Acute exercise restores insulin clearance in diet-induced obese mice

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Abstract

The aim of this study was to investigate the insulin clearance in diet-induced obese (DIO) mice submitted to acute endurance exercise (3 h of treadmill exercise at 60–70% VO₂max). Glucose-stimulated insulin secretion in isolated islets; ipGTT; ipITT; ipPTT; in vivo insulin clearance; protein expression in liver, skeletal muscle, and adipose tissue (insulin degrading enzyme (IDE), insulin receptor subunit β (IRβ), phospho-Akt (p-Akt) and phospho-AMPK (p-AMPK)), and the activity of IDE in the liver and skeletal muscle were accessed. In DIO mice, acute exercise reduced fasting glycemia and insulinemia, improved glucose and insulin tolerance, reduced hepatic glucose production, and increased p-Akt protein levels in liver and skeletal muscle and p-AMPK protein levels in skeletal muscle. In addition, insulin secretion was reduced, whereas insulin clearance and the expression of IDE and IRβ were increased in liver and skeletal muscle. Finally, IDE activity was increased only in skeletal muscle. In conclusion, we propose that the increased insulin clearance and IDE expression and activity, primarily, in skeletal muscle, constitute an additional mechanism, whereby physical exercise reduces insulinemia in DIO mice.

Introduction

Hyperinsulinemia is often associated with obesity and type 2 diabetes mellitus (T2DM) (Kahn et al. 2006). Chronic hyperinsulinemia impairs insulin receptor function, leading to insulin resistance (Kanety et al. 1994). Therefore, interventions to reduce hyperinsulinemia can efficiently treat and/or prevent diseases related to insulin resistance.

Plasma insulin levels are mainly controlled by two physiological processes: insulin secretion and insulin clearance. An imbalance between these two processes can lead to hyperinsulinemia. Although insulin secretion has been extensively studied (Mayhew et al. 1969, Meglasson & Matschinsky 1986, Prentki et al. 2013), less attention has been paid to hyperinsulinemia in obese patients (Erdmann et al. 2008, 2009).

The liver is the main organ responsible for insulin clearance, but other insulin-sensitive tissues, such as skeletal muscle and adipose tissue, seem to play a minor role in this process (Duckworth et al. 1998). Insulin clearance occurs in three steps: (i) binding of insulin to its receptor (IR), (ii) internalization of the insulin–IR complex, and (iii) degradation of the hormone by IDE (Duckworth et al. 1998).

IDE is the most important enzyme for insulin degradation; therefore, deficiency in this enzyme is an important risk factor for T2DM in humans (Groves et al. 2003, Rudovich et al. 2009). IDE knockout mice
display chronic hyperinsulinemia, glucose and insulin intolerance, and reduced expression of IR, suggesting a role for IDE in hyperinsulinemia-induced insulin resistance (Farris et al. 2003, Abdul-Hay et al. 2011). In addition, diet-induced obese (DIO) rodents display hyperinsulinemia as well as glucose and insulin intolerance (Ropelle et al. 2009, Brandimarti et al. 2013). The hyperinsulinemia observed in DIO mice was associated with reduced IDE expression (Brandimarti et al. 2013). These evidences suggest that IDE might be an important therapeutic target for diseases related to hyperinsulinemia and insulin resistance.

Endurance exercise is often indicated to obese and diabetic patients, as it has been shown to improve insulin sensitivity in rodents and humans (Henriksen 2002). This amelioration involves mechanisms such as reduction in the inflammatory state (Handschin & Spiegelman 2008), decreased phosphatase expression and activity (Khadir et al. 2014, Souza Pauli et al. 2014), and increased mitochondrial capacity (Joseph & Hood 2014). Endurance exercise-mediated reduction in insulinemia is explained, at least in part, by a decrease in insulin secretion (Aarnio et al. 2001). However, the role of exercise on insulin clearance and IDE expression and activity in pathological conditions, such as obesity, remains unclear.

Here, we investigated the role of the acute exercise on insulin clearance and the expression and activity of IDE in DIO mice. We showed that mice fed a high-fat diet, submitted to an acute exercise, displayed an increased insulin clearance and IDE activity in the skeletal muscle. These data might explain, at least in part, the reduced insulinemia in physical exercised DIO mice.

