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Short-term strength training reduces gluconeogenesis and NAFLD in obese mice

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Abstract

Non-alcoholic fatty liver disease (NAFLD) has a positive correlation with obesity, insulin resistance and type 2 diabetes mellitus (T2D). The aerobic training is an important tool in combating NAFLD. However, no studies have demonstrated the molecular effects of short-term strength training on the accumulation of hepatic fat in obese mice. This study aimed to investigate the effects of short-term strength training on the mechanisms of oxidation and lipid synthesis in the liver of obese mice. The short duration protocol was used to avoid changing the amount of adipose tissue. Swiss mice were separated into three groups: lean control (CTL), sedentary obese (OB) and strength training obese (STO). The obese groups were fed a high-fat diet (HFD) and the STO group performed the strength training protocol 1 session/day for 15 days. The short-term strength training reduced hepatic fat accumulation, increasing hepatic insulin sensitivity and controlling hepatic glucose production. The obese animals increased the mRNA of lipogenic genes *Fasn* and *Scd1* and reduced the oxidative genes *Cpt1a* and *Ppara*. On the other hand, the STO group presented the opposite results. Finally, the obese animals presented higher levels of lipogenic proteins (ACC and FAS) and proinflammatory cytokines (TNF- α and IL-1 β), but the short-term strength training was efficient in reducing this condition, regardless of body weight loss. In conclusion, there was a reduction of obesity-related hepatic lipogenesis and inflammation after short-term strength training, independent of weight loss, leading to improvements in hepatic insulin sensitivity and glycemic homeostasis in obese mice. Key points: (1) Short-term strength training (STST) reduced fat accumulation and inflammation in the liver; (2) Hepatic insulin sensitivity and HPG control were increased with STST; (3) The content and activity of ACC and content of FAS were reduced with STST; (4) STST improved hepatic fat accumulation and glycemic homeostasis; (5) STST effects were observed independently of body weight change.

Key Words

- ▶ strength training
- ▶ NAFLD
- ▶ obesity
- ▶ liver
- ▶ insulin sensitivity
- ▶ T2D

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is a pre-condition for most common liver diseases, and it can be developed from hepatic steatosis, non-alcoholic steatohepatitis (NASH), cirrhosis, to hepatocellular carcinoma (HCC) (Tilg *et al.* 2017). Insulin resistance, hyperinsulinemia and excess of circulating lipids may contribute to both increased liver lipid synthesis and hepatic insulin resistance (Marchesini *et al.* 2001, Roden 2006). Considering the liver is one of the primary organs responsible for glycemic control (Magnusson *et al.* 1992, Basu *et al.* 2005), NAFLD development may be linked to obesity and type 2 diabetes (T2D). Therefore, the discovery of strategies for the prevention and treatment of excessive accumulation of lipids in the liver is of great importance.

Although some therapeutic interventions such as the use of peroxisome proliferator-activated receptor (PPAR) γ agonists, vitamin E or liraglutide showed some efficacy in the treatment of NAFLD (Tilg *et al.* 2017), body fat reduction is still considered the primary form of treatment of this condition (Bacchi *et al.* 2013). Thus, aerobic physical exercise is considered an effective strategy in controlling NAFLD due to the reduction of both adiposity and hepatic lipids (Pereira *et al.* 2017, Muñoz *et al.* 2018, Sargeant *et al.* 2018). On the other hand, the effects of strength exercise in the fatty liver accumulation and its consequences have not yet been deeply investigated.

In 2013, the first randomized controlled study showed that diabetic subjects who underwent four months of aerobic or strength training reduced their hepatic fat accumulation (Bacchi *et al.* 2013). In 2015, Shamsoddini *et al.* showed 2 months of aerobic and resistance exercise training were enough to find a reduction in the circulating levels of alanine aminotransferase and aspartate aminotransferase (Shamsoddini *et al.* 2015). Recently, Botezelli *et al.* showed strength training was more efficient than aerobic training in reducing the content and activation of several proteins of pro-inflammatory activity in the liver of animals with hepatic steatosis (Botezelli *et al.* 2016). Moreover, the expression of lipogenic genes such as sterol regulatory element-binding protein-1c (SREBP-1c), acetyl-CoA carboxylase (ACC) and stearoyl CoA desaturase-1 (SCD1) was reduced in the liver of ovariectomized rats after 10 weeks of strength training (Domingos *et al.* 2012). Finally, a recent meta-analysis showed that strength training could also be an important strategy for the reduction of hepatic fat content (Medrano *et al.* 2018).

However, many studies investigating the effects of aerobic or strength training on the reduction of NAFLD

were accompanied by a reduction of body fat. Therefore, it has not yet been possible to determine the direct effects of exercise on NAFLD, independently of weight loss. Thus, this study aimed to investigate the influence of short-term strength training on the hepatic mechanisms of oxidation and lipid synthesis in obesity. The short-term training protocol was prescribed in order to avoid a reduction in the volume of adipose tissue.

Methods

Experimental animals

Male Swiss mice from the Unicamp Central Animal Facility (CEMIB) at 8 weeks old were used in the present study. The animal experiments were carried out respecting the Brazilian legislation on the scientific use of animals (Law No. 11.794, of October 8, 2008). The Ethics Committee on Animal Use (CEUA) of Biological Sciences (UNICAMP-Campinas-SP, number 4406-1) accepted all experiments. Four-week-old animals were maintained individually in polyethylene cages with the enriched environment (PVC pipes were sawed in the middle generating a shelter of 10×10 cm of the base and 5 cm of height) and under controlled conditions of the light–darkness cycle (12/12 h). The light switched on at 06:00 and off at 18:00 h, temperature controlled at 22±2°C, relative humidity maintained at 45–55% and on-site noises below 85 decibels. 100 W lamps were used during the clear period of the day (Phillips soft white light; 2700 K; 565–590 nm; 60 lux). Mice had free access to water and conventional feed.

