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Strength exercise reduces hepatic pyruvate carboxylase and gluconeogenesis in DIO mice

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Abstract

Obesity is linked to a reduction in the control of hepatic glucose production, which is the primary mechanism related to fasting hyperglycemia and the development of type 2 diabetes mellitus (T2DM). The main system involved in hepatic gluconeogenesis synthesis is controlled by pyruvate carboxylase (PC), which increases in obesity conditions. Recently, we showed that short-term strength training is an important tool against obesity-induced hyperglycemia. As aerobic exercise can reduce the hepatic PC content of obese animals, we hypothesized that strength exercise can also decrease this gluconeogenic enzyme. Therefore, this study investigated whether the metabolic benefits promoted by short-term strength training are related to changes in hepatic PC content. Swiss mice were divided into three groups: lean control (Ctl), obese sedentary (ObS), and obese short-term strength training (STST). The STST protocol was performed through one session/day for 15 days. The obese exercised animals had reduced hyperglycemia and insulin resistance. These results were related to better control of hepatic glucose production and hepatic insulin sensitivity. Our bioinformatics analysis showed that hepatic PC mRNA levels have positive correlations with glucose levels and adiposity, and negative correlations with locomotor activity and muscle mass. We also found that hepatic mRNA levels are related to lipogenic markers in the liver. Finally, we observed that the obese animals had an increased hepatic PC level; however, STST was efficient in reducing its amount. In conclusion, we provide insights into new biomolecular mechanisms by showing how STST is an efficient tool against obesity-related hyperglycemia and T2DM, even without body weight changes.

Key Words

- ▶ strength training
- ▶ pyruvate carboxylase
- ▶ obesity
- ▶ liver
- ▶ T2DM

Journal of Endocrinology
(2020) **247**, 127–138

Introduction

De novo synthesis of glucose from glycan precursors (non-sugars or non-carbohydrates), such as lactate, amino acids, and glycerol, is defined as gluconeogenesis. This synthesis is controlled by the liver and the cortex of the kidneys and is important for the maintenance of glycemic homeostasis, mostly under fasting conditions (Zhang *et al.* 2018). Due to insulin resistance, obese individuals present uncontrolled hepatic gluconeogenesis; thus, any derangement in this endogenous glucose production may contribute to the maintenance of fasting hyperglycemia and, consequently, to the development of type 2 diabetes mellitus (T2DM) (Magnusson *et al.* 1992, Gastaldelli *et al.* 2000, Basu *et al.* 2005). Therefore, it is of paramount importance to understand the mechanisms that underlie hepatic gluconeogenesis for the treatment and prevention of T2DM, providing new perspectives for glycemic control, since most of the current strategies are based on modulating insulin secretion and reducing insulin resistance.

Initially, there are mainly two mechanisms for hepatic gluconeogenesis to occur. First, inside the mitochondria, the main mechanism is controlled by the enzyme pyruvate carboxylase (PC), which forms oxaloacetate (OXA) from the carboxylation of pyruvate and provides substrate for the second step to start. In the cytosol, the gluconeogenic enzymes phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) continue the synthesis of a new glucose molecule from the OXA generated by the PC (Weber & Cantero 1954, McGilvery & Mokrasch 1956, Utter & Keech 1960). In 2001, the enzymes PEPCK and G6Pase were listed as the main ones responsible for increasing hepatic gluconeogenesis (Yoon *et al.* 2001). However, in 2009, it was shown that fasting hyperglycemia was not associated with these enzymes in humans (Samuel *et al.* 2009), highlighting that more attention should be directed to the role of PC in the liver gluconeogenesis process (Jitrapakdee *et al.* 2008). This is because PC, by providing the substrate, is an indispensable enzyme for gluconeogenesis.

