Treating fructose-induced metabolic changes in mice with high-intensity interval training: insights in the liver, white adipose tissue and skeletal muscle

Victor F. Motta, victormott@gmail.com

Thereza L. Bargut, therezabargut@gmail.com

Marcia B. Aguila, marciaguila@gmail.com

Carlos A. Mandarim-de-Lacerda¹, mandarim@uerj.br; mandarim.ca@gmail.com

Laboratory of Morphometry, Metabolism, and Cardiovascular Diseases, Biomedical Center, Institute of Biology, State University of Rio de Janeiro, RJ, Brazil

Running Title: Beneficial effects of HIIT with fructose intake

¹ Corresponding author: Laboratório de Morfometria, Metabolismo e Doença Cardiovascular, Centro Biomédico, Instituto de Biologia, Universidade do Estado do Rio de Janeiro. AV. 28 de Setembro 87 (fds) 20551-030 Rio de Janeiro, RJ, Brasil. Phone: (+55 21) 2868.8316; FAX: 2868.8033. E-mail: mandarim@uerj.br; mandarim.ca@gmail.com; URL: www.lmmc.uerj.br.
Abstract

Fructose-rich caloric sweeteners induce adverse changes in the population metabolism. The study evaluated the effects of high-intensity interval training (HIIT) on a fructose feeding model, focusing on the liver, white adipose tissue (WAT), skeletal muscle, and their interplay. Male C57BL/6 mice were fed for 18 weeks one of the following diets: control (C; 5 % of total energy from fructose), or fructose (F; 55 % of total energy from fructose). In the 10th week, for an additional eight-week period the groups were divided into non-trained (NT) or HIIT groups, totaling four groups: C-NT, C-HIIT, F-NT, and F-HIIT. At the end of the experiment, fructose consumption in the F-NT group led to a high systolic blood pressure, high plasma triglycerides, insulin resistance with glucose intolerance, and lower insulin sensitivity. We also observed liver steatosis, adipocyte hypertrophy, and diminished gene expressions of peroxisome proliferator-activated receptor gamma coactivator 1-alpha and fibronectin type III domain containing 5 (FNDC5; irisin) in this F-NT group. These results were accompanied by decreased gene expressions of nuclear respiratory factor 1 and mitochondrial transcription factor A (markers of mitochondrial biogenesis), and peroxisome proliferator-activated receptor alpha and carnitine palmitoyltransferase 1 (markers of beta-oxidation). HIIT improved all these data in the C-HIIT and F-HIIT groups. In conclusion, in mice fed a fructose diet, HIIT improved body mass, blood pressure, glucose metabolism and plasma triglycerides. Liver, WAT, and skeletal muscle were positively modulated by HIIT, indicating HIIT as a coadjutant treatment for diseases affecting these tissues.

Keywords: fructose; HIIT; liver; white adipose tissue; skeletal muscle
New & Noteworthy

We investigated the effects of HIIT in mice fed a fructose-rich diet and the resulting severe negative effect on the liver, WAT, and skeletal muscle, reducing the expression of FNDC5 (irisin) and PGC1alpha and, consequently, affecting markers of mitochondrial biogenesis and beta-oxidation. Because HIIT may block these adverse effects in all the three tissues, it might be suggested that it functions as a coadjutant treatment in combating the alterations caused by the high-fructose intake.
**Introduction**

Fructose is a monosaccharide present in small quantities in fruits and honey and is used industrially to sweeten drinks and sodas (6). However, the consumption of fructose causes high blood pressure, and worsen insulin resistance, hepatic steatosis, among other comorbidities (14, 27), even without modifying the body adiposity (45).

The exercise training showed beneficial effects in the fructose-induced adverse changes in rodents such as raised blood pressure and alterations in the oxidative stress profile (15), preventing alterations of insulin signaling and endothelial nitric oxide synthesis (46), and inflammation (5). Likewise, walking was efficient in diminishing the postprandial insulin secretion in humans consuming a high-fructose diet (17).

In the fructose-induced adversities, the beneficial effects of exercise training were seen when low (46) or moderate intensity training (15) were applied, but little is known about the consequences of the high-intensity interval training (HIIT, high-intensity exercise periods combined with short rest intervals). The relevance is that HIIT is more and more considered as a possible replacement for traditional aerobic exercise (11), because of the diminished amount of time required for the activity and the combination of aerobic and anaerobic stimuli (18).

Although most studies have been focused on the cardiovascular effects of exercise (15, 46), the liver, white adipose tissue (WAT) and skeletal muscle are also natural targets of exercise training (5, 33). WAT and skeletal muscle have a biological crosstalk that occurs in an endocrine way through the secretion of adipokines (WAT) and myokines, (muscle). Common mediators that are released by both tissues called adipo-myokines have had an increased significance in literature (20), including irisin (derived from the cleavage of FNDC5, Fibronectin type III domain-containing 5 protein that increases energy expenditure) (39).

