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Effect of physical training on liver expression of activin A and follistatin in a nonalcoholic fatty liver disease model in rats

R.N. Silva[1], P.G. Bueno[2], L.R.S. Avó[3], K.O. Nonaka[2], H.S. Selistre-Araújo[2] and A.M.O. Leal[3]

[1]Departamento de Fisioterapia, Universidade Federal de São Carlos, São Carlos, SP, Brasil
[2]Departamento de Ciências Fisiológicas, Universidade Federal de São Carlos, São Carlos, SP, Brasil
[3]Departamento de Medicina, Universidade Federal de São Carlos, São Carlos, SP, Brasil

Abstract

Nonalcoholic fatty liver disease (NAFLD) is characterized by fat accumulation in the liver and is associated with obesity and insulin resistance. Activin A is a member of the transforming growth factor beta (TGF)-β superfamily and inhibits hepatocyte growth. Follistatin antagonizes the biological actions of activin. Exercise is an important therapeutic strategy to reduce the metabolic effects of obesity. We evaluated the pattern of activin A and follistatin liver expression in obese rats subjected to swimming exercise. Control rats (C) and high-fat (HF) diet-fed rats were randomly assigned to a swimming training group (C-Swim and HF-Swim) or a sedentary group (C-Sed and HF-Sed). Activin βA subunit mRNA expression was significantly higher in HF-Swim than in HF-Sed rats. Follistatin mRNA expression was significantly lower in C-Swim and HF-Swim than in either C-Sed or HF-Sed animals. There was no evidence of steatosis or inflammation in C rats. In contrast, in HF animals the severity of steatosis ranged from grade 1 to grade 3. The extent of liver parenchyma damage was less in HF-Swim animals, with the severity of steatosis ranging from grade 0 to grade 1. These data showed that exercise may reduce the deleterious effects of a high-fat diet on the liver, suggesting that the local expression of activin-follistatin may be involved.

Key words: Activin A; Follistatin; Steatosis; Nonalcoholic fatty liver disease; Obesity; Exercise

Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common type of chronic liver disorder and includes a broad spectrum of liver damage, ranging from simple steatosis to steatohepatitis (NASH) and cirrhosis (1,2). Evaluation of these histopathologic characteristics is possible through a liver biopsy (1,2). NAFLD is closely associated with obesity and increased lipolysis due to loss of insulin sensitivity and impaired antilipolytic action of insulin, the main factor responsible for the development of hepatic steatosis (2).

Activins, members of the transforming growth factor beta (TGF-β) superfamily, are bioactive dimeric proteins composed of two beta subunits. Activin A, a homodimer composed of two beta A subunits, is involved in the pathogenesis of a variety of inflammatory diseases ranging from rheumatoid arthritis to inflammatory bowel disease and atherosclerosis, and also in several liver disorders, including NAFLD and liver fibrosis (3-5). Activin A is considered to be a negative regulator of liver growth by inhibiting the replication of hepatocytes and inducing apoptosis (3). The biological actions of activin are counteracted by follistatin, an unrelated glycoprotein that binds mature dimeric activin A with high affinity and blocks activin signaling (3,6). Follistatin is associated with liver regeneration after partial hepatectomy (7).

Activin signal transduction occurs through transmembrane serine/threonine kinase receptors. Activin binds to the activin type II receptors (ActRII), transphosphorylates type I receptors (activin receptor-like kinases, ALKs) (1-7) and forms the ActRII-IB/(ALK) 4 complex. This activated activin receptor complex recruits the receptor-associated Smads, which transmit the activin signals to the cell nucleus where they regulate the transcription of target genes (3,5,6).

Activin A seems to have multiple roles in NAFLD. It has been demonstrated that serum levels of activin A and follistatin and the activin A/follistatin mRNA ratio in liver are increased in patients with NAFLD, potentially

Correspondence: A.M.O. Leal, Departamento de Medicina, Universidade Federal de São Carlos, Rod. Washington Luís, km 235, 13565-905 São Carlos, SP, Brasil. E-mail: angelaleal@ufscar.br

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reflecting increased activin A bioactivity. Activin A contributes to the pathogenesis of NAFLD by inhibiting insulin sensitivity and metabolism of glucose and fatty acids. It has been demonstrated that activin A promotes collagen 3 and TGF-β1 expression and matrix metalloproteinase (MMP) activity, suggesting that activin A may promote hepatic fibrosis. On the other hand, activin A induces mitochondrial β-oxidation and downregulates *de novo* fatty acid synthesis, suggesting that activin A may reduce lipid accumulation in hepatocytes (4,6).