Gluconeogenesis, hepatic glucose production (HGP), and hepatic PC might be regulated by an indirect effect of insulin on suppressing white adipose tissue (WAT) lipolysis, which in turn decreases hepatic acetyl-CoA (an allosteric activator of PC), leading to reduced hepatic PC (Perry *et al.* 2015). On the other hand, high fat-fed rats showed insulin resistance in WAT, and increased PC activity; however, when these animals were treated with atglistatin, an inhibitor of adipose triglyceride lipase,

they showed normalized hepatic acetyl-CoA content, PC activity, and HPG (Perry *et al.* 2015). An elegant study showed that diabetic animals have around 2.5 times more PC than control lean animals (Weinberg & Utter 1980). In 2013, Kumashiro and collaborators showed that obese animals had increased hepatic PC levels and, after reducing its synthesis (using a specific antisense oligonucleotide – ASO), it was possible to observe an improvement in insulin sensitivity, a reduction in endogenous glucose production, and, consequently, a reduction in hyperglycemia in obese rodents (Kumashiro *et al.* 2013). Furthermore, the authors showed that the relative hepatic PC levels were directly correlated with serum glucose and glycated hemoglobin levels in humans (Kumashiro *et al.* 2013), unlike the association of PEPCK and G6Pase with serum glucose (Samuel *et al.* 2009). Therefore, as PC has been highlighted as the main molecule involved in hepatic gluconeogenesis, studies aiming to reduce its activity are crucial in trying to control fasting hyperglycemia in diabetic subjects.

In this context, it is known that the regular practice of aerobic exercise is an important non-pharmacological tool that assists in the control of fasting hyperglycemia. Muñoz and colleagues showed that an 8-week aerobic training protocol decreased body weight, fasting hyperglycemia, and hyperinsulinemia in obese mice, as well as improving insulin signaling. Interestingly, these data were accompanied by a reduction in hepatic PC levels (Muñoz *et al.* 2018). On the other hand, the effects of strength physical exercise and body weight changes on PC levels were not addressed. Recently, we verified that a short-term strength exercise protocol reduced HGP, ameliorating glycemic homeostasis without body weight changes (Pereira *et al.* 2019). However, it was not described whether this improvement in glucose metabolism is linked to PC levels. Therefore, the present study evaluated the effects of short-term strength exercise on the hepatic PC levels and glucose homeostasis of diet-induced obese (DIO) mice, with no differences in body adiposity.

Material and methods

Animals and diet

All the animal procedures were previously approved by the Ethics Committee on Animal Use (CEUA) in Biological Sciences (UNICAMP – Campinas – SP, case number 4406-1) and carried out according to the Brazilian legislation on the scientific use of animals (Law No. 11,794, of October 8, 2008). In the present study, 8-week-old male

Swiss mice were used, provided by the Multidisciplinary Center for Biological Research/UNICAMP. The animals arrived at 4 weeks old and were maintained in individual polyethylene cages with an enriched environment as previously described (Pereira *et al.* 2019). Briefly, animals were kept with a ratio of 12 h light:12 h darkness, the temperature was controlled at $22 \pm 2^\circ\text{C}$, and water and food (chow or high-fat diet) were offered ad libitum. More details about animal conditions were published before (Pereira *et al.* 2019).

The first step of this study was to induce obesity through diet, and at this point, the animals were distributed into two groups: control lean group (Ctl), fed a chow diet, and DIO group, fed a high-fat diet (HFD). The diet-induced obesity protocol lasted 14 weeks, and for the second step of this study, the obese group was equally redistributed according to the mice's body weight and fasting glycemia into two groups: (1) obese sedentary (ObS), consisting of obese mice that remained sedentary throughout the experiment, and (2) short-term strength training (STST), consisting of mice that performed the strength training protocol. The HFD was prepared according to the American Institute of Nutrition (AIN-93G) guidelines (Reeves *et al.* 1993), and it was modified to contain 35% fat (4% soy oil and 31% lard) (Oliveira *et al.* 2015).

Short-term strength training protocol

The short-term strength training protocol was already published in detail by our research group (Pereira *et al.* 2019). Briefly, the protocol was performed on a ladder where the mice carried the load apparatus fixed with adhesive tape to their tails. Before the strength training protocol, the animals were adapted to the ladder and load apparatus for 5 consecutive days.

After the adaptation protocol, the mice rested for 48 h. Then, the rodents were subjected to the maximal voluntary carrying capacity (MVCC) test to determine the maximum load with which each animal could climb the entire length of the ladder. The load for the first attempt at the MVCC test was equivalent to 75% of the animals' body weight. In the subsequent attempts, an incremental overload (5 g) was added in each further attempt to climb until the animal could no longer complete the entire course. The mice rested for 5 min in an individual cage between each attempt, and the heaviest overload carried in the last successful attempt was considered the animal's MVCC, and this value was used to prescribe the individual loads in the experiment.