Recently, the mRNA expression of FNDC5 was identified in the liver (26), as well as the serum irisin concentrations are inversely correlated with liver lipid accumulation (54).
Therefore, it is noteworthy to note that these mediators, including irisin, can be modulated by both obesity and exercise (20).

Taking the data into consideration, we were naturally encouraged to investigate the effects of HIIT in a model fed high-fructose. We hypothesized that HIIT might benefit the liver, WAT, and skeletal muscle against the fructose-induced adverse effects. Therefore, the current study was undertaken to assess the effects of HIIT on the liver, WAT and skeletal muscle in a high-fructose diet model of metabolic alterations.

**Materials and Methods**

**Animals**

We studied 3-months old male C57BL/6 mice, weighed 20-22 g, maintained under controlled conditions (Nexgen system Allentown Inc., PA, USA, 20 ± 2º C and 12 h/12 h dark/light cycle; 5 animals per cage in Xylan bedding), with free access to food and water. All procedures followed the standard guidelines for animal experimentation (NIH publication number 85-23, revised 1996) and were approved by the Ethics Committee on Animal Experimentation of the State University of Rio de Janeiro (protocol number CEUA/012/2015).

**Diets and exercise training**

The diets were manufactured by Pragsolucoes (Jau, SP, Brazil) following the American Institute of Nutrition (AIN 93) recommendations for rodents (37). Diets were offered to the animals during 18 weeks (animals were allocated to the groups at random): a) control diet (C), and b) fructose diet (F). The C diet contained 5 % of total energy from fructose (from sucrose) while the F diet had an addition of fructose (474.3 g/kg diet), totaling 55 % of total energy from fructose (Table 1). In the final eight weeks of the experiment (from week 10 to week 18), mice were submitted to HIIT, and the groups were:
a) Control diet, non-training mice (C-NT group);

b) Control diet, high-intensity interval training mice (C-HIIT group);

c) Fructose diet, NT mice (F-NT group);

d) Fructose diet, HIIT mice (F-HIIT group).

Translation the terminology used for monitoring and promoting physical activity and exercise to animal model is a challenge to be established (36). We observed a coding scheme that classifies specific physical activity by the rate of energy expenditure, specifying activities with its intensity, defined as the ratio of work metabolic rate for a standard resting metabolism (1). In the current study, the animals performed a VO\textsubscript{2} max and a maximum running speed test to estimate the intensity percentage (Oxylet equipment, Panlab/Harvard Apparatus, Barcelona, Spain). Therefore, to a mouse, we considered HIIT the protocol:

a) A test consisting of running (10 cm/s increase in speed every two minutes without inclination), measuring the VO\textsubscript{2} max when the mice reached and sustained the maximum running speed. The test ended when animal stopped responding to the running stimulus and refused to run. In a treadmill (LE8710 model - Panlab/Harvard Apparatus, Barcelona, Spain);

b) In the first four weeks, the animals ran a percentage of 80 % of VO\textsubscript{2} max as stipulated in the preliminary tests. At the beginning of the fifth week, the VO\textsubscript{2} max was measured again and, in the last four weeks of training, the animals ran a percentage of 90 % of VO\textsubscript{2} max. We used a 2:1 ratio scheme (two minutes of exercise per one minute of active recovery);

c) Mice performed HIIT three times per week, 12 min per session (starting at 2:00 p.m.) on Monday, Wednesday, and Friday, throughout the eight weeks.
Food intake, body mass, and oral glucose tolerance test

We measured the food intake (daily), body mass (BM, weekly), and the oral glucose tolerance test (OGTT, 24 h after the last HIIT session) (n=10/group for each analysis). We gave 1 g/kg of 25% glucose in 0.9% NaCl at time 0 (orogastric gavage). Then, glycemia was measured at 15, 30, 60 and 120 min (glucometer Accu-Check, Roche, SP, Brazil) and the curves were analyzed (the "area under the curve," AUC, was determined with GraphPad Prism version 7.03 for Windows, San Diego, CA, USA).

Blood pressure

We used the tail-cuff plethysmography (Letica LE 5001, Harvard/Panlab, Barcelona, Spain) to measure the systolic blood pressure (BP) weekly in conscious animals. The mice (n=10/group) were previously trained by two weeks in constraint conditions to minimize their stress.

