling is not completely understood. In this sense, unbalanced apoptosis and regeneration processes have been reported in pancreatic islet β-cells of diabetic subjects (29). Among possible factors, glucolipotoxicity, induced by high levels of blood nutrients as well as inflammation, could lead to several intracellular alterations, such as increased ROS production and endoplasmic reticulum stress, which lead to β-cell damage, dysfunction, and death, mainly by apoptosis. (9, 33, 36, 38).

Physical exercise induces several systemic adaptations, which in turn may benefit β-cell survival and function. Endurance training acts on insulin and leptin target tissues, enhancing their sensitivity to these hormones. In the hypothalamus, this effect leads to increased satiety and consequent reduction in food intake (39), lowering the levels of circulating nutrients. The increase in peripheral insulin sensitivity, induced by exercise, reduces hepatic glucose production (12). Moreover, endurance exercise decreases blood glucose and lipid content by enhanced liver and skeletal muscle uptake and oxidation of these substrates (17, 19). In addition, endurance training also reduces circulating pro-inflammatory and increases anti-inflammatory cytokine levels (4). Taken together, these alterations contribute to a more adequate environment for β-cell survival. The effects of exercise on β-cell intracellular adaptations are not well established. Insulin secretion by isolated islets, in response to different glucose concentrations, was lower in rats submitted to endurance training with increased insulin sensitivity (6, 22, 42). The decreased insulin response to glucose might, itself, contribute to β-cell survival by reducing the stress associated with the insulin secretory process (43). Furthermore, Choi and colleagues (8) showed that endurance training enhanced β-cell proliferation and mass, an effect attributed to the activation of the insulin/insulin-like growth factor I (IGF-1) signaling cascade. Moreover, ERK1/2 activation is also associated with β-cell function and survival (26). However, to date, the endurance training effect on these specific signaling cascades has not been addressed in pancreatic islets.

A correlation between oxidative stress and β-cell apoptosis has been already established (33). Whereas adequate ROS production seems to be required for normal glucose-stimulated insulin secretion (GSIS) (27), excessive amounts of these species may trigger β-cell dysfunction and apoptosis by increased cytochrome c leakage, which in turn could activate caspase-3 and the nuclear factor kappa B-transcription factor (NF-κB) (for review, see Refs. 18, 33). Thus an adequate balance between ROS production and antioxidant capacity...
proteins protects β-cell from oxidative stress-induced β-cell dysfunction and apoptosis.

It has been reported that the pancreatic β-cell mass increases after a program of endurance training in rats (8). However, the intracellular adaptations on survival and apoptotic pathways were not well established in islets from experimental models of exercise programs. Thus the present study sought to investigate the effects of endurance training protocols on growth and survival pathways, as well as apoptosis signaling in isolated pancreatic islets.

**MATERIAL AND METHODS**

Reagents. Anti-CuZn superoxide dismutase (CuZn-SOD) and anti-Se glutathione peroxidase (GPx-1) antibodies were obtained from Abcam (Cambridge, MA). Anti-catalase (CAT) was from Sigma (St. Louis, MO). Anti-α-tubulin, anti-phospho AKT 1/2/3 (pAKT), anti-total AKT, anti-total ERK, anti-phospho ERK (pERK), anti-AS160, anti-phospho AS160 (pAS160), anti-phospho mTOR (pmTOR), anti-total mTOR, anti-total p70s6k, anti-phospho mTOR (pmTOR), anti-total mTOR, anti-total p70s6k, anti-total AKT, anti-total ERK, anti-phospho ERK (pERK), anti-Bcl2, anti-Bax, anti-cleaved caspase-3, anti-anti proliferating cell nuclear antigen (PCNA), and Proto-oncogene tyrosine-protein kinase Fyn (Fyn kinase) were from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-phospho p70s6k (pp70s6k), and anti-total caspase-3 were from Cell Signaling (Beverly, MA). Anti-AS160, anti-phospho AS160 (pAS160), anti-phospho insulin and insulin-like growth factor 1 receptor (pIR/IGF1R) were from Upstate/Millipore (Billerica, MA). 2′,7′-Dichlorofluorescein diacetate (DCFH-DA) was from Sigma. =-Dichlorofluorescein diacetate (DCFH-DA) was from Sigma. Reagents for SDS-PAGE and immunoblotting were from Bio-Rad (Hercules, CA) and Sigma. SuperSignal West Pico Chemiluminescent substrate kit was from Pierce (Rockford, IL).Primers and TRizol reagent for real-time polymerase chain reaction (PCR) were purchased from Invitrogen (Carlsbad, CA).

