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Applied physiology, nutrition, and metabolism (ISSN 1715-5312, eISSN 1715-5320)

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Citation Details

Citation for the version of the work held in 'OpenAIR@RGU':

KAPRAVELOU, G., MARTINEZ, R., ANDRADE, A.M., NEBOT, E., CAMILETTI-MOIRON, D., APARICIO, V.A., LOPEZ-JURADO, M., ARANDA, P., ARREBOLA, F., FERNANDEZ-SEGURA, E., BERMANO, G., GOUA, M., GALISTEO, M. and PORRES, J.M. 2015. Aerobic interval exercise improves parameters of non-alcoholic fatty liver disease (NAFLD) and other alterations of metabolic syndrome in obese Zucker rats. *Applied physiology, nutrition, and metabolism*, 40(12), pages 1242-1252. Available from *OpenAIR@RGU*. [online]. Available from: <http://openair.rgu.ac.uk>

Citation for the publisher's version:

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1 **Aerobic interval exercise improves parameters of Non Alcoholic Fatty Liver Disease (NAFLD) and**
2 **other alterations of metabolic syndrome in obese Zucker rats.**

3

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20

21 **Abstract**

22 Metabolic syndrome (MS) is a group of metabolic alterations that increase the susceptibility to
23 cardiovascular disease and type II diabetes. Non Alcoholic Fatty Liver Disease (NAFLD) has been described
24 as the liver manifestation of MS. We aimed to test the beneficial effects of an aerobic interval training (AIT)
25 protocol on different biochemical, microscopic, and functional liver alterations related to the MS in the
26 experimental model of obese Zucker rat. Two groups of lean and obese animals (6 weeks old) followed a
27 protocol of aerobic interval training (4 min at 65-80% of VO_2 max, followed by 3 min at 50-65% of VO_2
28 max, 45-60 min, 5 days/week, 8 weeks of experimental period), whereas two control groups remained
29 sedentary. Obese rats had higher food intake and body weight ($P < 0.0001$), and suffered significant
30 alterations in plasma lipid profile, area under the curve (AUC) after oral glucose overload ($P < 0.0001$), liver
31 histology and functionality, and antioxidant status. The aerobic interval training protocol assayed
32 ameliorated the severity of alterations related to glucose and lipid metabolism, and increased the liver protein
33 expression of PPAR- γ , as well as the gene expression of Glutathione Peroxidase 4 ($P < 0.001$). The training
34 protocol also showed significant effects on the activity of hepatic antioxidant enzymes, although this action
35 was greatly influenced by rat phenotype. The present data suggest that AIT protocol is a feasible strategy to
36 improve some of the plasma and liver alterations featured by the MS.

37

38 *Key words:* metabolic syndrome, non-alcoholic fatty liver disease, aerobic interval training, aerobic capacity,
39 lipid metabolism, hepatic metabolic pathways, liver antioxidant status

40

41 **Introduction**

42 Metabolic syndrome (MS) is a cluster of interrelated metabolic conditions which increase the risk of
43 developing cardiovascular disease (Kaur 2014). MS is characterized by central obesity, dyslipidemia,
44 elevated blood pressure, and elevated plasma glucose (Grundy 2005). Patients with MS are also more
45 susceptible to develop type 2 diabetes mellitus (Reaven 2004).

46 Hepatic morphology and function can be adversely affected by MS leading to the development of Non
47 Alcoholic Fatty Liver Disease (NAFLD) (Marchesini et al. 2003) which is characterized by steatosis, lobular
48 and portal inflammation, hepatocyte ballooning, and fibrosis (Brunt and Tiniakos 2010). Furthermore, this
49 hepatic pathology is now considered as the liver manifestation of MS (Angelico et al. 2005). Although the
50 exact mechanisms leading to it are not yet completely understood, insulin resistance and chronic oxidative
51 stress have been reported to play a major role in liver damage and development of NAFLD (Polyzos et al.
52 2009; Rolo et al. 2012)