Euthanasia

The animals were fasted for six hours and had their blood glucose level measured (glucometer Accu-Check, Roche, SP, Brazil). Then, they were sacrificed (intraperitoneal sodium pentobarbital, 150 mg/kg). Blood samples were taken, and plasma was obtained (centrifugation at 120 G for 15 min at room temperature) and stored at -80°C. We studied the liver, epididymal WAT (dissected in the lower part of the abdomen and connected to the epididymis), and skeletal muscle (right gastrocnemius). Several fragments of each organ were kept in freshly prepared fixative (formaldehyde 4% w/v, 0.1 M phosphate buffer pH 7.2) for light microscopy, or frozen and stored at -80°C.
Plasma analyses

We measured total triglycerides (TG, automatic spectrophotometer and the commercial kit Bioclin System II, Quibasa, Belo Horizonte, MG, Brazil), and insulin levels (Single Plex kit, EZRMI-13K Rat/Mouse Insulin ELISA, Millipore Merck, Darmstadt, Germany) (n=10/group for each analysis). Further, we determined the homeostasis model assessment index (HOMA-IR) [(insulin x glucose)/22.5] (32), and the quantitative insulin sensitivity check index (QUICKI) [1 / log (fasting insulin µU / mL) + log (fasting glucose mg / dL)] (24).

Liver

Liver TG was measured as described (8). Briefly, frozen liver samples (approximately 50 mg) in 1 mL of isopropanol were placed in an ultrasonic processor. The homogenate was centrifuged at 120 G, and 5 mL of the supernatant was measured by an automatic analyzer (K55, Bioclin System II; Quibasa) using a kit for measuring TG (n=5/group).

Formalin-fixed liver specimens (n=5/group) were embedded in Paraplast Plus (Sigma-Aldrich, St. Louis, MO, USA), and 5-μm-thick sections were stained with hematoxylin and eosin. The digital images were acquired blindly and randomly (Nikon model 80i, digital camera DS-Ri1, Nikon Instruments, Inc., NY, USA). The stereological estimation of liver steatosis was determined by point counting, as mentioned previously (30).

Adipocyte size

Formalin-fixed WAT fragments (n=10/group) were embedded in Paraplast Plus (Sigma-Aldrich, St. Louis, MO, USA), and 5-μm-thick sections were stained with hematoxylin and eosin. The digital images (n=10/mice) were acquired blindly and randomly (Nikon model 80i, digital camera DS-Ri1, Nikon Instruments, Inc., NY, USA). Five sections per animal were used to estimate the mean cross-sectional area of adipocyte (the ratio between the volume
density of adipocytes, \( V_v \) [adipocyte], and twice the numerical density per area of adipocytes, \( Q_A \) [adipocyte]). We determined \( V_v \) [adipocyte] by point counting on a test system of 36 points, and \( Q_A \) [adipocyte] as the ratio between the number of adipocytes and the frame area (31).

**RT-qPCR**

We extracted total RNA with Trizol (Invitrogen, CA, USA) in 30 mg of the liver, WAT and gastrocnemius muscle (quantified with Nanovue spectroscopy, GE Healthcare Biosciences, Pittsburgh, USA) (n=6/group, the three tissues and all primers used). Then, 1 µg of RNA was treated with DNase I (Invitrogen). First-strand cDNA was synthesized using Oligo (dT) primers for mRNA and Superscript III reverse transcriptase (both Invitrogen). Quantitative real-time PCR was performed using a CFX96 thermocycler (Bio-Rad, Hercules, CA, USA) and SYBR Green mix (Invitrogen). Primers are demonstrated in Table 2, and their design followed the MIQE guidelines (7). We evaluated the gene expression of the following markers:

a) Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1alpha),
b) Peroxisome proliferator-activated receptor gamma coactivator 1-beta (PGC1beta),
c) Fibronectin type III domain containing 5 (FNDC5),
d) Nuclear respiratory factor 1 (NRF1),
e) Mitochondrial transcription factor A (TFAM),
f) Peroxisome proliferator-activated receptor alpha (PPARalpha),
g) Carnitine palmitoyltransferase 1 (CPT1).

We standardized the expression of the selected genes with the endogenous expressions of beta-actin and TATA-box protein (TBP).
Data analysis

We tested the data for normality (Shapiro–Wilk test) and equality of groups (Brown–Forsythe test) and then reported the data as mean and standard deviation of the mean. We examined the differences among groups with the one-way analysis of variance (ANOVA) and the posthoc test of Holm-Sidak. Also, we reviewed the effects of the diet and HIIT as independent factors and the possible interactions between them with the two-way ANOVA (GraphPad Prism version 7.03 for Windows, San Diego, California, USA). We considered statistically significant a $P$-value <0.05.

Results

Food intake and body mass

Food intake was not different in the groups throughout the experiment (Table 3). In the first ten weeks (before HIIT), BM showed no difference. After HIIT, BM was 12 % smaller in the C-HIIT group compared to the C group ($P < 0.001$), and 7 % less in the F-HIIT group compared to the F-NT group ($P = 0.001$) (Fig. 1).

Blood pressure

After the first ten weeks of the diet, systolic BP was 4 % higher in the F group than in the C group ($P = 0.0017$). After HIIT, systolic BP decreased 6 % in the C-HIIT compared to the C-NT group ($P < 0.001$) and decreased 9 % in the F-HIIT group compared to the F-NT group ($P < 0.001$) (Fig. 2).
Oral glucose tolerance test

There was a reduction of 19% in the OGTT of the C-HIIT group compared to the C-NT group ($P < 0.001$), and a decrease of 16% in the OGTT of the F-HIIT group compared to the F-NT group ($P < 0.001$). We showed the blood glucose evolution (Fig. 3A) and the area under the curve (Fig. 3B).