**Materials and methods**

**Animals and diet**

Male C57BL/6 mice from the State University of Campinas Facilities were maintained on a 12h light:12h darkness cycle at 20–21°C with controlled humidity during the entire experiment. The 4-week-old mice were distributed into three groups: control mice fed a standard chow diet (CTL), DIO mice fed a high-fat diet, and a group that was also fed a high-fat diet but that were submitted to an acute exercise at the end of the diet period (DIO+EXE). The compositions of diets are shown in Table 1. Mice from all groups were allowed to feed and drink tap water for 12 weeks *ad libitum*. All experimental procedures were approved by the State University of Campinas Ethics Committee.

**Table 1** Composition of diets.

<table>
<thead>
<tr>
<th>Ingredients (g/kg)</th>
<th>Chow diet</th>
<th>High-fat diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>140</td>
<td>140</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>465.7</td>
<td>208.7</td>
</tr>
<tr>
<td>Dextrinized cornstarch</td>
<td>155</td>
<td>100</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Fiber</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Mineral mix (AIN93G*)</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin mix (AIN93G*)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Lard</td>
<td>–</td>
<td>312</td>
</tr>
<tr>
<td>Energy (kcal/kg)</td>
<td>3.88</td>
<td>5.44</td>
</tr>
</tbody>
</table>

*See Reeves et al. (1993) for details.

**Oxygen consumption (VO₂) and exercise protocol**

Before measuring the VO₂max, all mice were submitted to 4 days adaptation to the treadmill and running (8 cm/s for 5 min each day). We measured VO₂max in all mice that were attached to a gas analyzer (Oxybet System, Panlab/ Harvard Apparatus, Barcelona, Spain) and placed on an individual sealed treadmill inclined at 25°. The treadmill exercise included a warm-up period of 8 min at 15 cm/s. Subsequently, we increased the treadmill speed by 10 cm/s each min until the mice were unable to maintain the necessary effort level. We recorded the oxygen uptake data continuously until the mice reached exhaustion at 1 s intervals using Metabolism software (Panlab/Harvard Apparatus, Barcelona, Spain). We defined exhaustion as the point when the mice were unable to keep pace with the set treadmill speed, as described previously (Rezende et al. 2006). After 10 days, the DIO+EXE group was submitted to an acute exercise on a treadmill inclined at 25° for 3 h at 60–70% of VO₂max. All measurements made in these mice, *in vivo* or *ex vivo*, were performed 30 min after the acute exercise. For the other mice (CTL and DIO), all measurements made were performed 30 min after 3 h of nonexercise.

**Intraperitoneal glucose, insulin, and pyruvate tolerance tests (ipGTT, ipITT, and ipPTT)**

For the ipGTT, after a 10-h fast (Supplementary Fig. 1A, see section on supplementary data given at the end of this article), mice received an i.p. administration of 1 g/kg glucose (Labsynth, Sao Paulo, Brazil) dissolved in saline solution (0.9% NaCl wt/vol) and the blood glucose was measured before (0 min) and 15, 30, 60, and 120 min after the administration. For the ipITT, after a 3-h fast (Supplementary Fig. 1B),
mouse received an i.p. administration of 1 U/kg insulin dissolved in saline solution and the blood glucose was measured before (0 min) and 5, 10, 15, 20, 25, 30, and 60 min after the administration. For the ipITT, after a 16-h fast (Supplementary Fig. 1C), mice received an i.p. administration of 1 g/kg sodium pyruvate (Merck) dissolved in saline solution and the blood glucose was measured before (0 min) and 15, 30, and 60 min after the administration, as described previously (Rodgers & Puigserver 2007) with minor modifications (1 g/kg instead of 2 g/kg sodium pyruvate and 16-h instead of 18-h fast). Glucose levels were evaluated in blood samples collected from tail tip. Blood glucose was measured using glucose strips on an Accu-Chek Performa II glucometer (Roche).