A recent review of the relationship between exercise and the liver and its implications for the therapy of fatty liver disorders concluded that the effect of exercise on liver fat reduction compared favorably with most of the available pharmacological therapies (8). Physical exercise is linked to improved glucose homeostasis and enhanced insulin sensitivity, and is a basic means for the prevention of type 2 diabetes and the management of both type 1 and type 2 diabetes (9). Physical exercise has also been shown to have positive effects in the prevention and attenuation of hepatic steatosis (10).

Studies in diabetic and obese rats have shown that exercise can modulate follistatin in muscle and fat tissue (11,12). A recent study also demonstrated that plasma follistatin increased in response to exercise, most likely originating from the liver (13). However, there is no evidence of a relationship between exercise and activin.

We hypothesized that activin A/follistatin may participate in the beneficial effect of exercise on NAFLD, which still has not been elucidated. In the present study, we evaluated the patterns of activin A and follistatin expression in the livers of obese rats subjected to swimming exercise.

Material and Methods

Animals and experimental groups

All procedures were approved by the Ethics Committee of Universidade Federal de São Carlos (#048/2007). Male Wistar rats (190 ± 18.4 g) were acclimatized in individual cages under controlled temperature, humidity and lighting (12-h dark/light cycle) conditions, and with free access to water and standard rodent chow. After 7 days, animals were randomly assigned to two groups: control rats (C, n = 15) fed standard rodent chow, and high-fat diet-fed rats (HF, n = 15), fed 60% kcal as fat (Research Diets, USA). Animals and food intake were weighed daily. After 12 weeks, animals in both groups were randomly divided into two groups, a swimming training group (C-Swim and HF-Swim) and a sedentary group (C-Sed and HF-Sed) with 6-7 rats/group.

Exercise protocol

Rats in the exercise groups (C-Swim and HF-Swim) were exercised by swimming 1.5 h/day, 45 min at 9:00 am and 45 min at 5:00 pm, 5 days per week, for 4 weeks. The swimming exercise was performed in 30 cm diameter plastic barrels filled with water to a depth of 50 cm and maintained at 32°-36°C. The protocol started with 3 days of adaptation. On the first and second days of adaptation, the rats swam for 1 h/day, 30 min at 9:00 am and 30 min at 5:00 pm without the addition of a weight to the tail. On the third day of adaptation, the rats swam for 1.5 h/day, 45 min at 9:00 am and 45 min at 5:00 pm again without the addition of a weight. On the fourth day, an external weight corresponding to 1% of the animal's body mass was attached to the base of the tail, and rats swam for 90 min/day. During the following weeks, rats swam for 90 min/day with the tail-weight increased weekly until reaching 3% and then 5% of the animal's body mass (14-16). All animals were towel dried before being returned to their cages. The sedentary groups were placed in shallow water for 5 min, towel dried and then returned to their cages at the same time as the exercised groups. Both the control rats and the HF diet rats were able to carry out the exercise protocol similarly.

After the training period, i.e., 48 h after the last exercise session, rats were sacrificed by decapitation. Blood plasma was collected and stored at –20°C; the liver was dissected, weighed, frozen immediately in liquid nitrogen, and stored at –80°C until analysis.

Glucose and insulin tolerance tests

Glucose tolerance tests (GTTs) were performed after the acclimatization period, at 3, 4, 8, and 12 weeks of dietary treatment, and after exercise training by the 16th week. After an overnight fast, unanesthetized rats were injected intraperitoneally (ip) with 50% glucose solution, 1.5 g/kg body weight (BW). Blood samples were obtained from the tail vein before and at 30, 60, 90, and 120 min after glucose injection. Insulin tolerance tests (ITTs) were performed after the acclimatization period and at 8 and 12 weeks of dietary treatment. After an overnight fast, unanesthetized rats were injected *ip* with Novolin R human insulin (Novo Nordisk, Brazil), 0.75 U/kg BW. Blood samples were obtained from the tail vein before and at 15, 30, 60, and 90 min after the insulin challenge. Blood glucose concentrations were measured with an Accu-Check glucose meter (Roche Diagnostic, USA).

Histological analysis

Formalin-fixed and paraffin-embedded liver sections were stained with hematoxylin and eosin (HE) and Masson's trichrome for histopathological examination. The severity of steatosis was graded by the extent of parenchymal involvement. Grade 0, <5% of the parenchyma was involved; grade 1, 5 to 33%; grade 2, 34 to 66%; grade 3, >66%. Other parameters assessed were the presence or absence of inflammation and fibrosis score (17).