Animals. Experiments were performed on male Wistar rats (7–8 wk old) obtained from the State University of Campinas Animal Breeding Center. The animals were kept at 22 ± 2°C on a 12:12-h light/dark cycle with free access to food and water. The experimental protocols were approved by the institutional State University of Campinas Committee for Ethics in Animal Experimentation, and all the recommendations for ethical usage of animals were followed.

**Table 1. Sequences of the primers used for real-time PCR gene expression quantification**

<table>
<thead>
<tr>
<th>Gene name</th>
<th>GeneBank Access Number</th>
<th>Primer Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKT NM_017093.1</td>
<td>Fw: 5’TGGCTCTGGATGAGTAC-3’</td>
<td>Rv: 5’TCCACATCGAGAGCGAAG-3’</td>
</tr>
<tr>
<td>AS160 XM_001074183.1</td>
<td>Fw: 5’-AGGAGTGACGACATTGTG-3’</td>
<td>Rv: 5’-GAGGCTTTAAGACATTGCGTGG-3’</td>
</tr>
<tr>
<td>p70 s6k NaM_031985.1</td>
<td>Fw: 5’-TGGACAACAGAGCGGAAAG-3’</td>
<td>Rv: 5’-GGGCTGTCAGGATGAGAAGC-3’</td>
</tr>
<tr>
<td>mTOR NM_019906.1</td>
<td>Fw: 5’-ATGTCCTAATCTGGTCGCC-3’</td>
<td>Rv: 5’-TGGCAATAGAGACCGGATG-3’</td>
</tr>
<tr>
<td>Bax NM_017059.1</td>
<td>Fw: 5’-GAAAGGAGGTCCATCTCG-3’</td>
<td>Rv: 5’-GTATGATAACCGGGAGATCG-3’</td>
</tr>
<tr>
<td>Bcl-2 NM_016993.1</td>
<td>Fw: 5’-TGGCAATAGAGACCGGATG-3’</td>
<td>Rv: 5’-GGGCTGTCAGGATGAGAAGC-3’</td>
</tr>
<tr>
<td>CAT NM_012520.1</td>
<td>Fw: 5’-GATGAGAATGAGAAGGAC-3’</td>
<td>Rv: 5’-GGGCTGTCAGGATGAGAAGC-3’</td>
</tr>
<tr>
<td>GPx1 NM_030826.3</td>
<td>Fw: 5’-TGCAATCTCCTGGTGAAA A-3’</td>
<td>Rv: 5’-TCATTTTGGTCTGGTGAAAA-3’</td>
</tr>
<tr>
<td>CuZnSOD NM_017050.1</td>
<td>Fw: 5’-GTAAGAGGGGATTTGGGAG-3’</td>
<td>Rv: 5’-TGGCAATAGAGACCGGATG-3’</td>
</tr>
<tr>
<td>MnSOD NM_017051.2</td>
<td>Fw: 5’-TGGCAATAGAGACCGGATG-3’</td>
<td>Rv: 5’-GGGCTGTCAGGATGAGAAGC-3’</td>
</tr>
<tr>
<td>GAPDH XR_009170.1</td>
<td>Fw: 5’-GGAGAATGAGAAGGAC-3’</td>
<td>Rv: 5’-GGGCTGTCAGGATGAGAAGC-3’</td>
</tr>
</tbody>
</table>

Fw, forward sequence (sense); Rv, reverse sequence (antisense) primer. All amplicons were in the size ranging from 100 to 180 bp.

*J Appl Physiol* • doi:10.1152/japplphysiol.00318.2011 • www.jappl.org

**Evaluation of the maximal physical capacity of each rat.** Rats were familiarized with the treadmill by carrying out five mild exercise sessions (at 5 m/min) of 5 min each for 2 wk before the beginning of the training protocol. After a familiarization period, rats were randomly assigned into the sedentary control group (CTL) and trained group (TRE5×). The physical capacity of each rat was evaluated through a maximal effort test (Tmax) on the treadmill, starting at 5 m/min and increasing the speed in 5 m/min at each 3-min stage. The maximal physical capacity was assumed to be the speed at which the rat stopped running spontaneously (40).