53 The effects of different types of exercise on MS have been studied. In 2009, Haram et al. (2009) reported
54 that high-intensity aerobic interval training was more effective at reducing cardiovascular disease risk in rats
55 with MS than moderate-intensity continuous training. Aerobic interval exercise has also been described as a
56 feasible and efficient strategy to restore mitochondrial dysfunction in rats after myocardial infarction by
57 inhibiting dynamic pathological remodelling (Jiang et al. 2014). With regard to liver metabolism, several
58 authors have studied the effect of moderate or vigorous intensity exercise on different aspects of NAFLD.
59 Moderate intensity exercise training showed beneficial effects on intrahepatic triglyceride content, although
60 it did not improve hepatic lipoprotein kinetics in obese individuals with NAFLD (Sullivan et al. 2012). On
61 the other hand, vigorous exercise in humans was associated with a decreased adjusted odds of having non-
62 alcoholic steatohepatitis (NASH), whereas doubling the recommended time of vigorous exercise was
63 associated with a decreased adjusted odds of advanced fibrosis (Kistler et al. 2011). Furthermore, Linden et
64 al. (2015) have reported that vigorous-intensity interval exercise training (40 m/min, 15% incline, 6 × 2.5
65 min bouts/day, 5 days/week treadmill running) was as effective as a longer moderate intensity protocol in
66 lowering hepatic triglycerides, serum alanine aminotransferase (ALT), perivenular fibrosis, and hepatic
67 collagen 1 α 1 mRNA expression in OLETF rats.

68 Although a direct relationship has not been established, insufficient aerobic capacity is the basis of
69 several cardiovascular and metabolic diseases (Tjønnå et al. 2008). Therefore, an improvement in such
70 capacity could result in health benefits reported for aerobic interval training.

71 Oxidative stress is responsible for part of the initiation of obesity associated co-morbidities including
72 NAFLD and NASH (Rolo et al. 2012; Tariq et al. 2014). The obese status is characterised by oxidative stress
73 partly caused by insulin resistance and partly by low chronic inflammation (Al Rifai et al. 2015). Conditions
74 in which antioxidant status is altered by prevailing oxidative forces can be reflected in altered activity or
75 expression of liver antioxidant enzymes (Soltys et al. 2001; Videla et al. 2004). The regulation of glucose
76 and lipid metabolism at hepatic level can be significantly affected by metabolic alterations such as those
77 related to the development of NAFLD. Several molecular pathways are involved in glucose and lipid
78 metabolism. There are specific components of the former pathways like 5' AMP-activated protein kinase
79 (AMPK) or Peroxisome Proliferator Activator Receptor (PPAR) that play a key role in their activation.
80 AMPK is a regulator of energy homeostasis that down-regulate the expression of gluconeogenic and
81 lipogenic enzymes (Galisteo et al. 2010; Lochhead et al. 2000) in energetic deficiency situations. PPARs are
82 a family of nuclear transcription factors related to the management of lipogenic and lipolytic pathways in
83 liver and adipose tissue (Souza-Mello 2015). The up-regulation of PPAR- γ isoform has been related to
84 different factors such as AMPK pathway (Sakai et al. 2014) or reactive oxygen species (ROS) generation
85 (Ristow et al. 2009). Animal experimental models are an accepted tool to study the multifactorial effects of
86 exercise on MS associated conditions. In this context, the obese Zucker rat model shares many similarities
87 with humans affected by MS, including obesity, dyslipidaemia, insulin resistance, hepatomegalia, altered
88 antioxidant status, and inflammatory process (Galisteo et al. 2010; Hey-Mogensen et al. 2012). According to
89 Kucera and Cervinkova (2014), this experimental animal model exhibits the initial stages of NAFLD mainly
90 characterized by steatosis, but does not spontaneously progress to stage 2 of the disease. This study aimed
91 therefore: 1) to assess the potentially beneficial effects of AIT protocol on aerobic capacity, glucose and lipid
92 metabolism parameters, liver histology and functionality, and hepatic antioxidant status in an animal
93 experimental model of MS, the obese Zucker rat, that presents hepatic alteration related to early stages of
94 NAFLD, 2) to study the role of AMPK and PPAR- γ in the signaling pathways involved in exercise-derived
95 effects.