Initially, the animals were divided into two groups: the control lean group (CTL) that were fed a chow diet and the Obesity group that were fed a high-fat diet (HFD). The high-fat diet was prepared according to the American Institute of Nutrition (AIN-93G) guidelines (Reeves *et al.* 1993), modified to contain 35% of fat (4% soy oil and 31% of lard) (Oliveira *et al.* 2015). After 14 weeks of exposure to HFD, the obese animals were equally distributed according to body weight and fasting glycemia into two groups: 1 – sedentary obese (OB), animals which remained sedentary throughout the experiment; 2 – strength training obese (STO), animals which underwent a short-term strength training.

Description of apparatus for performing the strength training for mice

A ladder of iron feet and stainless steel steps with 10 cm wide, 1.5 cm distance between the steps and an angle of

80 degrees to the ground was used (AVS projects). The ladder is 70 cm high, and the animals performed 12 ± 1 dynamic climbing movements with each of the hind legs, as proposed by [Frajacomo *et al.* \(2015\)](#). At the top of the ladder, there is a 30 cm² chamber that serves as a shelter for the animals during the rest period, between attempts to climb during the adaptation. A loading apparatus (i.e., a conical plastic tube with approximately 7.5 cm of height and 2.5 cm of diameter) was fixed with adhesive tape across the length of the tail of the animal, where the loads were coupled.

Adaptation of animals to the apparatus

The adaptation of animals to the apparatus, proposed by [Cassilhas *et al.*](#), was performed ([Cassilhas *et al.* 2013](#)). The procedures were carried out for five consecutive days. Before the start of the first attempt to climb and with the loading apparatus empty on its tail, the animal was kept inside the chamber at the top of the ladder for 60 s. For the first attempt at climbing, the animal was positioned on the ladder at 15 cm from the entrance of the chamber. For the second attempt, the animal was positioned 25 cm away from the chamber. For the third attempt onward, the animal was positioned at the base of the ladder, 70 cm away from the chamber. When the animal reached the chamber, an interval of 60 s was given. The attempts starting from the ladder base continued until the animal performed three successful attempts without the need for any stimulus.

Maximal voluntary carrying capacity (MVCC) determination

A test to determine the maximal voluntary carrying capacity (MVCC) proposed for rats ([Hornberger & Farrar 2004](#), [Speretta *et al.* 2016](#)) was adapted for mice in the present study. An incremental test to identify the individual maximum load in which the animal can perform one attempt of 70 cm climbing was performed. After the fifth day of adaptation to the apparatus, the animals remained at rest for one more day until the beginning of the test. During the test, the animals started from the base of the stairs, and the attempt was considered successful when the animals climbed a distance of 70 cm. The initial series was performed with an overload of 75% of the animal's body weight, and an incremental load of 5 g was added at each further attempt to climb until the animal could no longer complete the entire course. At each successful attempt, the animal was removed from the ladder and

placed in an individual cage, where it rested for 5 min until the start of the next attempt. The heaviest load in which the animal performed was considered the MVCC and was used for the prescription of the individual loads in the experiment.

Short-term strength training

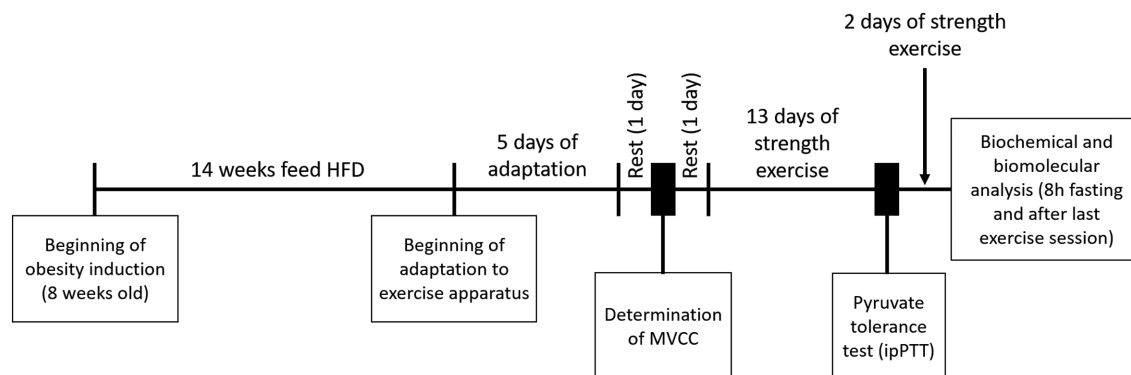
Forty-eight hours after the MVCC determination, the strength training protocol was initiated. The exercise sessions consisted of 20 climbing series with an overload of 70% of the MVCC and with a rest interval of 60–90 s between sets. After completing a series, the animal was removed from the ladder and placed in an individual cage during the resting time of 60 s. The animals were exercised for five consecutive days per week, followed by 2 days of rest, until they completed 13 sessions of physical exercise. Subsequently, mice were submitted to the pyruvate tolerance test. After 24 h, the animals performed two more sessions of exercise, totaling 15 sessions, as summarized in [Fig. 1](#) and more detailed in Supplementary Fig. 1 (see section on [supplementary data](#) given at the end of this article).

Intraperitoneal pyruvate tolerance test (ipPTT)

After an 8-hour fasting period and after the 13th exercise training session, the animals were submitted to an ipPTT (2.0 g of pyruvate/kg body weight) to estimate the hepatic glucose production (HGP). The pyruvate was injected intraperitoneally (i.p.), and the blood samples were collected at 30, 60 and 120 min from the tail for blood glucose determination. Glucose levels were determined using a glucometer (Accu-Chek; Roche Diagnostics). The results were evaluated determining the areas under the serum glucose curves (AUC) during the test by the trapezoidal method ([Matthews *et al.* 1990](#)), using Microsoft Excel.