The histological slides were analyzed and photographed with an Olympus® BX 51 optical microscope

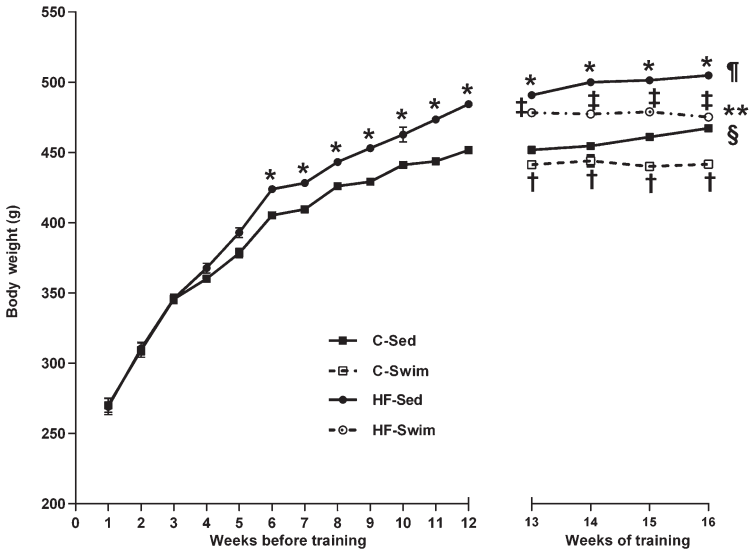

Figure 1. Body weight (g) of control and high-fat diet-fed rats. Data are reported as means ± SE for n = 6-7 rats/group. *P<0.05, sedentary high-fat diet group (HF-Sed) *vs* sedentary control group (C-Sed); †P<0.05, C-Sed *vs* exercised control group (C-Swim); ‡P<0.05, HF-Sed *vs* exercised high-fat diet group (HF-Swim); §P<0.05, C-Sed 12th week *vs* 16th week; ¶P<0.05, HF-Sed 12th week *vs* 16th week; **P<0.05, HF-swim 12th week (pre-exercise) *vs* 16th week (post-exercise) (*t*-test).

(Olympus Corporation, Japan). The pathologist who evaluated the sections and performed the histological assessments was unaware of the treatment groups.

Biochemical analysis

Alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transaminase (GGT), and plasma lipids, including total cholesterol and triglycerides, were measured enzymatically with commercially available assay kits (Katal Biotecnológica, Brazil).

RNA extraction and quantitative real-time PCR

Frozen liver samples (40 mg) were homogenized on ice in TRIzol reagent (Invitrogen Corporation, USA) following the manufacturer's instructions. The purity and concentration of total RNA were determined by measuring the absorbance of aliquots at a ratio of 260/280 nm. The integrity of RNA was checked on 2% agarose gels by inspecting the electrophoretic pattern of 28S and 18S ribosomal RNA. Total RNA (1 μg) from each sample was treated with DNase I (Invitrogen Corporation) to remove contaminating genomic DNA and reverse transcribed to synthesize cDNA using Moloney murine leukemia virus reverse transcriptase (Promega Corporation, USA). For real-time PCR, 20 ng cDNA and 0.4 μM of each primer were used in a 25 μL reaction volume containing SYBR Green PCR Master Mix (Applied Biosystems Inc., USA). The primer sequences used were: beta-A activin (forward primer: 5′-ATGGACCTAACTCTCAGCCAGA-3′; reverse primer: 5′-CTCTCCCCCTTCAAGCCCAT-3′); follistatin (forward primer: 5′-GGCGTACTGCTTGAAGTGAA-3′; reverse primer: 5′-GGGAAGCTGTAGTCCTGGTC-3′); GAPDH (forward primer: 5′-GATGCTGGTGCTGAGTAT GTCG-3′; reverse primer: 5′-GTGGTGCAGGATGCATT GCTGA-3′) (18,19). In addition, a rat liver cDNA standard was run in duplicate for every plate to produce a standard curve for quantification. The relative amounts of RNAs were calculated using the comparative Ct method. The expression of all genes was normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression.

Statistical analysis

Statistical analyses were performed by paired and unpaired Student *t*-tests and analysis of variance (ANOVA). The Tukey multiple comparisons test was used for *post hoc* analysis of between-group comparisons. Data are reported as means ± SE. P<0.05 was considered to be statistically significant. Data analyses were carried out using the STATISTICA data analysis software system, version 7 (StatSoft Inc., USA).

Results

Body weight

The body weight of HF diet-fed animals was significantly higher than that of control animals from week 6 until the end of the training period (Figure 1). At week 16, exercised animals (both control and HF diet-fed) had significantly lower body weights than sedentary animals (both controls and HF diet-fed). In HF diet-fed animals, post-exercise body weight at week 16 was significantly lower than pre-exercise body weight at week 12. Exercise training was thus able to prevent physiological weight gain associated with the standard rodent chow.