The short-term strength training protocol started 48 h after the MVCC determination. The STST consisted of 20 climbing series with an overload of 70% of the MVCC and with a rest interval of 60–90 s between sets. The mice were exercised for 5 consecutive days a week, followed by 2 days of rest, until they completed 13 sessions of physical exercise. After that, the mice underwent the pyruvate tolerance test or insulin tolerance test, and after 24 h, the animals performed two more training sessions, totaling 15 sessions, and then they were euthanized (Fig. 1).

Insulin tolerance test (ITT) and intraperitoneal pyruvate tolerance test (ipPTT)

Eight hours after the end of the 13th exercise session, some of the animals were subjected to the ITT and the rest were subjected to the ipPTT, after 8 h of fasting. For the ITT, the mice received an intraperitoneal injection of recombinant human insulin (Humulin R) from Eli Lilly at a concentration of 1.5 U/kg of body weight. Blood samples were collected from the tail at 0, 5, 10, 15, 20, 25, and 30 min to determine the glycemic levels using a glucometer (Accu-Chek; Roche Diagnostics). Time 0 was measured before the insulin injection. The rate constant for plasma glucose disappearance (kITT) was calculated using the formula $0.693/\text{biological half-life } (t_{1/2})$. Plasma glucose $t_{1/2}$ was calculated from the slope of the least squares analysis of the serum glucose concentration during the linear decay phase (Bonora *et al.* 1989). For the ipPTT, the animals were subjected to an intraperitoneal injection (i.p.) of pyruvate (2 g/kg of sodium pyruvate) (Azis Científica®, Cotia, SP, Brazil). The blood samples from the animals were drawn from the tail before the pyruvate injection, which was considered as time 0, and 30, 60, 90, and 120 min after the injection to determine the blood glucose concentration. The ITT and ipPTT results were evaluated based on the areas under the serum glucose curves (AUC) during the test using the trapezoidal method (Matthews *et al.* 1990) in Microsoft Excel (2013) (Microsoft Corporation).

Tissue extraction and immunoblotting analysis

The euthanasia was performed 8 h after the 15th exercise session, and during those 8 h the animals were also fasted. Ten minutes before the euthanasia, the animals received either human insulin (8 U/kg body weight Humulin-R; Lilly) or saline via i.p. The animals were anesthetized via i.p. by the injection of chloral hydrate of ketamine (100 mg/kg, Parke-Davis, Ann Arbor, MI, USA) and xylazine (10 mg/kg, Rompun, Bayer),

Figure 1

Experimental design and pipeline schematic regarding bioinformatics analysis. (A) Schematic representation of the experiments. The insulin tolerance test (ITT), intraperitoneal pyruvate tolerance test (ipPTT), and tissue extraction were performed 8 h after the exercise session and 8 h of fasting. (B) Pipeline schematic describing the steps performed.

and after verification and assurance of the lack of corneal reflexes, the liver was collected and rapidly snap-frozen in liquid nitrogen and stored at -80°C until analysis. The epididymal and retroperitoneal adipose tissue (right side) were removed and weighed to measure the fat depots. The liver was homogenized in extraction buffer (1% Triton-X 100, 100 mM Tris (pH 7.4), 100 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM EDTA, 10 mM sodium vanadate, 2 mM PMSF, and 0.1 mg aprotinin/mL) at 4°C with a TissueLyser II (QUIAGEN®) operated at maximum speed for 120 s. The lysates were centrifuged (Eppendorf 5804R) at $12,851\text{ g}$ at 4°C for 15 min to remove insoluble material, and the supernatant was used for the assay. The protein content was determined by the bicinchoninic acid method (Walker, 1994). The samples containing 60 μg of total protein were applied to a polyacrylamide gel for separation by SDS-PAGE and transferred to nitrocellulose membranes. The membranes were blocked with 5% dry milk at room temperature for 1 h and incubated with primary antibodies against the protein of interest. After that, a specific secondary antibody was used. The specific bands were labeled by chemiluminescence, and visualization was performed by the G:BOX photodocumentation system (Syngene). The bands were quantified using the

ImageJ 1.51s software. The primary antibodies used were: anti-Phospho-Akt ser473 (4060), anti-Akt (4685), and anti- β -actin (3700) from Cell Signaling Technology®, and anti-PC (sc271493) from Santa Cruz Biotechnology®. The secondary antibodies used were anti-rabbit and anti-mouse, from Cell Signaling Technology®. More details about the antibodies used can be found in Supplementary Table 1 (see section on [supplementary materials](#) given at the end of this article).