Tissue extraction and immunoblotting analysis

After the ipPTT, all animals were submitted to other two sessions of strength exercise and were anesthetized i.p. by the injection of chloral hydrate of ketamine (50 mg/kg, Parke-Davis, Ann Arbor, MI, USA) and xylazine (20 mg/kg, Rompun, Bayer, Leverkusen) respecting an 8-h fasting period and 8 h after the last exercise session. After the verification and assurance of the corneal reflexes, mice were injected i.p. with human insulin (8 U/kg body wt Humulin-R; Lilly, Indianapolis, IN, USA) or saline.

**Figure 1**

Experimental design. Summarized representation of the experiments during the short-term strength training protocol. The tests were performed 8 h after the exercise session respecting a period of 8-h fasting. MVCC, maximum voluntary carrying capacity.

After 10 min, the liver was rapidly removed and snap-frozen in liquid nitrogen and stored at -80°C until analysis and adipose tissue (right side) was removed and weighted. The liver was homogenized in an 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 of aprotinin/mL) at 4°C with a TissueLyser II (QIAGEN) operated at maximum speed for 120 s. The lysates were centrifuged (Eppendorf 5804R) at $12.851g$ 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 photo documentation system in G: box (Syngene). The bands were quantified using the software UN-SCAN-IT gel 6.1. The primary antibodies used were anti-Phospho-Akt ser473 (4060), anti-Akt (4685), anti-phospho-Acetyl-CoA Carboxylase ser79 (3661), anti-acetyl-coA carboxylase (3662), anti- α -tubulin (2144) from Cell Signaling Technology, anti-fatty acid synthase (sc-48357) from Santa Cruz Biotechnology and anti-TNF- α (Cat # 506101) and IL-1 β (Cat # 503501) from BioLegend. The secondary antibodies used were anti-rabbit IgG, HRP-linked antibody (7074) and anti-mouse IgG, HRP-linked antibody (7076) from Cell Signaling Technology.

Liver hematoxylin-eosin histology and oil red O staining

Liver samples were collected and fixed in isopentane for cryopreservation at -80°C . The tissue was sliced in a Leica Cryostat cryostat (CM1850) to a thickness of 10 μm and placed on identified adhesion slides. The slices were subjected to the hematoxylin-eosin (H&E) and oil red O staining methods. The slices were stained with hematoxylin for 10 min or with oil red O solution (Sigma-Aldrich) for 25 min, washed and stained with eosin (5 min). The slices stained with oil red O were used to analyses of lipid droplets area and red stained area using ImageJ (Schneider *et al.* 2012) program using the 40 \times image, in agreement with previous studies (Botezelli *et al.* 2016, da Rocha *et al.* 2017, Muñoz *et al.* 2018).

Triglyceride assay

Hepatic triglyceride (TG) content was determined using a commercial kit according to the manufacturer's instructions (Laborlab). TG values were normalized to total liver weight.

Real-time PCR

Total RNA was isolated using the PureZOLTM reagent (BIO-RAD). A 2 μg quantity of total RNA was used as a template for the synthesis of cDNA, according to the instructions of the kit (High Capacity cDNA Reverse Transcription, Applied Biosystems). Real-time PCR reactions were performed using 40 ng cDNA, 0.5 μL primers and 5 μL TaqMan Universal PCR Master Mix (Applied Biosystems). The primers used were *Fasn* (Mm00662319_m1),

Scd1 (Mm00772290_m1), *Cpt1a* (Mm01231183), *Ppara* (Mm00440939_m1) and *Gapdh* (Mm99999915_g1). The relative content of mRNAs was determined after normalization with GAPDH using the $\Delta\Delta\text{CT}$ method.

Bioinformatics analysis

Correlation analyses were performed as previously described (Andreux *et al.* 2012) using hepatic mRNAs (EPFL/LISP BXD HFD Liver Affy Mouse Gene 1.0 ST (Aug18) RMA), proteome (bvEPFL/ETHZ BXD Liver, High Fat Diet (Jun16) Top100 SWATH) and phenotypes (BXD Published Phenotypes) of BXD inbred mice families fed with high-fat diet and are accessible on Genenetwork (<http://www.genenetwork.org>).

Statistical analysis

All results were presented as the mean \pm standard error of the mean (S.E.M.). The Gaussian distribution of the data was assessed using a Kolmogorov–Smirnov test. Data were analyzed using Student's *t*-test to compare two groups or ANOVA to compare 3+ groups, for data with Gaussian distributions in each of the groups. When the data were found to be not following a Gaussian distribution, the Mann–Whitney test was used (if homoscedasticity) or Welch's *t*-test technique was used (if heteroscedasticity) to compare 2 groups and Kruskal–Wallis to compare 3+ groups. If found statistically significant, the one-way

ANOVA test was followed by Bonferroni's *post hoc* test and the Kruskal–Wallis test followed by Dunn's multiple comparisons tests to compare between the different groups. Two-way ANOVA, with Bonferroni's correction for multiple comparisons, when appropriate, was used to analyze each point of ipPTT. The level of statistical significance used was $P < 0.05$. The construction of the graphics and the statistical analysis were performed using GraphPad Prism 7.00.