**Endurance training protocol.** The TRE5× group ran on the treadmill at 60% of the maximal speed reached during Tmax (Tmax 1), 0% grade, 1 h/day, 5 days/wk, for 8 wk. After 4 wk of training a new Tmax (Tmax 2) was applied, and the relative running intensity was reset. At the end of 8 wk of training, another Tmax (Tmax 3) was applied to evaluate the physical capacity of the rats before starting sample collection and experiments. The CTL group was also submitted to the same three maximal effort tests (Tmax), as described for TRE5×; however, they remained sedentary during the entire experimental protocol.

**Body weight and circulating components.** Twenty-four hours after the last training session, rats were weighed, in the fasted state, in a digital scale and blood glucose was measured by the handheld Accu-Check Advantage II glycosimeter (Roche, Basel, Switzerland). After this, rats were immediately killed by exposure to CO2 in a sealed chamber, followed by decapitation. Blood samples were collected in heparinized Eppendorf tubes. Plasma was obtained by centrifugation at 15,300 g at 4°C. Plasma triglycerides were measured with a commercial kit (Roche Diagnostics, Mannheim, Germany), following the manufacturer’s guidelines, and insulin content was determined by radioimmunossay.

**Islet isolation, total islet insulin content, protein extraction, and immunoblotting.** Islets were then isolated by collagenase digestion of the pancreas, as described (21), with modifications (2). Pools of 600–1,000 islets were sonicated in ice-cold cell lysis buffer (Cell Signaling) using a cell homogenizer (Sonic & Materials, Newtown, CT). Protein concentration from tissue lysate was determined by the reducing agent compatible and detergent compatible protein assay method, according to the manufacturer (BioRad). Islet lysates were...
Table 2. Body weight, circulating parameters, and total islet insulin content

<table>
<thead>
<tr>
<th></th>
<th>Weight, g</th>
<th>Blood Glucose, mg/dl</th>
<th>Serum/Plasma Triglycerides, mg/dl</th>
<th>Serum/Plasma Insulin, ng/ml</th>
<th>Total Islet insulin content, % from CTL</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTL</td>
<td>503.7 ± 25.2</td>
<td>90.8 ± 3.9</td>
<td>94.2 ± 10.6</td>
<td>1.1 ± 0.2</td>
<td>100 ± 5</td>
</tr>
<tr>
<td>TRE5x</td>
<td>452 ± 18.1</td>
<td>84.3 ± 2.9</td>
<td>92.7 ± 6.0</td>
<td>1.2 ± 0.2</td>
<td>193 ± 18*</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SE (n = 5–10). Data from control (CTL) and trained (TRE5x) rats after 8 wk of training protocol in fasted state. *P < 0.05 Student’s t-test.
content in TRE5×, compared with CTL islets. When the phosphorylated protein levels were analyzed, we observed a significant increase in pAKT (60%), pERK1/2 (50%), pAS160 (100%), pmTOR (70%), and pp70s6k (90%) in TRE5× compared with CTL islets, which resulted in an increased phosphorylated-to-total ratio of all these proteins varying between 50 to 100% (Fig. 2, A–F; \( p < 0.05 \)). Also observed was a significant increase in Fyn kinase content (Fig. 2G), a protein

### Table 3. Real-time PCR gene expression quantification in islets of CTL and TRE5x rats

<table>
<thead>
<tr>
<th>Genes</th>
<th>CTL</th>
<th>AS160</th>
<th>p70 s6k</th>
<th>mTOR</th>
<th>Bax</th>
<th>Bcl-2</th>
<th>CAT</th>
<th>GPx1</th>
<th>CuZn SOD</th>
<th>Mn SOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>1.0 ± 0.02</td>
<td>1.0 ± 0.02</td>
<td>1.0 ± 0.05</td>
<td>1.0 ± 0.02</td>
<td>1.0 ± 0.05</td>
<td>1.0 ± 0.05</td>
<td>1.0 ± 0.05</td>
<td>1.0 ± 0.05</td>
<td>1.0 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>TRE5x</td>
<td>1.04 ± 0.03</td>
<td>0.88 ± 0.02</td>
<td>1.05 ± 0.18</td>
<td>1.04 ± 0.07</td>
<td>0.98 ± 0.16</td>
<td>1.46 ± 0.08*</td>
<td>1.01 ± 0.05</td>
<td>1.02 ± 0.06</td>
<td>1.13 ± 0.11</td>
<td>1.19 ± 0.12</td>
</tr>
</tbody>
</table>

Results are presented as fold ± SE, compared against control values and represent 4 independent experiments, normalized to the housekeeping gene GAPDH. Islets from CTL and TRE5x rats were collected and mRNA were isolated and used for real-time PCR analysis as described in MATERIALS AND METHODS. *\( p < 0.05 \); Student’s t-test.