Quantification of C-peptide/insulin ratio

During the ipGTt, blood samples were collected from the tail tip, before (0 min) and 15 and 30 min after glucose administration, and placed into microtubes containing anticoagulant heparin. The tubes were centrifuged at 1100 g, 15 min, 4°C, and the supernatant (plasma) was collected and stored at −80°C. Insulin was measured by RIA, as described (Rezende et al. 2007), and C-peptide was evaluated by Rat/Mouse C-Peptide 2 ELISA Kit (Cat. EZRMC2-21K, EMD Millipore) according to the manufacturer’s instructions.

In vivo insulin clearance

During the ipITT, blood samples were collected from the tail before (0 min) and 5, 15, 30, and 60 min after insulin administration to determine the concentrations of insulin (RIA) as described previously (Brandimarti et al. 2013) and the area under the curve was calculated.

Glucose-stimulated insulin secretion in pancreatic islets

Pancreatic islets were isolated as described (Boschero et al. 1995). Five islets per well were preincubated for 1 h in Krebs–Henseleit buffer solution (KHBS) containing 0.5 g/L BSA and 5.6 mmol/L glucose and were equilibrated (pH 7.4) with 95% O2 and 5% CO2 at 37°C. Afterward, the islets were incubated for an additional hour in 500 μL of KHBS containing 5.6, 11.2, or 22.4 mmol/L glucose. The supernatant was collected to evaluate insulin secretion, and the remaining islets were homogenized in an alcohol/acid solution to measure the total insulin content using an RIA.

Tissue samples

Mice were killed in a CO2-saturated atmosphere immediately followed by decapitation. Liver, gastrocnemius muscle, and perigonadal adipose tissue samples from the mice were extracted, snap-frozen in liquid nitrogen, and stored at −80°C for subsequent protein extraction by lystate buffer (10 mmol/L EDTA, 100 mmol/L Tris base, 100 mmol/L sodium pyrophosphate, 100 mmol/L sodium fluoride, 10 mmol/L sodium orthovanadate, 2 mmol/L phenylmethylsulphonyl fluoride, 1% Triton X-100, and 1 μg/mL aprotinin).

Akt phosphorylation assay

An i.p. administration of 100 μL (10 U) of insulin (Humulin, Eli Lilly) was performed in all groups (CTL, DIO, and DIO+EXE). Some of the CTL mice received 100 μL of saline solution, instead of insulin, as described previously (Ribeiro et al. 2012). Ten minutes following the administration of insulin or saline solution, mice were killed, and samples from the liver, gastrocnemius, and perigonadal adipose tissue were extracted, stored, and processed as described above. The p-Akt protein was performed by western blot analysis.

Western blot analysis

The Bradford method was performed to determine the protein concentration, using BSA as a standard. Fifty micrograms of the protein samples were boiled in Laemmli buffer, applied on 10% SDS-PAGE, and transferred to nitrocellulose membranes. These membranes were blocked in 10 mmol/L Tris base, 150 mmol/L NaCl, and 0.25% (vol/vol) of Tween 20 (TBS buffer) containing 5% (wt/vol) BSA for 1 h at room temperature. Membranes were then incubated with primary antibodies (anti-IDE, Abcam ab32216; anti-IRβ, Cell Signaling #3025; anti-p-Akt thr308, Santa Cruz Biotechnology sc-16646-R; anti-p-AMPK thr172, Cell Signaling #2531; anti-glyceroldehyde 3-phosphate dehydrogenase (anti-GAPDH, Sigma G9545) overnight at 4°C. Detection was performed by chemiluminescence (SuperSignal West Femto, Pierce Biotechnology) after incubation with horseradish peroxidase-conjugated secondary antibody, and bands were visualized using an Amersham Imager 600 (GE Healthcare Biosciences). Band intensities were quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA). GAPDH was used as a control loading, and Ponceau S Staining (Sigma-Aldrich) was also employed as a supplemental control (Supplementary Fig. 3).
IDE activity assay