Results

Short-term strength training reverses fasting hyperglycemia regardless of changes in body mass and adiposity

After 14 weeks of obesity induction, animals from the STO group started the short-term strength training. After 15 strength training sessions, the trained animals showed no statistical significant difference in body mass (Fig. 2A and B) and adiposity (Fig. 2E and F) when compared to the OB group. However, the hyperglycemia induced by obesity was reversed by strength training as observed in the STO animals, equating to that of the CTL group (Fig. 2C). The insulin concentration was higher for both obese groups. However, no statistical significant difference was observed between them (Fig. 2D). More details can be found in Supplementary Fig. 2.

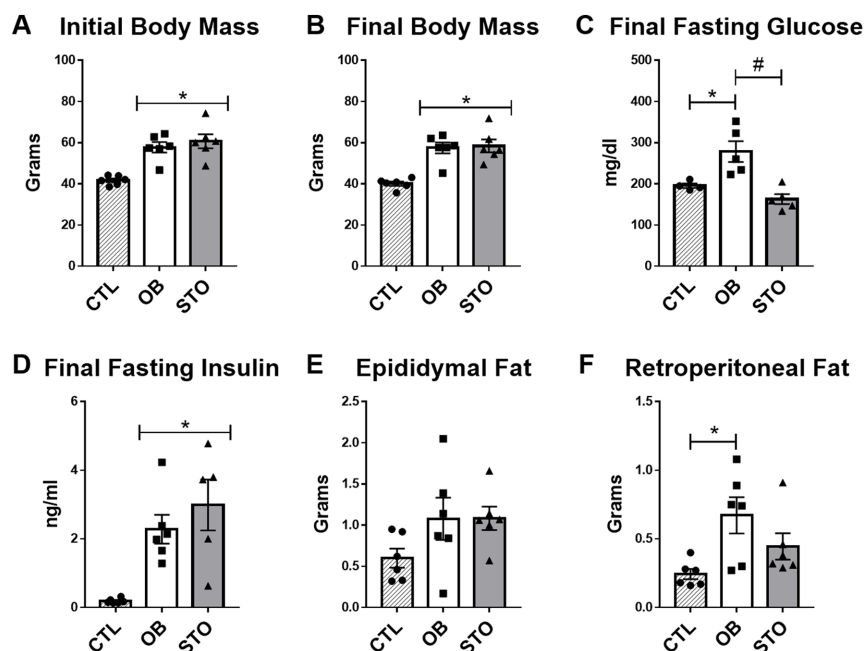


Figure 2

Physiological parameters of CTL, OB and STO groups. (A and B) Body mass of the animals at the beginning and at the end of the experiment. (C and D) Fasting glucose and insulinemia (after 8 h of fasting), respectively. (E and F) Adipose tissue weight of the epididymal and retroperitoneal regions, respectively. * $P < 0.05$ vs CTL; # $P < 0.05$ vs OB ($n = 4-6$ per group). We used Kruskal–Wallis test followed by Dunn's multiple comparisons tests in (B) and (D) and one-way ANOVA followed by Bonferroni's *post hoc* test in A, C, E and F.

Short-term strength training decreases the fatty liver accumulation

The next step was to evaluate the lipid deposits and TG levels in the mice liver after short-term strength training. Initially, by the analyses of hematoxylin and eosin staining, the OB group presented higher lipid stocks when compared to the CTL group (Fig. 3A). However, the STO group presented an expressive reduction in lipid droplets size (Fig. 3A and C). These results were confirmed with oil red O staining for detection of neutral lipids, with a reduction in the stained area (Fig. 3A and D). Similar data were observed for the hepatic TG concentrations, in which the strength training reduced the TG levels in the liver of obese animals (Fig. 3B). More details can be found in Supplementary Fig. 3.

Short-term strength training reduces hepatic glucose production (HGP) and increases hepatic insulin sensitivity

The ipPTT was carried out 8 h after the last strength session for HGP control evaluation. Initially, we observed that the glycemic values of OB group were higher than

CTL group at all times of the test (Fig. 4A). Moreover, obese trained animals presented lower glycemia at all points when compared to sedentary obese animals, with no statistically significant difference when compared to lean animals (Fig. 4A). Thus, the AUC of the OB group was higher during the test, while the STO group presented a reduction in these values compared to OB without significant differences compared to the CTL group (Fig. 4B). Interestingly, the increase in blood glucose during the test remained high until 90 min in the OB group but gradually increased and peaked at 60 min in the STO group and then gradually decreased (Fig. 4A), emphasizing the effectiveness of short-term strength training on glucose homeostasis.

Next, we evaluated the hepatic insulin sensitivity by Akt protein phosphorylation in response to insulin stimulus. The OB group reduced Akt phosphorylation at serine 473 residue, while the STO group reversed this result (Fig. 4C, D and E). More details can be found in Supplementary Fig. 4.

Once observed that HFD induced elevation in glycemia, reduced HGP control and decreased hepatic insulin sensitivity, bioinformatics analyses were performed to evaluate the relationship between fasting glycemia