GTT and ITT tests

Rats fed an HF diet had significantly higher glycemia in response to both glucose and insulin injections than

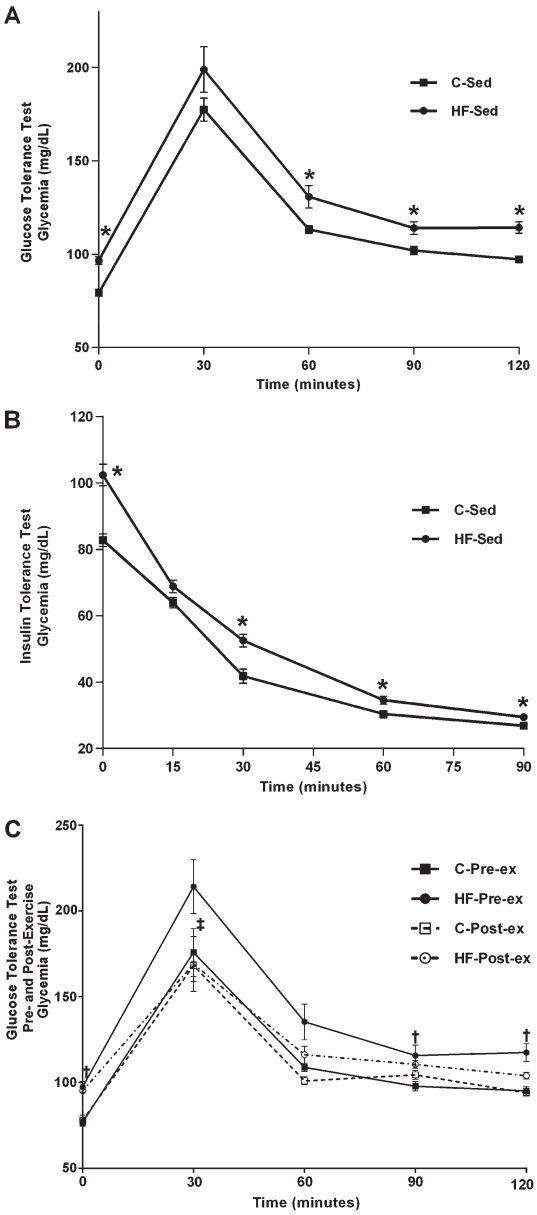

Figure 2. Glucose and insulin tolerance tests. Analysis of glycemia profile by the *A*, glucose tolerance test (GTT) and the *B*, insulin tolerance test (ITT) at the end of the 12th week in rats fed a high-fat diet. *C*, Analysis of glycemia profile by the GTT pre-exercise (12th week) and post-exercise (16th week) training. Data are reported as means ± SE for n = 15 (C and HF groups) and n = 6-7 rats/subgroup. *P < 0.05, high-fat diet group (HF) *vs* control group (C). †P < 0.05, exercised high-fat diet group pre-exercise (HF-Pre-ex) *vs* exercised control group pre-exercise (C-Pre-ex); ‡P < 0.05, HF-Pre-ex *vs* exercised high-fat diet group post-exercise (HF-Post-ex) (*t*-test).

control rats after 12 weeks of the diet regimen (Figure 2A and B). The post-exercise glycemic response to glucose injection was significantly lower than the pre-exercise test response in HF-Swim rats (Figure 2C). No difference was observed between pre- and post-exercise glycemia in response to glucose injection in C-Swim rats.

Liver enzymes and lipids

ALT levels were significantly higher in HF-Sed animals than in C-Sed animals (41.7 ± 2.7 *vs* 26.9 ± 1.4 U/L, P < 0.05) and significantly lower in HF-Swim animals than in HF-Sed animals (31.6 ± 2.1 *vs* 41.7 ± 2.7 U/L, P < 0.05). Plasma AST, GGT, total cholesterol and triglycerides levels did not vary among groups (data not shown).

Liver expression of mRNA

Activin A subunit mRNA expression was significantly higher in HF-Swim than in HF-Sed animals, but no difference was observed between C-Sed and C-Swim animals. Follistatin mRNA was significantly lower in C-Swim and HF-Swim animals than in either C-Sed or HF-Sed animals (Figure 3).

Liver morphology

Liver morphology varied significantly among groups. There was no evidence of steatosis or inflammation (grade 0) in rats fed standard rodent chow. In contrast, the severity of steatosis ranged from grade 1 to grade 3 in rats fed the HF diet. This was not associated with inflammation or fibrosis. After the training period, rats fed the HF diet showed improvement in the extent of damage to the liver parenchyma, with severity of steatosis ranging from grade 0 to grade 1. Representative images of liver sections are shown in Figure 4.

Discussion

The present data showed that exercise reduced the deleterious effects of an HF diet on the liver and altered both hepatic activin beta A subunit and follistatin mRNA expression in this rat model of NAFLD.