Plasma analyses (Table 3)

Fasting glucose: The F-NT group had a value 23% higher than the C-NT group ($P < 0.001$). The fasting glucose was 29% lower in the C-HIIT group than the C-NT group ($P < 0.001$). Similarly, the fasting glucose was 25% lower in the F-HIIT group than in the F-NT group ($P < 0.001$).

Fasting insulin: The F-NT group showed a value 24% higher than the C-NT group ($P < 0.001$). The fasting insulin was 55% lower in the C-HIIT group than in the C-NT group ($P = 0.0078$). The fasting insulin was 60% lower in the F-HIIT group than in the F-NT group ($P < 0.001$).

HOMA-IR: The F-NT group showed HOMA-IR 48% greater than the C-NT group ($P = 0.0168$). The HOMA-IR was 70% smaller in the C-HIIT group than the C-NT group ($P = 0.0004$). The HOMA-IR was 70% lower in the F-HIIT group than in the F-NT group ($P < 0.001$).

QUICKI: The F-NT group had a QUICKI index 14% lower than the C-NT group ($P < 0.001$). The QUICKI index was 36% higher in the C-HIIT group than in the C-NT group ($P < 0.001$). Similarly, the QUICKI index was 21% greater in the F-HIIT group than in the F-NT group ($P < 0.001$).

Triglycerides: The F-NT group showed a concentration of triglycerides 14% higher than the C-NT group ($P = 0.0469$). TG was 21% lower in the C-HIIT group compared to the C-NT
group \((P = 0.0016)\). TG was reduced in the F-HIIT group compared to the F-NT group (-19 \%; \(P = 0.0012\)).

Liver

Liver TG was increased in the F-NT group in comparison with the C-NT group (+17 \%; \(P = 0.0006\)). HIIT could decrease liver TG in the C-HIIT group compared to the C-NT group (-34 \%; \(P < 0.001\), and in the F-HIIT group in comparison with the F-NT group (-33 \%; \(P < 0.001\)) (Fig. 4A).

There was an increased lipid deposition in the F-NT group in comparison with the C-NT group (+498 \%; \(P < 0.001\)). Besides that, both HIIT groups showed reduced steatosis as compared with NT groups (-57 \%, for the C-HIIT group in comparison with the C-NT group; and -53 \%, for the F-HIIT group compared with the F-NT group; \(P < 0.001\)). The F-HIIT group also demonstrated elevated hepatic steatosis when compared to the C-HIIT group (+557 \%; \(P < 0.001\)) (Fig. 4B).

Adipocyte size

The adipocyte cross-sectional area was 24 \% greater in the F-NT group than in the C-NT group \((P < 0.001)\), but no difference was observed between the groups C-HIIT and C-NT. The adipocyte cross-sectional area was 17 \% smaller in F-HIIT group compared to the F-NT group \((P < 0.001)\) (Fig. 5).

PGC1alpha, PGC1beta, and FNDC5 gene expressions

Liver: PCG-1-alpha was 272 \% elevated in the C-HIIT group in comparison with the C-NT group \((P = 0.0002)\). On the other hand, in the F-HIIT group, PGC-1alpha was similar to the F-NT group and reduced in comparison with the C-HIIT group (-84 \%; \(P < 0.001\)) (Fig. 6A).
PGC-1beta was increased in the F-NT group in comparison to the C-NT group (+38 %, \( P = 0.0318 \)). It was reduced in the F-HIIT group when compared to the C-HIIT (-49 %, \( P = 0.0169 \)) and the F-NT (-66 %, \( P < 0.0001 \)) groups (Fig. 6B).

FNDC5 was 147 % higher in the C-HIIT group than in the C-NT group (\( P = 0.0023 \)), and it was 58 % lower in the F-HIIT group than in the C-HIIT group (\( P = 0.0024 \)) (Fig. 6E).

**WAT:** PGC-1alpha was 94 % lower in the F-NT group than in the C-NT group (\( P = 0.0024 \)). It was 463 % higher in the C-HIIT group than in the C-NT group (\( P = 0.0002 \)), and 6712 % higher in the F-HIIT group than in the F-NT group (\( P = 0.0013 \)) (Fig. 6C).

FNDC5 was 67 % lower in the F-NT group than in the C-NT group (\( P = 0.0052 \)), 206 % higher in the C-HIIT group than in the C-NT group (\( P < 0.001 \)), and 713 % greater in the F-HIIT group than in the F-NT group (\( P < 0.001 \)) (Fig. 6F).

**Gastrocnemius muscle:** PGC-1alpha was 85 % lower in the F-NT group than in the C-NT group (\( P = 0.0081 \)), 783 % higher in the C-HIIT than in the C-NT group (\( P < 0.001 \)), and 4759 % higher in the F-HIIT group than in the F-NT group (\( P < 0.001 \)) (Fig. 6D).