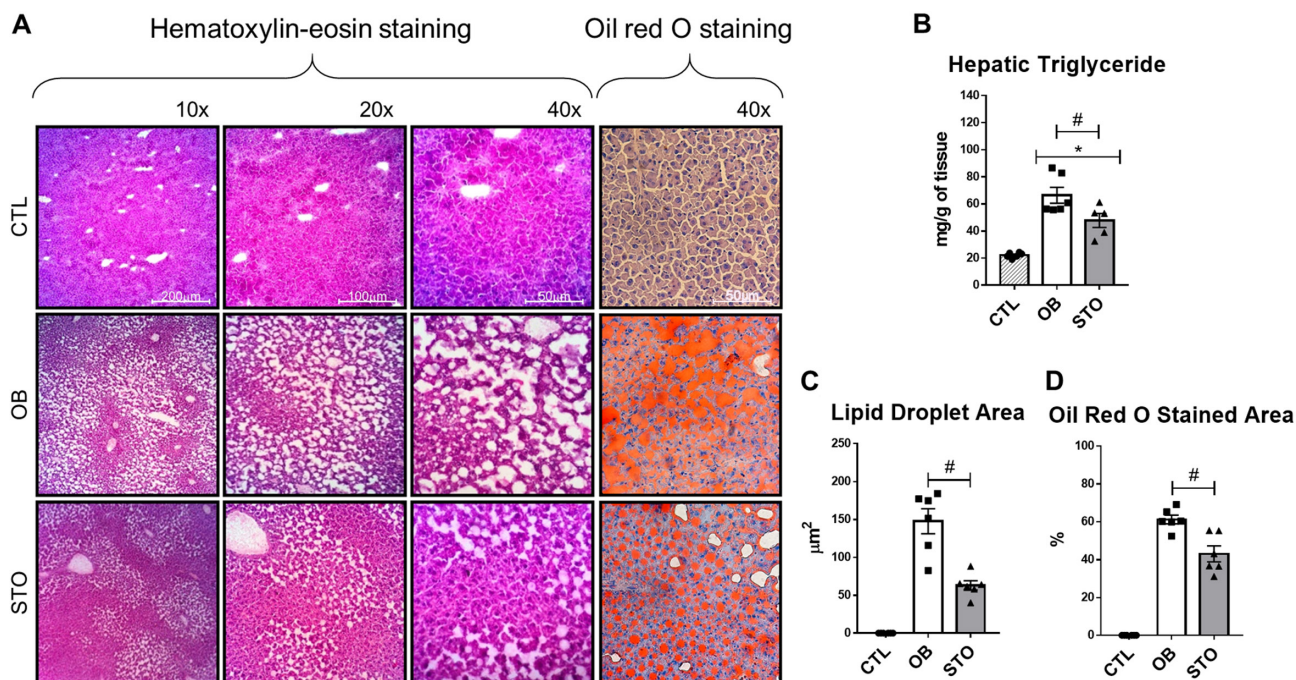
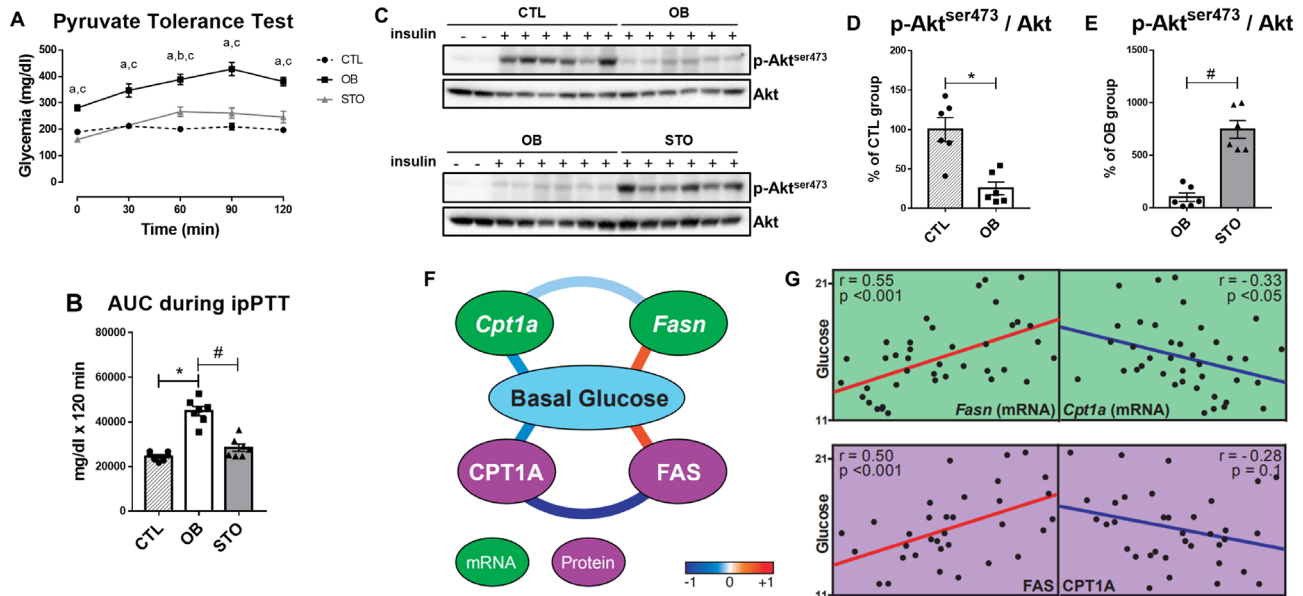


Figure 3

The hepatic fat content of CTL, OB and STO groups. (A) Hematoxylin and eosin staining and oil red O staining of the right lobe from three experimental groups. (B) TG content normalized for liver weight. (C) Liver lipid droplet area of the three groups, from oil red O staining. (D) Oil red O stained area of the three groups. * $P < 0.05$ vs CTL; # $P < 0.05$ vs OB ($n = 5-6$ per group). In (B), we used one-way ANOVA followed by Bonferroni's *post hoc* test. In (C) we used Mann-Whitney test between OB and STO groups. In (D) we used Student's *t*-test between OB and STO groups.