Decreased glucose tolerance has previously been shown to accompany obesity induced by an HF diet (20). Here, exercise training reduced insulin resistance in rats fed an HF diet. It is well established that exercise is linked to enhanced insulin sensitivity in diet-induced obese rats by activation of components of the insulin-signaling cascade pathway in both skeletal muscle and liver (21,22). However, it is important to consider the intensity, frequency and duration of the exercise. It has been reported that exercise training was able to improve insulin activity in fat tissue, and that a stronger effect was seen with high- than with moderate-intensity exercise (23,24).

In this study, we also demonstrated that moderate aerobic exercise reduces body weight and attenuates hepatic steatosis. Previous studies have reported that moderate aerobic exercise promotes a reduction of body weight and adiposity, improves blood lipid profiles and attenuates hepatic steatosis, suppressing HF

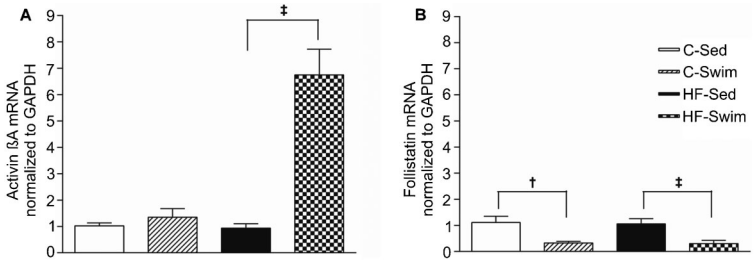

Figure 3. Liver expression mRNA. *A*, Activin βA and *B*, follistatin mRNA expression in liver of Wistar rats. Data are reported as means ± SE for n = 6-7 rats/group. †P<0.05, sedentary control group (C-Sed) *vs* exercised control group (C-Swim); ‡P<0.05, sedentary high-fat diet group (HF-Sed) *vs* exercised high-fat diet group (HF-Swim) (*t*-test).

diet-induced steatosis (10,25,26). It has long been observed that weight loss by caloric restriction alone or in combination with exercise prevents NAFLD (27). However, there is increasing evidence that exercise *per se* beneficially reduces hepatic steatosis independent of weight loss (28) and that exercise may offer additional benefits related to hepatic mitochondrial function compared to a restricted diet alone in rodents (29).

NAFLD comprises a continuum of liver diseases ranging from simple fat deposition (steatosis) to inflammation, extensive fibrosis and cirrhosis that develop in the absence of excessive alcohol intake (1). NAFLD is associated with obesity, insulin resistance and the metabolic syndrome (30). In this study, we used a previously described rodent model of NAFLD (11,31) and a validated histological scoring system to describe the histologic findings in this model (17). The results showed that an HF diet produced different degrees of fat accumulation but no inflammation or fibrosis. NAFLD is a complex disorder whose main initial pathological process is the accumulation of fat within the hepatocytes. It is believed to result from an imbalance between hepatic fatty acid synthesis/uptake and oxidation/export (6,30).

The results showed that swimming training minimized the liver tissue damage caused by an HF diet as previously demonstrated (10). The beneficial effects of exercise have been attributed to increased insulin sensitivity and fat oxidation and decreased lipid synthesis by the liver through activation of the AMP-activated protein kinase (AMPK) pathway (10,32). However, mechanistically, the effect of exercise on the pathogenesis of NAFLD is not understood completely.

We showed for the first time that activin and follistatin may be involved in the beneficial effects of exercise on NAFLD. We observed that the expression of activin A and follistatin mRNAs varied reciprocally in exercised and sedentary HF-fed animals, suggesting a local adaptive regulation promoting an increase in the biological effect of activin A. Activin A has been previously reported to be profoundly involved in liver function and in the pathogenesis of liver disease, including NAFLD (3,4,6). Follistatin is a binding protein that acts as an extracellular factor to bind and inactivate activin (33). It has recently been demonstrated that in mice with global inactivating