FNDC5 was 82 % lower in the F-NT group compared to the C-NT group (\( P = 0.0053 \)). However, FNDC5 was 130 % higher in the C-HIIT group than in the C-NT group (\( P = 0.0002 \)), and 818 % greater in the F-HIIT group than in the F-NT group (\( P < 0.001 \)) (Fig. 6G).

**Mitochondrial biogenesis**

**Liver:** NRF1 was reduced in the F-NT group than in the C-NT group (-70 %; \( P = 0.0067 \)), but NRF1 was elevated in the F-HIIT group than in the F-NT group (+255 %; \( P = 0.0038 \)) (Fig. 7A). TFAM was reduced in the F-NT group compared with the C-NT group (-86 %; \( P = 0.0002 \)). HIIT increased TFAM (+86 %; \( P = 0.0002 \) in the C-HIIT group than in the C-NT group; +213 %; \( P = 0.0157 \) in the F-HIIT group than in the F-NT group) (Fig. 7D).
WAT: NRF1 and TFAM were lower in the F-NT group than in the C-NT group (NRF1: -51 %, 
\( P = 0.0045 \); TFAM: -76 %, \( P = 0.0428 \)). In the F-HIIT group, NRF1 was 315 % higher (\( P < 
0.0001 \)), and TFAM was 832 % higher (\( P < 0.0001 \)) than in the F-NT group (Fig. 7B and 7E).

Gastrocnemius muscle: NRF1 and TFAM were lower in the F-NT group than in the C-NT 
group (NRF1: -93 %, \( P = 0.0053 \); TFAM: -89 %, \( P = 0.0002 \)). The NRF1 and TFAM were 
augmented in the F-HIIT group (NRF1: +1291 %, \( P = 0.0104 \); TFAM: +675 %, \( P = 0.0021 \)) 
than in the F-NT group (Figs. 7C and 7F).

Beta-oxidation

Liver: Both PPARalpha and CPT1 were reduced in the F-NT group compared to the C-NT 
group (PPARalpha: -76 %, \( P = 0.0005 \); CPT1: -60 %, \( P = 0.0019 \)). In contrast, these 
expressions were elevated in the C-HIIT group than in the C-NT group (PPARalpha: +67 %, 
\( P = 0.0025 \); CPT1: +280 %, \( P < 0.0001 \)), and in the F-HIIT group compared to the F-NT 
group (PPARalpha: +139 %, \( P = 0.0374 \); CPT1: +99 %, \( P = 0.0177 \)) (Figs. 8A and 8D).

WAT: PPARalpha and CPT1 were reduced in the F-NT group compared to the C-NT 
group (PPARalpha: -97 %, \( P = 0.0173 \); CPT1: -95 %, \( P = 0.0075 \)). PPARalpha and CPT1 were 
increased in the C-HIIT group than in the C-NT group (PPARalpha: +196 %, \( P = 0.0006 \); 
CPT1: +311 %, \( P < 0.0001 \)). PPARalpha and CPT1 were higher in the F-HIIT group than in 
the F-NT group (PPARalpha: +4777 %, \( P = 0.0017 \); CPT1: +3350 %, \( P = 0.0002 \)) (Figs. 8B 
and 8E).

Gastrocnemius muscle: PPARalpha and CPT1 were lower in the F-NT group compared to 
the C-NT group (PPARalpha: -58 %, \( P = 0.0121 \); CPT1: -76 %, \( P = 0.0236 \)), and were higher 
in the C-HIIT group than in the C-NT group (PPARalpha: +203 %, \( P < 0.0001 \); CPT1: +115 
\( P = 0.0021 \)). PPARalpha and CPT1 were augmented in the F-HIIT group compared to the 
F-NT group (PPARalpha: +631 %, \( P < 0.0001 \); CPT-1: +500 %, \( P = 0.0006 \)) (Figs. 8C and 
8F).
Two-way ANOVA

Table 4 summarizes the findings of the two-way ANOVA for diet vs. HIIT:

- **Biometry:** there was interaction in body mass and systolic blood pressure.
- **Biochemistry:** there was no significant interaction.
- **Muscle:** interaction occurred in genes related to mitochondrial biogenesis and PPARalpha.
- **eWAT:** interaction occurred in genes involved in mitochondrial biogenesis and CPT1.
- **Liver:** the interactions were in the genes of mitochondrial biogenesis, CPT1, PGC1alpha, PGC1beta, FNDC5, and in hepatic steatosis.