---

**Fig. 2.** Effects of endurance training on growth and survival signaling pathways. After 8 wk of endurance training, Western blot analysis of islets isolated from TRE5× revealed higher AKT (A) and ERK1/2 (B) phosphorylated-to-total ratio levels. Total (C) and phosphorylated (D) levels of AS160 were increased in TRE5× islets. The levels of phosphorylated-to-total ratios from mTOR (E) and p70s6k (F) were higher in TRE5× islets. Fyn kinase (G) and PCNA (H) were enhanced in TRE5×, whereas phosphorylated IR/IGF1R (I) were unaltered. Results are expressed as means ± SE from 2 independent experiments (\( n = 3–5 \)). *\( p < 0.05 \) Student’s t-test.
involved with pancreatic beta cell proliferation. Taken together, these results suggest a positive role for endurance training in islet growth and survival, which was paralleled by the marked increase (P < 0.0001) in TRE5× PCNA content (Fig. 2H). Noteworthy is that the upstream signal for all these proteins was not altered as judged by the similar levels of phosphorylated insulin and IGF-1 receptors in TRE5× islets (Fig. 2f).

With regard to apoptosis marker expression, endurance training resulted in significant alterations for all proteins studied. The anti-apoptotic Bcl-2 mRNA and protein content were 46 and 100% increased in TRE5× compared with CTL rats (Table 3 and Fig. 3B, respectively; p < 0.05). On the other hand, the pro-apoptotic proteins Bax and cleaved caspase-3 were 25 and 50% lower, respectively, whereas total caspase-3 was threefold increased in TRE5×, compared with CTL islets (Figs. 3, A, D, E, respectively; P < 0.05). The Bax/Bcl-2 protein ratio, an indirect indicator of β-cell apoptosis (4), was significantly reduced by >50% compared with CTL group, suggesting that endurance training has a positive action against β-cell apoptosis.

Endurance training favors the pancreatic islet redox balance by increasing antioxidant capacity and reducing ROS production. Endurance training did not alter the mRNA expression of any of the antioxidant enzymes studied. The CuZnSOD and GPX-1 protein contents were also similar between TRE5× and CTL islets (Fig. 4, A and B). However, catalase protein content was significantly increased by 50% in TRE5× islets (Fig. 4C; P < 0.05), demonstrating an enhanced ROS scavenging capacity in these islets. A significant reduction was also observed in ROS production under basal (2.8 mM) and stimulatory (22 mM) glucose concentrations (49 and 77%, respectively) in TRE5× compared with CTL islets (Fig. 4D).

DISCUSSION

It is widely accepted that endurance training favors glucose homeostasis through the amelioration of the systemic insulin effect (16, 17). This peripheral insulin-sensitizing effect and its consequence on reduction of blood nutrient levels, mainly glucose and lipids, seem to be associated with increased pancreatic islet survival and function (24). Here, we demon-

![Fig. 3. Effects of endurance training upon pro-apoptotic and anti-apoptotic markers. After 8 wk of endurance training, Western blot analysis of isolated islets from TRE5× showed reduced Bax (A), but increased Bcl-2 content (B), shifting the Bax/Bcl-2 ratio toward β-cell survival (C). Cleaved caspase-3 content (D) was reduced, whereas total caspase-3 increased in islets from TRE5× rats (E). Results are expressed as means ± SE of 2 independent experiments (n = 3–5). *P < 0.05 Student's t-test.](http://jap.physiology.org/doi/10.1152/japplphysiol.00318.2011)
stratified that these effects are probably mediated by the activation of growth and survival signaling pathways.

There is evidence that endurance training also modulates other endocrine pancreas functions. Endurance exercise has been reported to alter pancreatic islet glucose metabolism (22, 42), reducing GSIS, despite the higher levels of pancreatic islet glucose uptake and the total insulin content (34). Furthermore, endurance training has been found to increase \( \beta \)-cell mass by hyperplasia and reduction of apoptotic rate (8). However, the molecular mechanisms involved in these adaptations are still poorly understood. In light of this, we aimed to investigate the effects of endurance training on some pathways involved in \( \beta \)-cell growth and survival.