Tissues IDE activity was measured by SensoLyte 520 IDE Activity Assay Kit (Cat. AS-72231, AnaSpec, Fremont, CA, USA) according to the manufacturer’s instructions, using the fluorescence resonance energy transfer (FRET) substrate (Mca-GGFLRHKQEDDnp). When active IDE cleaves the FRET substrate and it results in an increase of 5-carboxyfluorescein (5-FAM) fluorescence, which was measured at an excitation wavelength of 490 nm and an emission wavelength of 520 nm, on a SpectraMax M3 plate reader (Molecular Devices, Ismaning, Germany). The total IDE activity was calculated using the following equation:

\[
\text{IDE activity} = \frac{A_1 - A_0}{T \times V} \times D
\]

Where \(A_1\) is the concentration of 5-FAM at 60 min and \(A_0\) at 0 min; \(T\) is the total time of the assay (60 min); \(V\) is the volume of samples; and \(D\) is the dilution. The kinetic concentration of 5-FAM and the total IDE activity were normalized per \(\mu\)g of total protein that was determined using Bradford reagent (Bio-Rad).

Interleukin-6 measurements

Interleukin (IL)-6 levels in plasma from mice were measured by Mouse IL-6 ELISA Ready-SET-Go (Cat. 88-7064-22, eBioscience, San Diego, CA, USA).

Statistical analysis

Groups were compared by one-way ANOVA using the unpaired Tukey’s post hoc test. Data are presented as the mean ± S.E.M. All data were considered significantly different if \(P\) value was ≤0.05.

Results

Effects of acute exercise on the metabolic parameters of DIO mice

As expected, DIO mice had an increased body and perigonadal fat pad weight as well as higher levels of blood glucose and insulin, compared with CTL mice (Table 1). A single bout of acute exercise did not alter the body, fat, and skeletal muscle masses, but reduced the fasting blood glucose and insulin in DIO mice (Table 2).

Table 2  Metabolic parameters of DIO mice after acute exercise.

<table>
<thead>
<tr>
<th></th>
<th>CTL</th>
<th>DIO</th>
<th>DIO + EXE</th>
</tr>
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<tbody>
<tr>
<td>Body weight (g)</td>
<td>26.94±0.45</td>
<td>36.75±1.46</td>
<td>35.42±1.07</td>
</tr>
<tr>
<td>Fat pad weight (g)</td>
<td>0.23±0.02</td>
<td>1.15±0.16</td>
<td>1.01±0.22</td>
</tr>
<tr>
<td>Skeletal muscle pad weight (g)</td>
<td>0.15±0.005</td>
<td>0.15±0.007</td>
<td>0.16±0.002</td>
</tr>
<tr>
<td>Fasting glycemia (mg/dL)</td>
<td>93.75±4.54</td>
<td>149.80±21.11</td>
<td>85.14±14.34</td>
</tr>
<tr>
<td>Fasting insulinemia (ng/mL)</td>
<td>0.12±0.015</td>
<td>1.06±0.27</td>
<td>0.3193±0.06</td>
</tr>
</tbody>
</table>

\(\text{a}P\leq0.05\ vs\ \text{CTL}; \text{b}P\leq0.05\ vs\ \text{high-fat diet.}

Acute exercise improves glucose homeostasis in DIO mice

In general, hyperglycemia results from impaired glucose and/or insulin tolerance. We performed ipGTT and ipITT in all groups of mice and observed that DIO mice displayed impaired glucose and insulin tolerance (Fig. 1A, B, C and D). As expected, acute exercise improved glucose and insulin tolerance (Fig. 1A, B, C and D). In addition, higher hepatic glucose production (HGP) also contributes to hyperglycemia in obesity. To determine the role of acute exercise on HGP, we performed an ipPTT in all groups. As shown in Fig. 1E, the blood glucose was higher in DIO than CTL mice, and a single bout of acute exercise reestablished the blood glucose during the ipPTT in DIO mice, as evidenced by the glucose area under the curve (AUC) (Fig. 1F).