Discussion

The study of chronic consumption of fructose is relevant today because fructose-containing products may cause severe adverse metabolic effects (49). In the model we analyzed, we found high BP, greater insulin resistance, elevated plasma TG, hepatic steatosis, and hypertrophied adipocytes in mice fed the fructose diet, as reported (9, 30). Furthermore, we observed the adverse effects of fructose associated with altered expressions in PGC1alpha, PGC1beta, FNDC5, and gene expressions of beta-oxidation and mitochondrial biogenesis markers. The effects of fructose on these markers are more studied in the liver, where fructose reduced PGC1alpha in association with an increased TG accumulation (23). In accordance, fructose also showed an inhibitory effect on PPARalpha and CPT1, corroborating for the lipid accumulation (34) as well as the knockdown of PGC1beta was shown to prevent fructose-induced hypertriglyceridemia (35).
The effects of fructose in WAT are related with the adipocyte’s enlargement, a known effect of fructose on the adipocyte (30), and hypertrophied adipocytes have decreased beta-oxidation and mitochondrial biogenesis (2). In fructose-fed rats, the beta-oxidation was dropped in the skeletal muscle (52). However, regarding mitochondrial biogenesis, the effects of fructose on skeletal muscle are still a matter of debate. Fructose-fed rats showed increased mitochondrial biogenesis in the skeletal muscle (13), but the exposure of L6 myotubes to fructose led to a mitochondrial injury (21).

In mice fed a fructose-rich diet, the significant benefits of HIIT were confirmed in diminishing BM, BP, hepatic steatosis, and the adipocyte size (in agreement with the literature in different animal models (12, 33). HIIT also improves glucose tolerance/insulin resistance and plasma TG, as reported elsewhere in other models (30, 44). Also, HIIT increased PGC1alpha and FNDC5 expression, leading to augmented markers of mitochondrial biogenesis and beta-oxidation in the liver, WAT, and skeletal muscle. Therefore, we can consider the beneficial effects of HIIT parallel to those of a drug treatment (metformin), which even continuing the fructose intake, reversed, at least partially, the liver injury and prevented NAFLD progression to more severe stages of liver disease (22).

Overall, HIIT also benefits the C animals, in agreement with previous reports demonstrating an amelioration of body mass, glucose tolerance, and hepatic lipid accumulation, associated with increased beta-oxidation in the liver and skeletal muscle in the C group (33). Similarly, healthy women submitted to HIIT protocol showed increased whole-body and skeletal muscle fat oxidation (48).

PGC1alpha is a molecule that regulates a range of gene expressions (42). Exercise training stimulates the production of PGC1alpha, especially in skeletal muscle, which is significant because PGC1alpha is secreted into the bloodstream and reach other tissues like adipose tissue (42). WAT itself can express PGC1alpha (47), as we have shown here in WAT and skeletal muscle of fructose-fed mice submitted to HIIT. Likewise, in rodents (47), and in humans, endurance training promotes increased expression of PGC1alpha in skeletal
muscle (41). In the liver of endurance trained rats (16), or voluntary physical activity (43),
PGC1alpha mRNA expression and protein expression were higher than in the sedentary
animals.
Exercise training and PGC1alpha stimulate the production of FNDC5 in skeletal muscle,
which is cleaved and secreted as irisin, with effects on the muscle itself and in WAT (4, 39).
FNDC5 can also be secreted by WAT (39), and liver (26). Irisin may induce browning in
subcutaneous WAT and muscle accretion, with an associated increase in energy expenditure
and body mass loss (4, 40). In the liver, FNDC5 reduces hepatic lipid accumulation and
stimulates fatty acid oxidation (28). Circulating irisin leads to benefits in metabolism, being
inversely associated with the visceral fat mass. Therefore, irisin is linked with reduced
obesity and improved glucose metabolism (19).
In the current study, we have seen greater FNDC5 in WAT and skeletal muscle in exercising
mice (C-HIIT and F-HIIT groups), which agrees with data from a swimming intervention in
obese rats, which restored plasma levels of irisin and increased FNDC5 protein expression in
skeletal muscle (53). The endurance training in obese rats stimulated FNDC5 production by
skeletal muscle (38). Furthermore, the enhancement in plasma levels of irisin by exercise
training is associated with improvements in adiposity and TG levels (29).
Irisin, together with PGC1alpha, may improve the PPARalpha expression (4, 41), which is
relevant because PPARalpha is the master regulator of beta-oxidation, responsible for the
transcription of CPT1. CPT1 allows fatty acid entrance into mitochondria and the consequent
degradation of fatty acids (10). HIIT has restored both PPARalpha and CPT1 in the skeletal
muscle (10) and liver (33) of a high-fat-induced obese mouse. These benefits corroborate
current findings in a fructose-fed model. Contrarily, we have seen a lower gene expression of
PGC1beta in the liver from F-HIIT animals. PGC1beta is an essential regulator of lipid
metabolism related to and increased hepatic de novo lipogenesis and triglyceride synthesis
(35). Therefore, HIIT, not only induced beta-oxidation but also appeared to reduce
lipogenesis.
Sustained activation of beta-oxidation requires a primary content of mitochondria (3). Interestingly, irisin has also been linked to the induction of mitochondrial biogenesis in C2C12 myocytes, including PGC1alpha, NRF1 and TFAM genes (51). Likewise, PGC1alpha is also responsible for the transcription of genes involved in mitochondrial biogenesis, such as NRF1 and TFAM (47), and PPARalpha upregulation induces mitochondrial biogenesis (34). NRF1 is the primary target of PGC1alpha, involved in the regulation of other mitochondrial genes, while TFAM is required for mitochondrial DNA maintenance and replication (25).