Reverse transcription and quantitative polymerase chain reaction (RTqPCR)

Total RNA was extracted from the tissues using the TRIzol methodology (Invitrogen). The cDNA was synthesized using the High Capacity cDNA RT Kit (Thermo Fisher Scientific). The qPCR was performed using SYBR Green PCR Master Mix (Applied Biosystems). The following primers were evaluated: *PC* – forward: GACGGCGAGGAGATAGTGTC; reverse: CATGGACTGTTCGGAACCTCA and *Actb* – forward: TGTCGAGTCGCGTCCA; reverse: TCATCCATGGCGAACTGGTG (used as the normalizing gene). Samples for this reaction, in triplicate, were made in a 96-well plate (MicroAmp, Applied Biosystems) for amplification and reading in the Step One Plus Real-Time

PCR System (Applied Biosystems). The primers used were designed using the Primer-BLAST software (NCBI).

Bioinformatics analysis

All the data used in this bioinformatics analysis were provided by GeneNetwork (<http://www.genenetwork.org/>) using the EPFL/LISP BXD CD Liver Affy Mouse Gene 1.0 ST (Aug 18) data set. The Pearson's correlation was calculated using Prism (8.0.1) GraphPad Software with phenotypes provided by previous experiments available in GeneNetwork (Philip *et al.* 2010, Andreux *et al.* 2012, Williams *et al.* 2016) and heat maps were made using the Morpheus software (<https://software.broadinstitute.org/morpheus/>). To better describe the bioinformatics analysis, a pipeline illustration was drawn and can be found in Fig. 1B. Briefly, the same data set was used to devise the correlations and the heat map. The PC levels were correlated with phenotypes related to body composition, glucose metabolism, and locomotor activity. Moreover, to make the heat map, all data used were distributed in values between 0 and 1 using the z-score distribution method, and then the data were organized in ascending order and the three lowest and highest values of the first row (locomotor activity or fat mass) were used to create the heat map.

Statistical analysis

The results were shown as the mean $\pm$ S.E.M.. The Shapiro-Wilk test was used to evaluate the Gaussian distribution of the data, and Student's *t*-test was used to compare the two groups with parametric data when necessary. Furthermore, to compare more than two groups, the one-way ANOVA test was performed, followed by Bonferroni's post hoc test. To analyze the points of the ITT and ipPTT, the two-way ANOVA (with repeated measures when appropriate) was used, with Bonferroni's correction for multiple comparisons. The statistical significance level considered was $P < 0.05$. The graphics were created and the statistical analysis performed using Prism (7.00) GraphPad Software.

Results

Short-term strength training reduces blood glucose levels and increases insulin sensitivity in obese mice, even without changes in body adiposity

After obesity induction with a HFD, the animals in the ObS group presented increased body mass, fasting glycemic levels, and body adiposity compared to the Ctl animals (Fig. 2A, B, C and D). However, after 15 strength training

Figure 2

Physiological parameters of experimental groups. (A) Body weight at beginning of HFD, before and after STST protocol. (B and C) Weight of epididymal and retroperitoneal fat, respectively. (D) Fasting glucose levels after 8 h of fasting. (E) Glucose levels during ITT. (F) Area under the curve during ITT. (G) Rate constant for plasma glucose disappearance. In A and E: ^a $P < 0.05$ for Ctl vs ObS; ^b $P < 0.05$ for Ctl vs STST; ^c $P < 0.05$ for ObS vs STST. In B, C, D, F, and G: * $P < 0.05$ vs Ctl; # $P < 0.05$ vs ObS ($n = 6$ per group).

sessions, the animals in the STST group showed identical fasting glycemic levels to the Ctl group, even without changes in body composition (Fig. 2A, B, C and D). After insulin injection, the animals in the ObS group showed higher glycemic levels and impaired kITT compared to the Ctl group, reinforcing the insulin resistance status in these animals (Fig. 2E, F and G). But the STST group showed the same pattern as the lean animals, with no difference in the AUC and kITT (Fig. 2E, F and G). More details about Fig. 2 can be found in Supplementary Figure 2.