The activation of the insulin receptor substrate 2 (IRS-2)/phosphoinositide 3-kinase (PI3K) pathway plays a central role in the maintenance of \( \beta \)-cell mass and function (14). Among the effectors of the IRS-2/PI3K pathway, the phosphorylation of downstream AKT and ERK1/2 proteins seems to be mainly responsible for the regulation of \( \beta \)-cell growth and survival.

AKT is phosphorylated by the phosphatidylinositol (3,4,5)-trisphosphate-dependent protein kinase 1 (PDK-1). Once activated, AKT phosphorylates several downstream substrates, including those associated with cell growth (mTOR and glycogen synthase kinase 3 (GSK3)), survival (Bad and GSK3), and differentiation [Forkhead box O1 (FoxO1)], and cAMP-responsive element-binding protein (CREB) (13). In addition, ERK1/2 is also phosphorylated by the IRS-2/PI3K signaling pathway, through the activation of the small G protein, Ras (26). ERK1/2 directly regulates the activity of transcription factors associated with \( \beta \)-cell function and survival. As such, ERK1/2 activation concomitantly stimulates insulin and pancreatic duodenum homeobox 1 (PDX-1) gene expression and reduces the pro-apoptotic CCAAT/enhancer-binding protein beta-homologous protein (CHOP) gene transcription and protein content (26).

Our data provide further evidence that exercise training also exerts its effects by activating islet growth and survival pathways by favoring the phosphorylation of key proteins associated with these processes. Increased levels of phosphorylation were observed for several AKT pathway components, as well as for the ERK1/2 protein in islets from TRE5× rats. It is important to note that we were unable to detect either enhanced gene expression or protein content for most proteins studied. AS160 protein content and phosphorylation were also increased in TRE5× islets. Although little is known concerning its role in this tissue, an involvement in \( \beta \)-cell survival and function has been demonstrated. AS160 is also reported to act independently of AKT phosphorylation because it is a substrate of the AMP-activated kinase (AMPK) (3, 7). Accordingly, in the MIN6 cell line, AS160 knockdown resulted in altered basal and stimulated GSIS, as well as increased apoptosis rate (3). It was suggested that AS160 modulates GSIS, controlling insulin vesicle trafficking through its Rab GTPase activity, whereas its function on \( \beta \)-cell survival might be processed by its AKT downstream signaling effect. With regard to islet growth and survival, increased AS160 content and phosphorylation sup-

---

**Fig. 4.** Effects of endurance training on pancreatic islets redox balance parameters. After 8 wk of endurance training, Western blot analysis of isolated islets did not reveal alterations in CuZnSOD (A) and GPX-1 (B) protein contents. Catalase levels were higher in TRE5× islets (C). ROS, measured by DCFH-DA oxidation, was reduced in islets from the TRE5× rats under basal (2.8 mM) or stimulatory (22 mM) glucose concentrations (D). Results are expressed as means ± SE from two independent experiments (n = 6–10). *P < 0.05, Student’s t-test. ROS production analysis was conducted by comparing both groups within the same glucose incubation condition.
ports the AKT cascade activation hypothesis, leading to higher levels of mTOR and phosphorylation of its substrate, p70s6k, as observed in our TRE5× rats. Indeed it has been demonstrated that activation of mTOR and p70s6k results in increased protein synthesis in β-cells (31).

We also demonstrated herein that endurance exercise training is associated with increased islet levels of ERK1/2 phosphorylation. This data goes in line with others demonstrating increase in islet PDX-1 protein and insulin levels after exercise training protocols (8, 34). Furthermore, the improvement in anti-apoptotic and suppression of pro-apoptotic protein expression might also be related to increased levels of pERK. The activation of ERK1/2 seems to be involved with the increase of Bcl2 mRNA and protein content, as well as the inactivation of caspase-3, mediated by an enhanced CREB expression (11). In islets from TRE5× rats, we also found a significant decrease in the Bax/Bcl-2 ratio, a well known predictor of β-cell survival (20, 25). This observation agrees with the previous description of reduced β-cell apoptosis in islets from trained rats (8) and provides evidence that endurance training improves β-cell survival through the activation of the AKT and ERK1/2 signaling pathways.