C-peptide/insulin ratio during ipGTT

After glucose administration (ipGTT), DIO mice displayed higher plasma insulin and C-peptide levels, compared with CTL mice (Fig. 2A, B, C and D). However, the increase in insulin levels was proportionally higher than C-peptide, resulting in an overall reduced C-peptide/insulin ratio in DIO mice (Fig. 2E and F). Acute exercise reduced plasma insulin in DIO + EXE mice, returning to values similar to those of CTL mice (Fig. 2A and B). However, plasma C-peptide levels were not reduced at the same proportion than insulin in DIO + EXE mice (Fig. 2C and D). Therefore, the lower C-peptide/insulin ratio, found in DIO mice, was attenuated by the acute exercise (Fig. 2E and F).
Acute exercise decreased glucose-stimulated insulin secretion, but did not alter pancreatic islet insulin content in DIO mice

Hyperinsulinemia induced by a high-fat diet could be explained by alterations in insulin release and/or insulin removal. First, we evaluated glucose-stimulated insulin secretion (GSIS) in isolated pancreatic islets. The islets from DIO mice released more insulin and had increased total insulin content compared with CTL islets (Fig. 3A and B). Acute exercise did not alter islet insulin content (Fig. 3B); however, it reduced GSIS in isolated islets from DIO mice (Fig. 3A).

Acute exercise normalized in vivo insulin clearance in DIO mice

Because insulin removal is an important component in the regulation of plasma insulin levels, we assessed the in vivo insulin clearance in all groups. As shown in Fig. 4A and B, insulin clearance was lower in DIO mice compared with CTL. Acute exercise normalized the insulinemia in DIO mice, as evidenced by the insulin AUC (Fig. 4B), indicating increased insulin clearance in this group.

Effects of acute exercise on IDE and IRβ protein expression in DIO mice

IDE and IR are important proteins involved in insulin clearance. Therefore, we evaluated the levels of these two proteins in various tissues of all groups. The IDE and IRβ protein levels were reduced in the liver (Fig. 5A and D) and were unchanged in the gastrocnemius (Fig. 5B and E) and in perigonadal adipose tissue from DIO mice compared with CTL (Fig. 5C and F). Interestingly, a single bout of acute exercise increased IDE and IRβ protein levels not only in the liver, but also in the gastrocnemius from DIO mice (Fig. 5D and E). However, the acute exercise did not alter IDE and IRβ protein expression in perigonadal adipose tissue in these obese mice (Fig. 5F).
Effects of acute exercise on p-Akt and p-AMPK protein expression in DIO mice

The beneficial effects of acute exercise on glucose homeostasis are, at least in part, due to increased phosphorylation and activation of Akt (Arias et al. 2007). Thus, we evaluated the protein expression of p-Akt in several tissues of all groups. After insulin administration, p-Akt protein levels were reduced in the liver, but not in gastrocnemius and adipose tissue from DIO mice compared with CTL (Fig. 6A, B and C). Acute exercise increased insulin-stimulated p-Akt protein levels in the liver and gastrocnemius in DIO mice (Fig. 6A and B). We also measured p-AMPK in all groups because this protein is phosphorylated and activated by exercise in an insulin-independent manner. We observed that p-AMPK is reduced in the liver and adipose tissue of DIO mice compared with CTL; acute exercise did not restore p-AMPK levels in these tissues, but significantly increased p-AMPK in the gastrocnemius (Fig. 6D, E and F).

Effects of acute exercise on IDE activity in liver and gastrocnemius from DIO mice

We also measured IDE activity in the liver and gastrocnemius from mice. As expected, mice fed a high-fat diet had a decreased IDE activity in liver, compared with CTL mice (Fig. 7A and B). Despite an increase in IDE expression (Fig. 5A), acute exercise decreased IDE activity in the liver from DIO + EXE mice, compared with CTL and DIO mice (Fig. 7A and B). However, in gastrocnemius, IDE activity was increased in DIO + EXE mice compared with DIO mice (Fig. 7C and D).

Discussion

It has been proposed that exercise reduces insulinemia primarily by decreasing insulin secretion (Aarnio et al. 2001). Here, we provide evidence to suggest that the increase in insulin clearance, induced by an acute exercise, also contributes to the reestablishment
of plasma insulin levels in hyperinsulinemic DIO mice. This increase in insulin clearance is probably due to increased IDE activity in the skeletal muscle, demonstrating the importance of this tissue on insulin removal and degradation, during exercise. Based on these results, we propose that the increased insulin clearance and IDE activity, in skeletal muscle, may be an additional mechanism, whereby acute exercise reduces plasma insulin levels in obese mice.