Obese trained animals showed increased hepatic insulin sensitivity and better control of HGP compared to sedentary obese animals

In the next step, the effects of obesity and short-term strength training were checked using parameters related to hepatic insulin sensitivity and control of HGP. Eight hours after the 13th strength training session, the animals from the three experimental groups were subjected to the ipPTT, receiving an intraperitoneal injection of pyruvate solution and having their glycemic values checked every

30 min, from 0 to 120 min into the experiment. As shown in Fig. 3A, the animals in the ObS group showed increased glycemic values compared to the animals in the Ctl group throughout the 120 min of the test. However, the animals in the STST group showed a reduction in these values at all points. Consequently, the AUC during the test was higher for the ObS group compared to the other two groups, while the STST group showed no difference from the Ctl group (Fig. 3B).

To assess the hepatic insulin sensitivity, we measured hepatic pAkt^{ser473} levels after an intraperitoneal insulin injection, since Akt activation is a critical step of the insulin-signaling pathway in the liver (Kubota *et al.* 2017). We observed that the ObS group animals had significant hepatic insulin resistance, with reduced levels of pAkt^{ser473} even without changes in total Akt levels (Fig. 3C, D and E). On the other hand, the trained animals presented increased Akt levels and phosphorylation compared to the animals in the ObS group (Fig. 3F, G and H), showing that STST is a new strategy to increase hepatic insulin sensitivity in obese animals. More details about Fig. 3 can be found in Supplementary Figure 3.

Figure 3

ipPTT and hepatic insulin sensitivity. (A) Glucose levels during ipPTT. (B) Area under the curve during ipPTT. (C) Bands of hepatic pAkt^{ser473} and total Akt of Ctl and ObS groups after insulin injection. (D) Quantification of total Akt levels of Ctl and ObS groups. (E) Quantification of hepatic pAkt^{ser473}/Akt of Ctl and ObS groups. (F) Bands of hepatic pAkt^{ser473} and total Akt of ObS and STST groups after insulin injection. (G) Quantification of total Akt levels of ObS and STST groups. (H) Quantification of hepatic pAkt^{ser473}/Akt of ObS and STST groups. Only the bands of the animals stimulated with insulin were quantified. The quantification of total Akt levels were normalized by Ponceau staining, and pAkt^{ser473} was normalized by total Akt quantification. In A: ^a*P* < 0.05 for Ctl vs ObS; ^b*P* < 0.05 for Ctl vs STST; ^c*P* < 0.05 for ObS vs STST. In B, D, E, G, and H: ^{*}*P* < 0.05 vs Ctl; [#]*P* < 0.05 vs ObS (*n* = 6 per group).

Figure 4

Bioinformatics analyses and hepatic PC levels. (A) locomotor activity; (B) muscle mass; (C) blood glucose levels in GTT 60 min after glucose intraperitoneal injection; (D) fat mass; (E) perirenal WAT mass. Blue lines represent negative correlations and red lines represent positive correlations. (F) Heat map showing how the lipogenic genes decrease with more activity stimulus and increase with fat accumulation. (G) Hepatic *PC/Actb* mRNA levels of Ctl and ObS groups ($n = 4-5$ per group). (H) Western blot analysis of hepatic PC and β -actin of Ctl and ObS groups ($n = 6$ per group). (I) Quantification of bands of hepatic PC/ β -actin of Ctl and ObS groups. (J) Hepatic *PC/Actb* mRNA levels of ObS and STST groups ($n = 4-5$ per group). Only the bands of the animals stimulated with insulin were quantified. (K) Western blot analysis of hepatic PC and β -actin of ObS and STST groups ($n = 6$ per group). (L) Quantification of bands of hepatic PC/ β -actin of ObS and STST groups. * $P < 0.05$ vs Ctl; # $P < 0.05$ vs ObS. A full color version of this figure is available at <https://doi.org/10.1530/JOE-20-0193>.