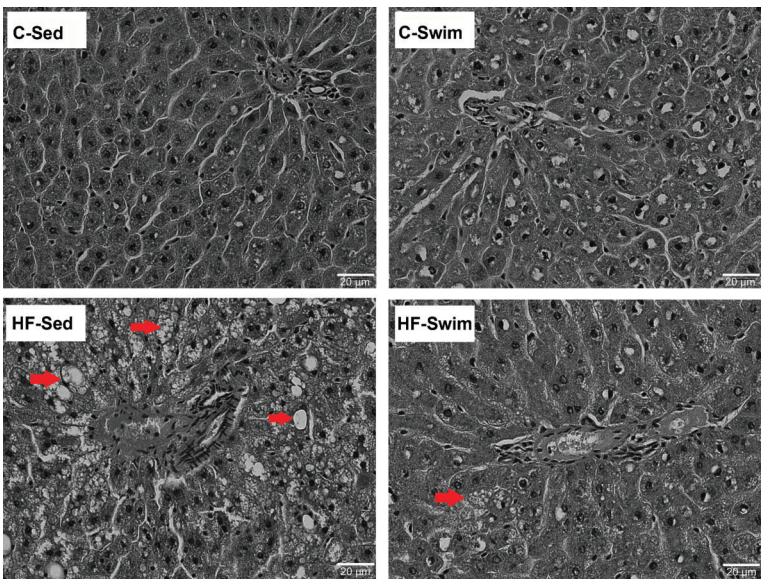

Figure 4. Histological analysis of liver from rats fed standard rodent chow and a high-fat diet. Representative microphotographs of hematoxylin-eosin-stained sections (bar = 20 µm) are shown. Red arrows indicate fat accumulation. C-Sed: sedentary control group; C-Swim: exercised control group; HF-Sed: sedentary high-fat diet group; HF-Swim: exercised high-fat diet group.

mutations that deplete the circulating activin antagonists follistatin-like-3 or the follistatin 315 isoform, insulin signaling in liver was enhanced, and paradoxically, hepatic steatosis developed and phosphoenolpyruvate carboxykinase (PEPCK) expression was not suppressed, suggesting dual actions of activin in hepatocytes (34).

The activin/follistatin circuitry is complex, with local autocrine/paracrine functions, and at the same time may be self-modulated (35,36). Many studies have described potential mechanisms to explain the role of activin/follistatin in exercise-induced liver adaptation (4,5). In this regard, activins have been shown to be associated with insulin production and insulin sensitivity, as well as with fat oxidation in liver and other tissues (37). In addition, activin A can act as an anti-inflammatory cytokine (38), and inflammation is an important mechanism involved in both insulin resistance and NAFLD (5,39). Moreover, it has recently been shown that exercise training reduces hepatic inflammation, injury and fibrosis by suppressing macrophage infiltration in diet-induced obesity in mice (40).

In spite of their novelty, the data presented here should be considered along with the study limitations. The modulation of activin and follistatin expression by exercise was shown only at the transcriptional level, thus post-transcriptional regulation cannot be ruled out. In addition, the mechanisms involved in the action of activin/follistatin on hepatic steatosis were not investigated in the present study. However, the findings may offer insight into the development of pharmacological strategies contributing to the control of obesity comorbidities such as NAFLD.

In conclusion, the results showed that exercise may reduce the deleterious effects of an HF diet on the liver and suggest that the local expression of activin and follistatin may be involved. Further studies are necessary to investigate the mechanisms of regulation of the activin/follistatin circuitry in metabolic and liver diseases.