Herein, HIIT has enhanced the gene expressions of NRF1 and TFAM in both WAT and liver, despite the continuous fructose ingestion. In a different model (eNOS -/- mice), the mitochondrial biogenesis and mitochondrial DNA content were augmented in WAT after a swim training period (50). In rats, the endurance training or voluntary activity increased mitochondrial biogenesis, including the protein expression of TFAM (43).

In summary, mice fed a fructose diet have shown significant adverse changes in BM, BP, glucose metabolism, plasma triglycerides, and liver steatosis. These alterations were associated with a reduced expression of FNDC5 (irisin) and PGC1alpha and, consequently, with downregulation of genes related to mitochondrial biogenesis and beta-oxidation. HIIT has improved all the data with benefits to all three organs: liver, WAT, and skeletal muscle. Therefore, we conclude that HIIT is a coadjutant treatment when the health condition of the individual allows it, to combat metabolic syndrome-related abnormalities.
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Disclosures

The authors declare they have no conflict of interests.

References


11. Christensen PM, Jacobs RA, Bonne T, Fluck D, Bangsbo J, and Lundby C. A short period of high-intensity interval training improves skeletal muscle mitochondrial function


Table 1. Composition and energy content of the experimental diets (AIN-93M-based diets).

<table>
<thead>
<tr>
<th>Ingredients (g/Kg)</th>
<th>Control</th>
<th>Fructose</th>
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</thead>
<tbody>
<tr>
<td>Casein</td>
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<tr>
<td>Corn starch</td>
<td>620.692</td>
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<tr>
<td>Sucrose</td>
<td>100.0</td>
<td>100.0</td>
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<tr>
<td>Fructose</td>
<td>-</td>
<td>474.3</td>
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<tr>
<td>Soy oil</td>
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<tr>
<td>Fiber</td>
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<tr>
<td>Vitamin mix</td>
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<tr>
<td>Mineral mix</td>
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<tr>
<td>Cysteine</td>
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<td>Choline</td>
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<td>2.5</td>
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<td>Antioxidants</td>
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<tr>
<td>Total mass</td>
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<tr>
<td>Carbohydrates (% energy)</td>
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<tr>
<td>Protein (% energy)</td>
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<td>14</td>
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<td>Lipids (% energy)</td>
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<tr>
<td>Total energy (kcal/kg)</td>
<td>3804</td>
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Table 2. RT-qPCR primers and their respective sequences.

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<th>Gene</th>
<th>(5' - 3')</th>
<th>Primers</th>
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<td>RV GGGGTGTTGAAGGTCTCAAA</td>
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<td></td>
<td>FW GCAGAGCACGGCAAAATGA</td>
<td>RV GGCTTTGACCCGAGAAGAC</td>
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<td>CPT-1a</td>
<td>FW GGCTGCCGTGGGACATT</td>
<td>RV TGCCTTGCACTTTGGTGAC</td>
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<td>CPT-1b</td>
<td>FW GGTGCTGATCATTGGTGAGG</td>
<td>RV CGCTCTGGTTTTCTCCTT</td>
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<td>FNDC5</td>
<td>FW GTTGCTAGGGGGCAACAGT</td>
<td>RV GTAACGTTGGCCAGTTTGT</td>
</tr>
<tr>
<td>NRF1</td>
<td>FW AACCACACCCACAGGATCAGA</td>
<td>RV TCTTCGCTTTATGCTCCATGA</td>
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<td>PGC-1α</td>
<td>FW CCATGGCAGAAGAGGTGAGA</td>
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<td>PGC-1β</td>
<td>FW CCAGGCCTCAGGGTACACTAC</td>
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<tr>
<td>PPARα</td>
<td>FW CAGCCTTCACCCTTGCTC</td>
<td>RV TTGCTGCTGTCGCTTTGTT</td>
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<td>TBP</td>
<td>FW GAAGAAGAGATGGAGAGAG</td>
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</tbody>
</table>

Abbreviations: CPT1, Carnitine palmitoyltransferase 1; FNDC5, Fibronectin type III domain-containing 5; NRF1, Nuclear respiratory factor 1; PGC-1, Peroxisome proliferator-activated receptor gamma coactivator 1; PPAR-α, Peroxisome proliferator-activated receptor alpha; TBP, TATA binding protein; TFAM, Mitochondrial transcription factor A.
Table 3. Carbohydrate metabolism.