Bioinformatics analysis and effects of obesity and short-term strength training on hepatic PC levels

To check the relevance of PC in overall metabolic control, we first evaluated the correlation between hepatic PC

levels and several phenotypes using a public database with a large panel of isogenic BXD mice strains. We found that the expression of this PC gene was positively correlated with glucose levels, fat mass, and peritoneal WAT, revealing the harmful effects of obesity on PC levels

(Fig. 4A, B, C, D and E). On the other hand, the *PC* expression was negatively correlated with locomotor activity and muscle mass, indicating that physical activity might be an alternative to reduce *PC* levels. Moreover, the first heat map (Fig. 4F) shows that with an increase in locomotor activity, both *PC* expression and lipogenic genes (*Fasn* and *Srebp1*) decrease. In contrast, the opposite effect is shown in the second heat map, where the expression of those genes increases with the highest fat mass levels.

Finally, we investigated whether obesity and STST can change *PC* levels in mice hepatocytes. Initially, we observed that obesity increased the hepatic *PC* protein levels of obese mice (Fig. 4H and I), even without changing the mRNA levels of *PC* (Fig. 4G). In contrast, after 15 sessions of strength training, the trained animals presented reduced *PC* protein levels (Fig. 4K and L), also without changes in *PC* mRNA levels (Fig. 4J). More details about Fig. 4 can be found in Supplementary Figure 4.

Discussion

Insulin resistance is indicated as the main factor responsible for the onset of T2DM and cardiovascular diseases related to hyperglycemia (Patel & Goyal 2019). By 2050, it is estimated that 33% of the US population will be diagnosed with insulin resistance, and it will be one of the main causes of death (Boyle *et al.* 2010). In this context, hepatic insulin resistance plays a fundamental role in hepatic glucose production (HGP), since it is known that the failure of insulin to inhibit HGP is a crucial factor in the perpetuation of hyperglycemia in diabetic patients (Magnusson *et al.* 1992, Petersen & Shulman 2017). Several studies demonstrate that obesity is strongly associated with hepatic insulin resistance, and despite the significant advances of medicine (Tilg *et al.* 2017, Foretz *et al.* 2019), lifestyle changes such as physical training, leading to increased energy expenditure and reduced body adiposity, are still the most promising interventions with the best results (Bacchi *et al.* 2013, Sargeant *et al.* 2018, Pereira *et al.* 2019). However, the biomolecular mechanisms involved in the increased hepatic insulin sensitivity in obese patients provided by physical exercise remains poorly understood. In the present study, we demonstrated that a short period of strength training was able to increase insulin sensitivity and hepatic Akt phosphorylation in response to insulin in obese mice, culminating in the improved control of HGP and normalization of fasting blood glucose levels.

Furthermore, we also showed that the training reversed the increment in hepatic *PC* content induced by obesity, indicating a new mechanism by which strength training can be classified as an essential strategy against hepatic insulin resistance. Another important point of our study is that we did not observe any reduction in fat deposits after the exercise protocol, showing that the aforementioned effects were directly caused by the training, and were not side effects related to the decrease in body adiposity after the long-term exercise protocol.

Previously to short-term strength training, the animals were subjected to DIO. We observed that the sedentary obese animals showed an increase in both body mass and body adiposity, providing an increase in fasting glucose. However, after 15 sessions of strength training, the glycemic values of the trained animals returned to normal levels, showing no difference from the lean animals, thus corroborating data previously found both in rodents subjected to the same conditions (Pereira *et al.* 2019) and in women with liver insulin resistance who performed strength training for 4 months (Honka *et al.* 2016). We also observed that the animals in the ObS group showed reduced insulin sensitivity, with higher glycemic levels and less glucose uptake after intraperitoneal insulin injection, as is well described in the literature (Oliveira *et al.* 2015, Roden & Shulman 2019). Previous studies have already shown that strength training can be an important tool against obesity-associated insulin resistance (Klimcakova *et al.* 2006, Tang *et al.* 2014). After 8 weeks of strength training, obese rats showed both an improvement in insulin action and higher glucose tolerance, assessed by the ipITT and oral glucose tolerance test, respectively (Tang *et al.* 2014). Similar, data were found in a study involving obese male subjects that showed increased insulin sensitivity in the euglycemic-hyperinsulinemic clamp after 3 months of dynamic strength training (Klimcakova *et al.* 2006). In the present study, we showed that a short period of strength training is also able to increase insulin action in obese mice, providing higher glucose uptake and reversing hyperglycemia.