**Figure 4**

Hepatic glucose production during ipPTT and hepatic insulin sensitivity. (A) Glycemic curve during ipPTT. (B) The area under the curve during ipPTT. (C) Bands of p-Akt^{ser473} levels in the liver after insulin stimulus. (D) Quantification of hepatic p-Akt/Akt of CTL and OB groups. (E) Quantification of hepatic p-Akt of OB and STO groups. Only the bands of the animals stimulated with insulin were quantified. (F and G) Interaction network and correlation plots showing correlations between basal glucose levels (fasted state), hepatic mRNAs (shown in green) and proteins (shown in purple) of BXD mice fed with high-fat diet. Positive and negative Pearson's correlation coefficients are indicated by red and blue lines, respectively. Correlation plots of each analysis are also displayed, with Pearson's *r* and *P*-values indicated. In (A): ^a*P* < 0.05 for CT vs OB; ^b*P* < 0.05 for CT vs STO; ^c*P* < 0.05 for OB vs STO. In (B, D and E): ^{*}*P* < 0.05 vs CT; [#]*P* < 0.05 vs OB (*n* = 7 per group in A and B; *n* = 6 per group in D and E). In (A), we used two-way ANOVA test with Bonferroni's correction for multiple comparisons. In (B), we used Kruskal–Wallis test followed by Dunn's multiple comparisons tests. In (D and E), we used Student's *t*-test.

of obese animals with genes and proteins involved in hepatic lipid oxidation and synthesis. For this, families of isogenic mice were used as a reference to integrate transcriptome, proteome and phenotypes (Andreux *et al.* 2012). Fasting glycemia presented a negative correlation with lipid oxidation, which was assessed through analysis of the protein levels and mRNA of CPT1A. Coherently, fasting glycemia presented a positive correlation with hepatic lipogenesis, as assessed by the FAS (fatty acid synthase) content (Fig. 4F and G, Bioinformatics analysis – Supplementary Table 1).

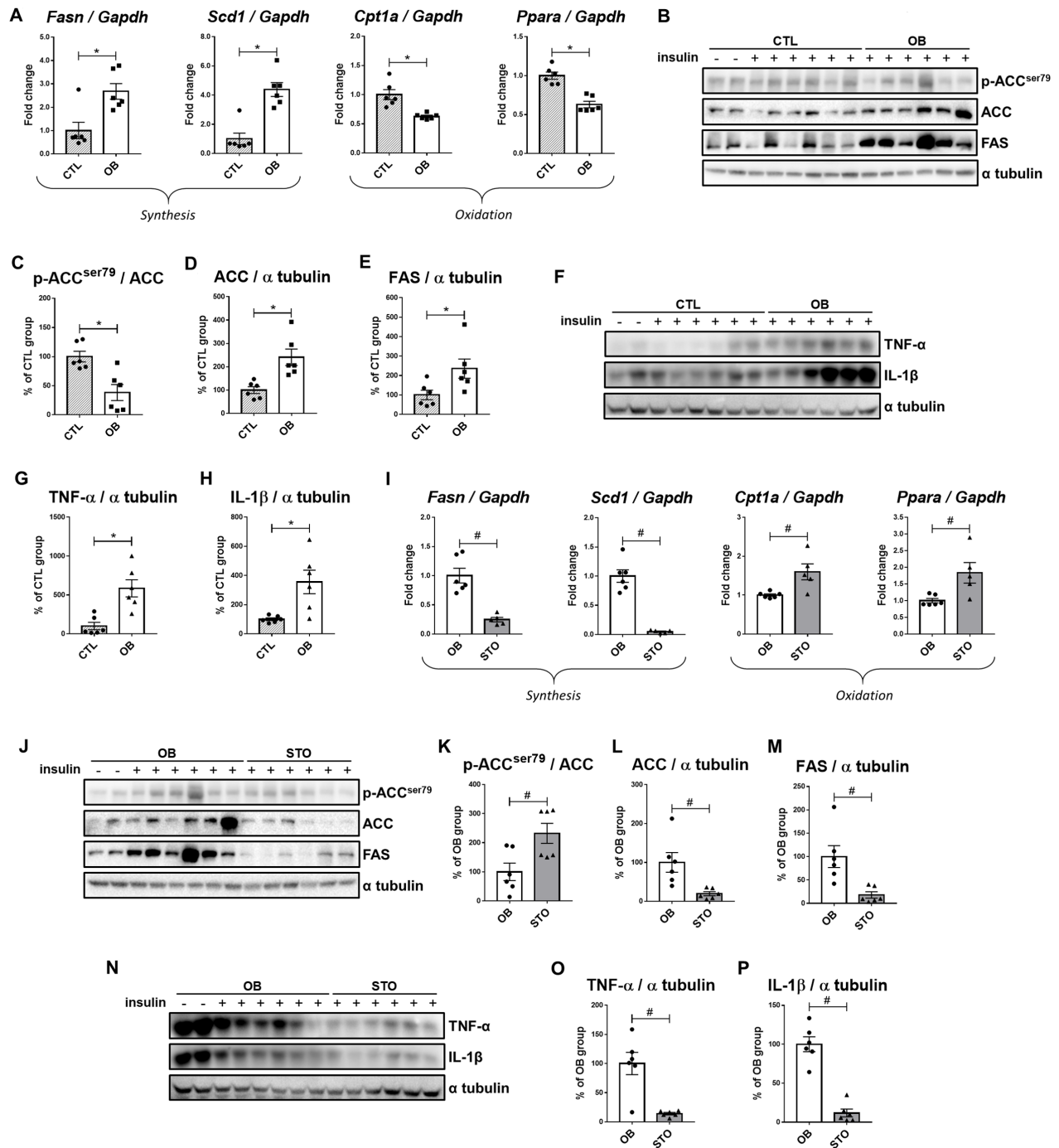
Short-term strength training reduces lipogenesis, increases lipid oxidation and reduces inflammation in the liver

Finally, we investigated whether the short-term strength training led to molecular changes related to the synthesis and oxidation of lipids in the liver of obese mice. Initially, the obesity state increased the mRNA levels of lipogenic genes *Fasn* and *Scd1*, while the mRNA of oxidative genes *Cpt1a* and *Ppara* were reduced (Fig. 5A). Also, an obesity-induced reduction in ACC phosphorylation and an increase in total ACC and FAS content (Fig. 5B, C, D and E)

were observed. However, the STO group reduced *Fasn* and *Scd1* and increased the *Cpt1a* and *Ppara* mRNA levels (Fig. 5I). Also, this group increased ACC phosphorylation and reduced ACC and FAS content (Fig. 5J, K, L and M). We also observed that obesity increased the proinflammatory cytokines tumor necrosis factor alpha (TNF- α) and interleukin 1 beta (IL-1 β) levels (Fig. 5E, G and H) and the short-term strength training reversed this condition (Fig. 5N, O and P). More details can be found in Supplementary Fig. 5.