In DIO rodents, reduced insulinemia following exercise has been reported (Chiarreotto-Ropelle et al. 2013, Souza Pauli et al. 2014). Here, by analyzing insulin secretion in isolated islet from DIO+EXE mice, we confirm that the secretion was reduced (Fig. 3A). This reduced secretion may be due to a neural modulation, for example, activation of the sympathetic nervous system that inhibits insulin secretion (Aarnio et al. 2001) and/or from possible cross-talk between the skeletal muscle and the pancreas (Mizgier et al. 2014).

Although the insulin secretion was reduced in DIO mice after acute exercise, we cannot exclude the participation of insulin clearance on this phenomenon. Indeed, as previously reported (Brandomarti et al. 2013), we found an impairment in the insulin clearance in DIO mice compared with CTL (Fig. 4), which was confirmed by the reduced C-peptide/insulin ratio (Fig. 2E and F). Interestingly, an acute exercise reestablished insulin clearance (Fig. 4) and attenuated the reduced C-peptide/insulin ratio in obese mice (Fig. 2E and F). Plasma C-peptide/insulin ratio during ipGTT is not the gold standard for measured insulin clearance, but it is well accepted as an indicator of this process. C-peptide is co-secreted with insulin (1:1 ratio), but C-peptide is cleared from plasma in a very lower rate.
Therefore, changes in C-peptide/insulin ratio are mainly associated with alterations on insulin clearance.

Previous studies have shown a reduction in the insulin clearance in obese humans (Erdmann et al. 2009, 2012) and in DIO animal models (Strömblad & Björntorp 1986, Kim et al. 2007, Brandimarti et al. 2013), and a downregulation in the expression and/or activity of liver IDE seems to play an important role in the impairment of insulin clearance. In cafeteria diet-fed mice, the reduced insulin clearance was related to a downregulation of hepatic expression of IDE (Brandimarti et al. 2013), a phenomenon also observed in our DIO mice. In addition, we also observed a decreased IDE activity in the liver from our DIO mice that could explain, at least in part, the reduced insulin clearance and hyperinsulinemia, observed in this group. However, different results from ours were previously reported. An increase in the expression and activity of IDE in the liver (Wei et al. 2014) and increased insulin clearance (Castell-Auví et al. 2012), in DIO rodents, were also observed. These differences between studies are most likely due to a large variety of animals (Kim et al. 2007, Castell-Auví et al. 2012, Brandimarti et al. 2013), diet type (Castell-Auví et al. 2012, Wei et al. 2014), or the diet feeding period (Wei et al. 2014).

There are also discrepancies among studies that explored the role of exercise upon insulin clearance. Treadmill endurance exercise training, performed by lean mice, decreased insulin clearance probably by reducing IDE expression in the liver, despite an augmented IDE expression in the skeletal muscle (Costa-Júnior et al. 2015). On the other hand, short-term swimming exercise training increased IDE protein expression in the liver and reduced insulinemia in lean rats (Kim et al. 2011). These data suggest that different results may be obtained by different types of exercise and/or rodent strain. The short-term swimming exercise training also increased IDE expression in the liver of Goto-Kakizaki (GK) rats, a nonobese diabetic rodent, which has an IDE polymorphism that results in 31% less insulin degradation (Fakhrai-Rad et al. 2000). This increased IDE expression in trained GK rat also might be associated with an increased insulin clearance, because a reduced insulinemia was observed without any alteration in the plasma C-peptide levels. However, these effects were
accompanied by reduced body weight, impeding to separate the direct effect of exercise from the effect of weight loss. Here, we show for the first time that a single session of treadmill endurance exercise, a protocol which does not have any impact on the body composition, increased IDE expression and activity in the skeletal muscle from insulin-resistant and prediabetic DIO mice, which was related to an increased insulin clearance.

Although insulin clearance occurs primarily in the liver, the skeletal muscle seems to play an important role on insulin removal and degradation, during the exercise. The increase in insulin clearance by acute exercise is not explained by alteration in the liver IDE. This affirmation is based on the fact that IDE activity in the liver was reduced in DIO+EXE mice, despite an increase in its expression. However, a higher IDE protein expression and activity was observed in gastrocnemius of DIO+EXE mice explaining, at least in part, the increased insulin clearance induced by acute exercise.