Similarly, the trained animals also showed more activation of Akt and better control of HGP compared to the animals in the ObS group, reversing obesity-induced liver insulin resistance. There are already a large number of studies showing that aerobic training provides several benefits to the liver in obesity conditions (Pereira *et al.* 2017, Sargeant *et al.* 2018). After 8 weeks of swimming, obese mice showed higher phosphorylation of hepatic Akt after insulin injection, also with reduced fasting glycemia and independently of any adiposity reduction

(Marinho *et al.* 2012). The authors also observed improved control of HGP in the trained animals, with lower glycemic values at all points in the ipPTT (Marinho *et al.* 2012). A recent meta-analysis involving 94 participants from six different studies showed that aerobic training can increase insulin action in the liver of overweight subjects (Sargeant *et al.* 2018). Another important finding was that strength training also provided an increase in total Akt levels. It is widely demonstrated that despite the lower activation of Akt in the liver of obese animals with non-alcoholic fatty liver disease (NAFLD), their total protein levels remain unchanged (Xiao *et al.* 2018, Xu *et al.* 2020). Similarly, several studies using obese rodents subjected to aerobic exercise have not observed changes in Akt levels, not even after an acute session (Ropelle *et al.* 2009) such as long-term aerobic training (Oliveira *et al.* 2011, Muñoz *et al.* 2018). However, in the present study, the animals subjected to the short-term strength training protocol showed an increase in total hepatic Akt content, revealing another mechanism by which strength training contributes to an increase in insulin action in NAFLD. However, despite the initial evidence showing that strength training provides significant metabolic improvements in obesity conditions (Klimcakova *et al.* 2006, Pereira *et al.* 2017), the mechanisms related to these benefits remain poorly explored.

Recently, our research group demonstrated that after 15 sessions of strength training, obese mice showed reduced hepatic lipogenesis, fatty acid synthase (FAS), and acetyl-CoA carboxylase (ACC) contents, and increased ACC phosphorylation, suppressing their lipogenic activity (Pereira *et al.* 2019). Thus, the hepatic lipid content was reduced, increasing insulin action and the control of HGP. However, the mechanisms that control hepatic gluconeogenesis in these animals have not been explored. We suggest that short-term strength training can provide better control of HGP, reducing hepatic PC content. As previously discussed, PC is known to be an essential protein in HGP control, mediating the conversion of pyruvate to OXA and initiating gluconeogenesis (Utter & Keech 1960). Kumashiro and colleagues observed that HFD-induced obesity increased hepatic PC content, although their transcripts did not change (Kumashiro *et al.* 2013). However, the use of ASO to reduce PC expression concomitant with a HFD provided better control of HGP in rodents, since they showed reduced glycemic values at all points during the ipPPTT, increased Akt phosphorylation, and lower basal endogenous glucose production, confirming the relevant participation of PC in maintaining healthy blood glucose levels (Kumashiro

et al. 2013). Similarly, our bioinformatics analysis, which includes a large panel of isogenic BXD mice strains, revealed that mice with higher hepatic PC transcription had elevated glycemic values during a glucose tolerance test, thus corroborating our data showing increased PC levels and impaired insulin sensitivity in sedentary obese mice. Furthermore, our bioinformatics analysis also demonstrated a strong negative correlation between PC levels and both locomotor activity and muscle mass, two phenotypes commonly associated with physical exercise (Ross *et al.* 2019), thus reinforcing our hypothesis that the metabolic benefits provided by strength training could be mediated by mechanisms involving PC.

Finally, we assessed whether strength training was able to alter the protein content of hepatic PC. Muñoz and colleagues demonstrated that aerobic exercise can lead to an improvement in the hepatic metabolism of obese rodents by reducing PC levels (Muñoz *et al.* 2018). However, the effects of strength training in this context and without changes in body adipose remain unexplored. In the mitochondria, when there is an excess of energy, high levels of Acetyl-CoA activate PC and stimulate the conversion of pyruvate to OXA to initiate the formation of a new glucose molecules. When there are high levels of mitochondrial NADH, the mitochondrial malate dehydrogenase converts OXA to malate so it can be carried to the cytoplasm. Then, in the reduction process (NAD⁺ to NADH), cytosolic malate dehydrogenase converts malate into OXA again (Jitrapakdee *et al.* 2008). Furthermore, this cytoplasmic OXA is converted to phosphoenolpyruvate by PEPCK and, after passing through a reverse glycolysis process, the G6Pase enzyme is able to remove a phosphate group from the glucose-6-phosphate molecule, facilitating its access to the circulatory system (Weber & Cantero 1954). In the present study, it was possible to observe that only 15 strength exercise sessions were sufficient to reduce the HGP of obese animals. According to the data obtained in this study, we suggest that this improvement in the animals' fasting hyperglycemia was mediated by the reduction in PC levels. Because the exercised obese animals showed a reduction in PC content, consequently, it is estimated that there was a reduction in the intracellular OXA levels and, then, lower substrate availability for gluconeogenesis. Nevertheless, these animals showed an improvement in hepatic insulin sensitivity, which may have favored the attenuation of PEPCK and G6Pase synthesis, thus contributing to a reduction in gluconeogenesis.