<table>
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<th>Data</th>
<th>C-NT</th>
<th>C-HIIT</th>
<th>F-NT</th>
<th>F-HIIT</th>
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<td>Food intake (g)</td>
<td>2.9±0.06</td>
<td>2.7±0.46</td>
<td>2.8±0.44</td>
<td>2.6±0.84</td>
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<td>Glucose (mmol/L)</td>
<td>8.2±0.5</td>
<td>5.8±0.4†</td>
<td>10.1±0.4†</td>
<td>7.6±0.3 ‡§</td>
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<tr>
<td>Insulin (µU/ml)</td>
<td>22.0±11.0</td>
<td>9.9±7.9†</td>
<td>27.4±8.1†</td>
<td>10.9±13.0§</td>
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<tr>
<td>HOMA-IR</td>
<td>8.3±1.9</td>
<td>2.5±1.2†</td>
<td>12.3±2.9†</td>
<td>3.7±1.5 ‡§</td>
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<tr>
<td>QUICKI</td>
<td>0.28±0.08</td>
<td>0.38±0.02†</td>
<td>0.24±0.03†</td>
<td>0.29±0.05‡§</td>
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<tr>
<td>Triglycerides (mg/dL)</td>
<td>69.0±2.2</td>
<td>54.6±2.3†</td>
<td>78.7±6.9†</td>
<td>63.8±10.4§</td>
</tr>
</tbody>
</table>

**Legend:** Data are mean ± SD, (n=10). One-way ANOVA and posthoc test of Holm-Sidak, *P < 0.05* when: † ≠ C-NT group, ‡ ≠ C-HIIT group, § ≠ F-NT group. **Groups:** C (control); F (fructose), NT (non-trained), HIIT (high-intensity interval training).
### Table 4. Two-way ANOVA: fructose vs. HIIT.

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<th>Interaction %</th>
<th>Fructose %</th>
<th>HIIT %</th>
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<td>ns</td>
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</tbody>
</table>
**Figure legends**

**Fig. 1:** Body mass evolution (mean ± SD, n=10). One-way ANOVA and posthoc test of Holm-Sidak, $P < 0.05$, when: † ≠ C-NT group, § ≠ F-NT group. Groups: C, control; F, fructose; NT, non-trained; HIIT, high-intensity interval training.

**Fig. 2:** Systolic blood pressure (mean ± SD, n=10). One-way ANOVA and posthoc test of Holm-Sidak, $P < 0.05$, when: † ≠ C-NT group, ‡ ≠ C-HIIT group, § ≠ F-NT group. Groups: C, control; F, fructose; NT, non-trained; HIIT, high-intensity interval training.

**Fig. 3:** Blood glucose evolution (mean ± SD, n=10). One-way ANOVA and posthoc test of Holm-Sidak, $P < 0.05$, when: † ≠ C-NT group, ‡ ≠ C-HIIT group, § ≠ F-NT group. Groups: C, control; F, fructose; NT, non-trained; HIIT, high-intensity interval training.

**Fig. 4:** Liver triglycerides and volume density of steatosis (Vv [liver, steatosis]) (mean ± SD, n=5). One-way ANOVA and posthoc test of Holm-Sidak, $P < 0.05$, when: † ≠ C-NT group, ‡ ≠ C-HIIT group, § ≠ F-NT group. Groups: C, control; F, fructose; NT, non-trained; HIIT, high-intensity interval training. Representative photomicrographs were stained with hematoxylin and eosin and are of the same magnitude.

**Fig. 5:** Adipocyte area dispersion (mean ± SD, n=10). One-way ANOVA and posthoc test of Holm-Sidak, $P < 0.05$, when: † ≠ C-NT group, ‡ ≠ C-HIIT group, § ≠ F-NT group. Groups: C, control; F, fructose; NT, non-trained; HIIT, high-intensity interval training.

**Fig. 6:** PGC1alpha and FNDC5 gene expressions (mean ± SD, n=6). One-way ANOVA and posthoc test of Holm-Sidak, $P < 0.05$, when: † ≠ C-NT group, ‡ ≠ C-HIIT group, § ≠ F-NT group. Groups: C, control; F, fructose; NT, non-trained; HIIT, high-intensity interval training.

**Fig. 7:** NRF1 and TFAM gene expressions (mean ± SD, n=6). One-way ANOVA and posthoc test of Holm-Sidak, $P < 0.05$, when: † ≠ C-NT group, ‡ ≠ C-HIIT group, § ≠ F-NT group. Groups: C, control; F, fructose; NT, non-trained; HIIT, high-intensity interval training.
Fig. 8: PPARalpha and CPT1 gene expressions (mean ± SD, n=6). One-way ANOVA and posthoc test of Holm-Sidak, $P < 0.05$, when: † ≠ C-NT group, ‡ ≠ C-HIIT group, § ≠ F-NT group. Groups: C, control; F, fructose; NT, non-trained; HIIT, high-intensity interval training.
<table>
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