Some molecules, induced by exercise, may be involved with the modulation of IDE expression. It was shown that chemical sensitization of insulin action, via pioglitazone, increased hepatic IDE expression through activation of peroxisome proliferator-activated receptor-gamma (PPAR-γ) (Wei et al. 2014). PPAR-γ is an important nuclear transcription factor activated by exercise (Spangenburg et al. 2009), suggesting a probable mechanism, whereby exercise may increase IDE expression. Another possible mechanism for the control of IDE expression is via nuclear respiratory factor-1, which is also activated by endurance exercise (Baar et al. 2002). This factor interacts with the IDE promoter region to increase the transcription of the IDE gene (Zhang et al. 2012). Furthermore, signaling molecules (myokines), secreted by the skeletal muscle during contractions (Pedersen & Febbraio 2012), might induce IDE expression in several tissues during exercise. It was reported that a cytokine member of the IL-6 family, the ciliary neurotrophic factor, can modulate IDE expression in the liver of diabetic mice (Rezende et al. 2012). Here, we show an increase in plasma concentration of IL-6 in DIO+EXE mice (Supplementary Fig. 2) and we thought that this...
myokine may be a candidate for the effects of exercise on IDE expression. Thus, a better understanding of the mechanism of action of this myokine could contribute to the knowledge regarding the control of insulin clearance, during physical activity. Other molecules may modulate the IDE activity (Ryan et al. 1985, Camberos et al. 2001, Hamel et al. 2003, Cordes et al. 2009). In particular, it was described that ATP reduces the ability of IDE to degrade insulin (Camberos et al. 2001). During physical exercise, skeletal muscle ATP content is reduced and this might explain the higher IDE activity in gastrocnemius from DIO mice (Fig. 7D).

Lower IDE expression and/or activity might induce hyperinsulinemia and insulin resistance in obese mice. It was demonstrated that IDE knockout mice, as well as GK rat, display chronic hyperinsulinemia that induces insulin resistance (Farris et al. 2004, Abdul-Hay et al. 2011). It is known that the hyperinsulinemia, present in obese and T2DM patients, may activate negative feedback pathways of insulin signaling such as mTOR/S6K1 (Ye 2007). Therefore, the increased expression and/or activity of IDE might prevent this negative feedback through a reduction in the circulating insulin, thus improving the sensitivity to this hormone (Wei et al. 2014). In fact, the increased IDE activity in the skeletal muscle, of DIO + EXE mice, may be associated with improved insulin sensitivity and glucose tolerance. In addition, previous study has shown that exercise training also improves glucose tolerance and increases IDE expression in the liver of GK rats (Kim et al. 2011). As we mentioned, these rats have a partial loss of function in IDE that affects its capacity to degrade insulin (Fakhrai-Rad et al. 2000). The authors did not measure the activity of the IDE and therefore it is very difficult to know the role of this enzyme in the improvement of glucose tolerance induced by exercise. Thus, this assumption deserves further investigation.

In summary, we demonstrated that after an acute endurance exercise, insulinemia is reestablished due to a reduction in insulin secretion and increased insulin clearance in DIO mice. The increased insulin clearance seems to be due to an increase in the IDE activity, primarily, in the skeletal muscle. Therefore, increased insulin clearance and IDE activity may constitute an additional mechanism, whereby the exercise reduces insulinemia in obese mice, and could be a new target for the prevention and/or treatment of diseases associated with hyperinsulinemia, such as obesity and T2DM.

**Supplementary data**

This is linked to the online version of the paper at http://dx.doi.org/10.1530/JOE-15-0483.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.
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Author contribution statement
M A K, J M C-J, and L F R were responsible for the study design. M A K, J M C-J, S M F, G J S, A O P P, and T R N were responsible for data acquisition. M A K, J M C-J, S M F, G J S, A O P P, and L F R contributed to data analysis and data interpretation. M A K and J M C-J wrote and revised the manuscript. All authors approved the final version.

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