In addition, the increased Fyn kinase content observed in TRE5× islets strengthens the evidence for the exercise-induced pancreatic β-cell growth. Fyn kinase is a nonreceptor tyrosine kinase, member of the Src family of tyrosine kinases, involved with several signaling pathways, that was reported to be associated with pancreatic β-cell growth (1). A weakness of the present study was not to investigate β-cell mass and proliferation morphometric parameters, which could reinforce our hypothesis of positive effect for exercise training in β-cell growth and survival. However, we found a marked increase in PCNA protein content in TRE5× islets. PCNA is a useful marker for pancreatic β-cell proliferation (21) and the increase in its content supports an action of exercise on β-cell proliferation in our model that corroborates the activation of growth and survival signaling pathways observed in our results.

In an attempt to establish how skeletal muscle and pancreatic islet adaptations correlate, it was proposed that the increase in pancreatic β-cell mass, induced by exercise training, is mediated by the activation of the insulin/IGF-1-induced IRS-2/P13K pathway, resulting in higher levels of phosphorylated AKT ser473 (pAKT) (8). Our results, however, do not support this proposal, as we were unable to detect differences in tyrosine phosphorylation levels of insulin and IGF-1 receptors between TRE5× and CTL islets. Although we did not evaluate the levels of islet tyrosine phosphorylation in response to insulin stimulation, we are tempted to suggest that activation of β-cell growth and survival pathways is not predominantly triggered by insulin/IGF-1 signaling. Therefore, the crosstalk involved with the modulation between contracting skeletal muscle and pancreatic islets needs to be further investigated.

Regarding the β-cell redox modulation, because ROS have a dual role depending on their intracellular content (45) and considering that pancreatic β-cells are highly sensitive to oxidative stress because low antioxidant enzyme expression and activity (28), we also evaluated their islet content in the present study. At low concentrations, ROS potentiates GSIS; however, at levels of oxidative stress, ROS provoke β-cell dysfunction and apoptosis (44). Oxidative stress-induced apoptosis begins by the increasing peroxidation of anionic phospholipid cardiolipin (35), which stimulates mitochondrial cytochrome c release, caspase-9 activation, and subsequent induction of caspase-3 (15, 30, 41). Endurance training protocols have been associated with increased skeletal muscle antioxidant capacity (37). Improvement of antioxidant enzymatic activity was reported in the endocrine pancreas from diabetic rats after exercise training (10). Accordingly, our results demonstrate that the expression of catalase was markedly increased in islets from healthy endurance-trained rats. Hence, ROS levels were reduced in islets from TRE5× rats, an effect that may be associated with an increased content of islet uncoupling protein 2 (6). Our results corroborate previous findings showing that higher levels of catalase enhance Bcl-2 and decrease Bax content in the RINm5F cell line (32), indicating that endurance training may be a physiological tool to induce these adaptations in pancreatic islets.

Taken together, the reduction in ROS production and the increase in catalase content in TRE5× islets favor pancreatic β-cell redox balance, preventing mitochondrial cytochrome c leakage, caspase-3 activation, and reducing apoptosis.

In summary, the present study suggests that exercise training exerts a positive role on pancreatic islet growth and survival in healthy rats, at least in part, by activation of the AKT and ERK1/2 pathways as well as by enhancing the anti-apoptotic protein, Bcl-2, and decreasing the pro-apoptotic proteins levels, cleaved caspase-3 and Bax, although these effects seem not to be triggered by the canonical insulin and IGF-1 signaling. TRE5× rats also exhibit improved redox balance attributable to reduced ROS production and increased antioxidant capacity. Therefore, our data provide new evidence to ensure exercise training as a potential tool for the prevention of β-cell dysfunction and apoptosis, which in turn would prevent or ameliorate Type 2 diabetes.

GRANTS
Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Instituto Nacional de Ciência e Tecnologia: Obesidade e Diabetes grants supported this study.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
Author contributions: V.C.C., L.R.S., A.R., E.M.C., J.R.B., A.C.B., and C.C.Z. performed experiments; V.C.C., L.R.S., A.R., E.M.C., J.R.B., A.C.B., and C.C.Z. analyzed data; C.C.Z. drafted manuscript.

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES

J Appl Physiol • doi:10.1152/japplphysiol.00318.2011 • www.jappl.org