Discussion

Previous studies indicated hepatic insulin resistance was strongly associated with NAFLD (Petersen & Shulman 2017, Tilg *et al.* 2017). Approximately 70% of T2DM individuals (Leite *et al.* 2009) and 94% of obese diabetics (Silverman *et al.* 1990) are diagnosed with NAFLD. Here, we found that 14 weeks of exposure to HFD was efficient in generating our background of interest (obesity, T2DM and NAFLD). Also, we demonstrated strength training could be an effective alternative for reducing hepatic fat accumulation, reflecting a reduction in fasting glycemia,

**Figure 5**

Parameters of hepatic lipogenesis, fat oxidation, and inflammation profile. (A) Levels of mRNA genes related to lipogenesis (*Fasn* and *Scd1*) and oxidation (*Cpt1a* and *Ppara*) of CTL and OB groups. (B) Bands of the lipogenic proteins in the liver of mice from CTL and OB groups after insulin stimulus. (C) Quantification of hepatic p-ACC^{ser79}/ACC of CTL and OB groups. (D and E) Quantification of hepatic ACC and FAS content, respectively, of CTL and OB groups. (F) Bands of the proinflammatory proteins in the liver of mice from CTL and OB groups after insulin stimulus. (G and H) Quantification of hepatic TNF- α and IL-1 β content, respectively, of CTL and OB groups. (I) Levels of mRNA genes related to lipogenesis (*Fasn* and *Scd1*) and oxidation (*Cpt1a* and *Ppara*) of OB and STO groups. (J) Bands of the lipogenic proteins in the liver of mice from OB and STO groups after insulin stimulus. (K) Quantification of hepatic p-ACC^{ser79}/ACC of OB and STO groups. (L and M) Quantification of hepatic ACC and FAS content, respectively, of OB and STO groups. (N) Bands of the proinflammatory proteins in the liver of mice from OB and STO groups after insulin stimulus. (O and P) Quantification of hepatic TNF- α and IL-1 β content, respectively, of OB and STO groups. Only the bands of the animals stimulated with insulin were quantified. * $P < 0.05$ vs CTL; # $P < 0.05$ vs OB ($n = 5-6$ per group). In (A) (*Fasn*) and I (*Fasn*) we used Welch's *t*-test. In (A) (*Cpt1a*), (C, D, E, H and I) (*Scd1* and *Cpt1a*), (K, L, O and P) we used Student's *t*-test. In the others, we used Mann-Whitney test.

an increase in hepatic insulin sensitivity and, consequently, an improvement in the control of HGP. We observed the reduction of hepatic lipids occurred by the reduction in the protein content of ACC and FAS. All these results were observed independently of the body mass and adiposity reductions.

The literature provides evidence that aerobic training reduces NAFLD (Shen *et al.* 2015, Wu *et al.* 2015). Shen *et al.* (2015) observed reductions in liver fat depots in obese rodents which underwent aerobic exercise for 10 weeks. Also, aerobic training reduced TG and total cholesterol levels in hepatic and serum samples (Wu *et al.* 2015). However, the effects of strength training, with no change in adiposity on these parameters, had not yet been explored in the literature. Herein, the short-term strength training was effective in reducing both the size of fat deposits and TG levels in the liver. These results reinforce strength training may be another strategy for the treatment of NAFLD.

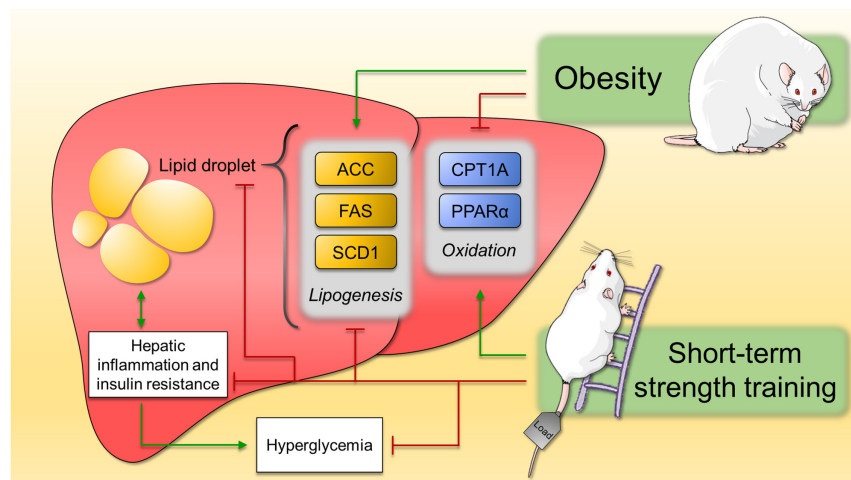
The reduction of hepatic lipogenesis in the STO group was linked to increased hepatic insulin sensitivity and reduced HGP. Previous studies have found lower glycemic values during the ipPTT for obese mice that swam for 8 weeks compared to the sedentary control group (Marinho *et al.* 2012, Souza Pauli *et al.* 2014). Nevertheless, both aerobic training and the acute aerobic session can improve hepatic insulin sensitivity (de Moura *et al.* 2013, Muñoz *et al.* 2018). However, little is known about strength training in this context. Recently, Botezelli *et al.* (2016) observed that long-term strength training (8 weeks) reduced the levels of hepatic steatosis and inflammation in the liver of rodents fed a high fructose diet. These animals showed better glycemic control in glucose tolerance and insulin tests. However, the control of hepatic glucose production during ipPTT and the activation of insulin pathway proteins in the liver of trained animals were not evaluated. In our study, we showed short-duration strength training was efficient in reversing inflammation (TNF- α and IL-1 β) and hepatic insulin resistance provided by HFD-induced obesity, improving the HGP control during ipPTT.