Therefore, we provide fresh evidence about a new mechanism by which strength training acts against

Figure 5

Schematic mechanism of the effect of short-term strength training on hepatic PC levels, independent of body weight changes. Obesity increases hepatic PC levels and reduces hepatic insulin action and HGP control, causing hyperglycemia. Short-term strength training reduces hepatic PC levels and counteracts these adverse metabolic effects in obese mice, even without changes in adiposity. This is a new biomolecular mechanism by which short-term strength training is an efficient tool against obesity-related hyperglycemia and T2DM. A full color version of this figure is available at <https://doi.org/10.1530/JOE-20-0193>.

metabolic complications. As already aforementioned, previous studies have shown that strength training can reduce the protein content and the activity of lipogenic proteins in obese mice (Pereira *et al.* 2019), corroborating previous data observed in humans (Bacchi *et al.* 2013), obese rats (dos Santos *et al.* 2019), and ovariectomized rats (Domingos *et al.* 2012) after long-term strength training. With the reduction in liver fat, the insulin action and the control of HPG in these animals were improved (Pereira *et al.* 2019); however, the relationship between NAFLD and liver insulin resistance remains poorly understood. As shown in the heat map in Fig. 4E, mice with reduced levels of PC mRNA also showed a reduction in the expression of lipogenic genes (*Fasn* and *Srebf1*). Therefore, these data allow us to conclude that changes in the hepatic content of PC mediated by obesity may be related to the control of both HGP and fat liver accumulation. On the other hand, even though our bioinformatics analysis showed a positive correlation between hepatic levels of PC mRNA and both total fat mass and perineal white adipose tissue, the STST animals showed reduced PC protein content without reducing adiposity, indicating that strength exercise might change PC metabolism through a non-adiposity related mechanism. Interestingly, we found no changes in PC mRNA levels in the Obs and STST groups, confirming previous data found in human livers (Kumashiro *et al.* 2013) and suggesting that changes in liver PC protein levels caused by obesity and strength training are related to post-transcriptional changes. However, more studies are needed to understand these mechanisms better.

In conclusion, short-term strength training reduced hepatic PC levels in obese mice, increasing insulin action

in the liver, and counteracting HFD-induced insulin resistance and hyperglycemia, as summarized in Fig. 5. Therefore, we suggest a new mechanism by which short-term strength training can be an efficient tool against hepatic metabolic disorders associated with obesity. Importantly, all these results were observed independently of a reduction in body adiposity.

Supplementary materials

This is linked to the online version of the paper at <https://doi.org/10.1530/JOE-20-0193>.

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.

Funding

The present work received financial support from the São Paulo Research Foundation (FAPESP; case numbers 2016/12569-6, 2016/24406-4, and 2015/07199-2).

Author contribution statement

L P M designed the paper. R M P and K C C R wrote the paper and had the overall responsibilities for the experiments and the truth of the results in this study. R M P, K C C R, M R S, G F P, and A P M performed the experiments and data collection. R M P performed the statistical analysis. A P M performed the RTqPCR analysis. G F P performed the bioinformatics analysis. L P M contributed to the financial support. F M S, A S R S, D E C, E R R, J R P, and L P M contributed to the discussion and provided laboratory support. All the authors have read and approved this manuscript.

Acknowledgements

The authors would like to thank FAPESP (2015/07199-2, 2016/12569-6, 2016/24406-4), CNPq, FAEPEX, and CAPES for their financial support.

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Received in final form 31 July 2020

Accepted 14 August 2020

Accepted Manuscript published online 17 August 2020