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References

1. Brunt EM. Nonalcoholic steatohepatitis: definition and pathology. *Semin Liver Dis* 2001; 21: 3-16, doi: 10.1055/s-2001-12925.
2. Smith BW, Adams LA. Non-alcoholic fatty liver disease. *Crit Rev Clin Lab Sci* 2011; 48: 97-113, doi: 10.3109/10408363.2011.596521.
3. Rodgarkia-Dara C, Vejda S, Erlach N, Losert A, Bursch W, Berger W, et al. The activin axis in liver biology and disease. *Mutat Res* 2006; 613: 123-137, doi: 10.1016/j.mrrev.2006.07.002.
4. Yndestad A, Haukeland JW, Dahl TB, Bjoro K, Gladhaug IP, Berge C, et al. A complex role of activin A in non-alcoholic fatty liver disease. *Am J Gastroenterol* 2009; 104: 2196-2205, doi: 10.1038/ajg.2009.318.
5. Hashimoto O, Funaba M. Activin in glucose metabolism. *Vitam Horm* 2011; 85: 217-234, doi: 10.1016/B978-0-12-385961-7.00011-1.
6. Yndestad A, Haukeland JW, Dahl TB, Halvorsen B, Aukrust P. Activin A in nonalcoholic fatty liver disease. *Vitam Horm* 2011; 85: 323-342, doi: 10.1016/B978-0-12-385961-7.00015-9.
7. Ooe H, Chen Q, Kon J, Sasaki K, Miyoshi H, Ichinohe N, et al. Proliferation of rat small hepatocytes requires follistatin expression. *J Cell Physiol* 2012; 227: 2363-2370, doi: 10.1002/jcp.22971.
8. Johnson NA, Keating SE, George J. Exercise and the liver: implications for therapy in fatty liver disorders. *Semin Liver Dis* 2012; 32: 65-79, doi: 10.1055/s-0032-1306427.
9. Colberg SR, Albright AL, Blissmer BJ, Braun B, Chasan-Taber L, Fernhall B, et al. Exercise and type 2 diabetes: American College of Sports Medicine and the American Diabetes Association: joint position statement. Exercise and type 2 diabetes. *Med Sci Sports Exerc* 2010; 42: 2282-2303, doi: 10.1249/MSS.0b013e3181eeb61c.
10. Schultz A, Mendonca LS, Aguila MB, Mandarim-de-Lacerda CA. Swimming training beneficial effects in a mice model of nonalcoholic fatty liver disease. *Exp Toxicol Pathol* 2012; 64: 273-282, doi: 10.1016/j.etp.2010.08.019.
11. Bueno PG, Bassi D, Contrera DG, Carnielli HM, Silva RN, Nonaka KO, et al. Post-exercise changes in myostatin and actRIIB expression in obese insulin-resistant rats. *Mol Cell Endocrinol* 2011; 339: 159-164, doi: 10.1016/j.mce.2011.04.006.
12. Dutra DB, Bueno PG, Silva RN, Nakahara NH, Selistre-Araujo HS, Nonaka KO, et al. Expression of myostatin, myostatin receptors and follistatin in diabetic rats submitted to exercise. *Clin Exp Pharmacol Physiol* 2012; 39: 417-422, doi: 10.1111/j.1440-1681.2012.05690.x.
13. Hansen J, Brandt C, Nielsen AR, Hojman P, Whitham M, Febbraio MA, et al. Exercise induces a marked increase in plasma follistatin: evidence that follistatin is a contraction-induced hepatokine. *Endocrinology* 2011; 152: 164-171, doi: 10.1210/en.2010-0868.
14. Matsakas A, Bozzo C, Cacciani N, Caliaro F, Reggiani C, Mascarello F, et al. Effect of swimming on myostatin expression in white and red gastrocnemius muscle and in cardiac muscle of rats. *Exp Physiol* 2006; 91: 983-994, doi: 10.1113/expphysiol.2006.033571.
15. Leme JA, Gomes RJ, de Mello MA, Luciano E. Effects of short-term physical training on the liver IGF-I in diabetic rats. *Growth Factors* 2007; 25: 9-14, doi: 10.1080/08977190701210693.
16. Voltarelli FA, Gobatto CA, de Mello MA. Determination of anaerobic threshold in rats using the lactate minimum test. *Braz J Med Biol Res* 2002; 35: 1389-1394, doi: 10.1590/S0100-879X2002001100018.
17. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological

scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; 41: 1313-1321, doi: 10.1002/hep.20701.
18. Gold EJ, Francis RJ, Zimmermann A, Mellor SL, Cranfield M, Risbridger GP, et al. Changes in activin and activin receptor subunit expression in rat liver during the development of CCl4-induced cirrhosis. *Mol Cell Endocrinol* 2003; 201: 143-153, doi: 10.1016/S0303-7207(02)00417-3.
19. Ferreira MC, Cavallo IK, Florio P, Petraglia F, Reis FM. Activin betaA subunit, follistatin and follistatin-like 3 are expressed in the endometrium of ovariectomized rats and regulated by estrogen replacement. *J Mol Histol* 2008; 39: 535-541, doi: 10.1007/s10735-008-9194-x.
20. Woods SC, Seeley RJ, Rushing PA, D'Alessio D, Tso P. A controlled high-fat diet induces an obese syndrome in rats. *J Nutr* 2003; 133: 1081-1087.
21. Yaspelkis BB, III, Lessard SJ, Reeder DW, Limon JJ, Saito M, Rivas DA, et al. Exercise reverses high-fat diet-induced impairments on compartmentalization and activation of components of the insulin-signaling cascade in skeletal muscle. *Am J Physiol Endocrinol Metab* 2007; 293: E941-E949, doi: 10.1152/ajpendo.00230.2007.
22. De Souza CT, Frederico MJ, da Luz G, Cintra DE, Ropelle ER, Pauli JR, et al. Acute exercise reduces hepatic glucose production through inhibition of the Foxo1/HNF-4alpha pathway in insulin resistant mice. *J Physiol* 2010; 588: 2239-2253, doi: 10.1113/jphysiol.2009.183996.
23. Wisloff U, Najjar SM, Ellingsen O, Haram PM, Swoap S, Al-Share Q, et al. Cardiovascular risk factors emerge after artificial selection for low aerobic capacity. *Science* 2005; 307: 418-420, doi: 10.1126/science.1108177.
24. Wisloff U, Najjar SM, Ellingsen O, Haram PM, Swoap S, Al-Share Q, et al. Cardiovascular risk factors emerge after artificial selection for low aerobic capacity. *Science* 2005; 307: 418-420, doi: 10.1126/science.1108177.
25. Estadella D, Oyama LM, Damaso AR, Ribeiro EB, Oller Do Nascimento CM. Effect of palatable hyperlipidic diet on lipid metabolism of sedentary and exercised rats. *Nutrition* 2004; 20: 218-224, doi: 10.1016/j.nut.2003.10.008.
26. Marques CM, Motta VF, Torres TS, Aguila MB, Mandarim-de-Lacerda CA. Beneficial effects of exercise training (treadmill) on insulin resistance and nonalcoholic fatty liver disease in high-fat fed C57BL/6 mice. *Braz J Med Biol Res* 2010; 43: 467-475, doi: 10.1590/S0100-879X2010007500030.
27. Ueno T, Sugawara H, Sujaku K, Hashimoto O, Tsuji R, Tamaki S, et al. Therapeutic effects of restricted diet and exercise in obese patients with fatty liver. *J Hepatol* 1997; 27: 103-107, doi: 10.1016/S0168-8278(97)80287-5.
28. Johnson NA, George J. Fitness versus fatness: moving beyond weight loss in nonalcoholic fatty liver disease. *Hepatology* 2010; 52: 370-381, doi: 10.1002/hep.23711.
29. Rector RS, Uptergrove GM, Morris EM, Borengasser SJ, Laughlin MH, Booth FW, et al. Daily exercise *vs* caloric restriction for prevention of nonalcoholic fatty liver disease in the OLETF rat model. *Am J Physiol Gastrointest Liver Physiol* 2011; 300: G874-G883, doi: 10.1152/ajpgi.00510.2010.
30. Utzschneider KM, Kahn SE. Review: The role of insulin resistance in nonalcoholic fatty liver disease. *J Clin Endocrinol Metab* 2006; 91: 4753-4761, doi: 10.1210/jc.2006-0587.
31. London RM, George J. Pathogenesis of NASH: animal models. *Clin Liver Dis* 2007; 11: 55-74, viii, doi: 10.1016/j.cld.2007.02.010.
32. Park H, Kaushik VK, Constant S, Prentki M, Przybytkowski E, Ruderman NB, et al. Coordinate regulation of malonyl-CoA decarboxylase, sn-glycerol-3-phosphate acyltransferase, and acetyl-CoA carboxylase by AMP-activated protein kinase in rat tissues in response to exercise. *J Biol Chem* 2002; 277: 32571-32577, doi: 10.1074/jbc.M201692200.
33. Shimonaka M, Inouye S, Shimasaki S, Ling N. Follistatin binds to both activin and inhibin through the common subunit. *Endocrinology* 1991; 128: 3313-3315, doi: 10.1210/endo-128-6-3313.
34. Ungerleider NA, Bonomi LM, Brown ML, Schneyer AL. Increased activin bioavailability enhances hepatic insulin sensitivity while inducing hepatic steatosis in male mice. *Endocrinology* 2013; 154: 2025-2033, doi: 10.1210/en.2012-1844.
35. Leal AM, Blount AL, Donaldson CJ, Bilezikjian LM, Vale WW. Regulation of follicle-stimulating hormone secretion by the interactions of activin-A, dexamethasone and testosterone in anterior pituitary cell cultures of male rats. *Neuroendocrinology* 2003; 77: 298-304, doi: 10.1159/000070896.
36. Bilezikjian LM, Blount AL, Leal AM, Donaldson CJ, Fischer WH, Vale WW. Autocrine/paracrine regulation of pituitary function by activin, inhibin and follistatin. *Mol Cell Endocrinol* 2004; 225: 29-36, doi: 10.1016/j.mce.2004.02.010.
37. Mine T, Yasuda H, Fujita T, Hasegawa Y. Effects of inhibin on activin A-Induced glucose metabolism in rat hepatocytes. *Endocrine* 1996; 5: 41-46, doi: 10.1007/BF02738654.
38. Jones KL, Mansell A, Patella S, Scott BJ, Hedger MP, de Kretser DM, et al. Activin A is a critical component of the inflammatory response, and its binding protein, follistatin, reduces mortality in endotoxemia. *Proc Natl Acad Sci U S A* 2007; 104: 16239-16244, doi: 10.1073/pnas.0705971104.
39. Schenk S, Saberi M, Olefsky JM. Insulin sensitivity: modulation by nutrients and inflammation. *J Clin Invest* 2008; 118: 2992-3002, doi: 10.1172/JCI34260.
40. Kawanishi N, Yano H, Mizokami T, Takahashi M, Oyanagi E, Suzuki K. Exercise training attenuates hepatic inflammation, fibrosis and macrophage infiltration during diet induced-obesity in mice. *Brain Behav Immun* 2012; 26: 931-941, doi: 10.1016/j.bbi.2012.04.006.