The ACC protein catalyzes the acetyl-CoA carboxylation for the synthesis of malonyl-CoA and FFA, playing a key role in triacylglycerol synthesis. On the other hand, the ACC inhibits the oxidative activity of the cell by reducing carnitine palmitoyltransferase 1 A (CPT1A) (Kobayashi *et al.* 2010, Bechmann *et al.* 2012). Consistently, knockout mice for the isoform 2 of the ACC gene were protected from NAFLD induced by

both diets high in saturated fat as high carbohydrate diets (Abu-Elheiga *et al.* 2012). Nevertheless, *ob/ob* mice with the deletion of ACC, specifically in the liver, did not present an increase in the accumulation of liver fat (Kim *et al.* 2017). Finally, subjects with hepatic steatosis treated with MK-4074 (liver-specific inhibitor of ACC1 and ACC2) had a 36% reduction in lipid content after 4 weeks of intervention (Kim *et al.* 2017). Thus, therapies that reduced the content levels activity of ACC in the liver were the subject of several studies for the prevention and treatment of NAFLD (Nuñez-Durán *et al.* 2018, Romier *et al.* 2018). In this context, aerobic training is known to provide an increase in the phosphorylation of ACC in the liver of obese animals, inhibiting its lipogenic action (Rector *et al.* 2008). Here, we showed for the first time the short-term strength training was able to reduce the activity and amount of ACC, increasing its phosphorylation and reducing its expression and total content.

In turn, the FAS protein is responsible for the synthesis of palmitic acid (Chakravarty *et al.* 2004), and it is identified as another key protein in the development of NAFLD (Dorn *et al.* 2010). Dorn *et al.* observed that *Fasn* expression was increased in mice with fatty liver (Dorn *et al.* 2010). The same study showed the hepatic expression of *Fasn* was positively correlated with NAFLD degree in humans. As ACC, FAS inhibition was also investigated to combat NAFLD. Chronic use of FAS inhibitor reduced lipogenesis in both mice and monkeys (Singh *et al.* 2016). Herein, the short-term strength training reduced the levels of mRNA and FAS content, reinforcing the efficiency of this protocol in downregulating the synthesis of lipids in the liver of obese animals, regardless of body weight change.

Furthermore, besides the reduction of lipid synthesis, we verified an increase in the transcription of genes involved in lipid oxidation *Cpt1a* and *Ppara* in the liver of trained animals. Therefore, the oxidative mechanism may be considered as another positive effect by which strength training acts against obesity-induced NAFLD. Several studies investigated approaches to combat hepatic steatosis with these strategies. Recently, Hsiao *et al.* (2017) observed that the treatment of obese mice with pioglitazone, described as peroxisome proliferator-activated receptor gamma (PPAR γ) agonist, increased hepatic levels of mRNA *Cpt1a* even without a significant increase in *Ppara*. These animals had hepatic steatosis attenuated. Similarly, a period of 8 weeks of aerobic training increased *Ppara* and *Cpt1a*, reducing the levels of hepatic lipids in obese mice (Muñoz *et al.* 2018).

**Figure 6**

Role of short-term strength training on lipid metabolism in the liver, independent of body weight change. Obesity provides insulin resistance, fasting hyperglycemia and increase of lipid synthesis mechanisms in the liver, increasing inflammation and protein level of ACC and FAS, *Fasn* and *Scd1* transcription, and ACC activity. At the same time, this condition reduces the transcription of oxidative genes *Ppara* and *Cpt1a*. On the other hand, short-term strength training provided the opposite effects even without a reduction in body adiposity. Therefore, we demonstrated for the first time that the present short exercise protocol could be an important tool in the combat and/or treatment of NAFLD. A full colour version of this figure is available at <https://doi.org/10.1530/JOE-18-0567>.

Here, we bring the first evidence that the short-term strength training provides an increase in lipid oxidation in the liver, countering obesity-induced NAFLD.

In summary, short-term strength training reduced hepatic fat accumulation and inflammation, increased hepatic insulin sensitivity and provided better control of the HGP, contributing to the reduction of obesity-induced hyperglycemia. These phenomena occurred through the reduction of both activity and content of the proteins involved with lipogenesis in the liver as well as the increase of transcription of genes involved with lipid oxidation, independently of changes in body mass and adiposity (Fig. 6). In summary, we provided new evidence supporting the practice of strength training as a strategy for the prevention and treatment of NAFLD.

Supplementary data

This is linked to the online version of the paper at <https://doi.org/10.1530/JOE-18-0567>.

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

L P M designed the paper. R M P wrote the paper and had the overall responsibilities of the experiments in this study. K C C R, C P A, M R S,

T D P C, R S C and D G M performed the experiments and data collection. C P A performed the histological experiments. M R S performed the PCR analysis. R S G performed the bioinformatics analysis. R A M and R S G performed the statistical analysis. A S R S, D E C, E R R, J R P and L P M contributed to discussion and supported the financial costs. All the authors have read and approved this manuscript.

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