96 **Materials and methods**

97 **Animals and experimental design**

98 Twenty young male obese (fa/fa) (O) and 20 lean heterozygous (fa/+) (L) Zucker rats (6 weeks old) with
99 an initial mean body weight of 179 ± 2.8 and 148 ± 3.4 g, respectively, were allocated to four different
100 experimental groups (two obese and two lean groups, n=10 rats each). Two of the experimental groups (an
101 obese and a lean one) performed aerobic interval exercise according to an established training protocol (OE,
102 LE) while the two remaining groups were considered as sedentary groups (OS, LS). The experiment lasted
103 for 8 weeks, during which the animals were housed in a well ventilated, thermostatically controlled room
104 ($21\pm 2^\circ\text{C}$). A reversed 12:12 light/dark cycle was implemented so the animals would perform the training
105 protocol in darkness. Throughout the trial, animals had free access to type 2 water and consumed the
106 experimental diet (see below) *ad libitum*. Food intake was recorded daily whereas body weight was
107 measured once a week. At the end of experimental period, a glucose tolerance test following the protocol
108 described by Prieto et al. (2004) was performed 24 h after the last training session in order to re-establish the
109 normal physiological conditions altered in response to the energetic demand induced by the aerobic interval
110 exercise. Blood glucose concentration from the animals' tail was recorded at periods 0, 15, 30, 90 and 120
111 min after the glucose overload ingestion (BREEZE® 2, Bayer), and the area under the curve (AUC) was
112 determined. The animals were allowed to recover for 24 h prior being fasted for a further 8 h, anesthetized
113 with xylazine/ketamine and sacrificed. Blood was collected by puncture of the abdominal aorta (with heparin
114 as anticoagulant) and centrifuged at $1458 \times g$ for 15 min to separate plasma that was subsequently frozen in
115 liquid N₂ and stored at -80°C . The liver was extracted, weighed, photographed for macroscopic studies,
116 divided into various portions and immediately frozen in liquid N₂ and stored at -80°C . All experiments were
117 undertaken according to Directional Guides Related to Animal Housing and Care (EUC 2010) and all
118 procedures were approved by the Animal Experimentation Ethics Committee of the University of Granada,
119 Spain.

120 **Experimental diet**

121 The experimental diet was formulated following the guidelines of the American Institute of Nutrition
122 (AIN-93M; Reeves et al. 1993), in order to meet the nutritional recommendations of adult rats (NRC 1995),
123 with casein (70%) and whey (30%) as protein sources, to reach a 12% protein level. Dietary insoluble fiber
124 was added as cellulose to provide a dietary level of 10%, while 4% of fat was provided as sunflower oil.
125 There was no further addition of saturated fat or cholesterol.

126 **Exercise Protocol**

127 The exercise groups followed a protocol of aerobic interval training five days a week during the eight
128 weeks of the experimental period. The training protocol was performed in a motorized treadmill specially
129 designed for rats (Panlab Treadmills for five rats, LE 8710R), and all sessions were performed during the
130 dark cycle of the animals (active period). This training protocol was designed based on recent studies that
131 have demonstrated that a high intensity interval training (65-80% of VO_2 max combined with periods of 50-
132 65% of VO_2 max) promotes best results on weight reduction and blood lipid profile (Donnelly et al. 2009).
133 One week before the start of the study, the animals were adapted to the training procedures through a low
134 intensity running protocol every day for 20 min in the treadmill at 18m/min. The running sessions of 1 h
135 started with a 10 min warm up at 40% VO_2 max, and consisted of successive 4 min exercise periods at 65-
136 80% of VO_2 max, followed by 3 min recovery periods at 50-65% of VO_2 max. The intensities and length of
137 the training were gradually incremented every week (Table 1). To establish the velocity that would
138 correspond to the VO_2 max of each rat, a maximal incremental test was performed at the start of the study. A
139 final incremental test was performed 96 h prior the end of the study to test the maximal aerobic capacity and
140 physical performance achieved by the animals as a result of the intervention. The maximal incremental test
141 was carried out following the protocol described by Clemente et al. (2011) and Wisløff et al. (2001) with
142 slight modifications. This protocol ran by the computer software SeDaCom V2. (Panlab. Harvard apparatus),
143 first measures 5 min ambient air and then air within the treadmill to determine the appropriate ratio VO_2 :
144 VCO_2 . The test ends when the animal is visibly exhausted and rested on the shock bar for > 5 seconds. Basal
145 and final blood lactate concentrations were measured at the start and at the end of the incremental test in
146 blood obtained from the animals' tail (Lactate Pro, Arkray, The Netherlands).

147 During the experimental trial, the sedentary groups were subjected to a 15 min of low velocity (15 m/min)
148 training protocol twice a week, to reflect a human sedentary lifestyle (Morris et al. 2007).

149 **Plasma and liver biochemical analysis**

150 Biochemical parameters of glucose and lipid metabolism, and liver function were measured in plasma
151 using a Shenzhen Midray BS-200 Chemistry Analyzer (Bio-Medical Electronics) at the Bioanalysis Unit of
152 the Scientific Instrumentation Centre (Biomedical Research Park, University of Granada). Plasma insulin
153 concentration was quantified using a rat insulin enzyme immunoassay kit (Spibio, Montigny le Bretonneux,
154 France).

155 A portion of liver was lyophilized in order to determine the percentage of water. Hepatic lipids were
156 extracted from the freeze-dried liver portion using the method described by Folch et al. (1957) with slight
157 modifications (Kapraev et al. 2013). The extracted lipids were dissolved in 1mL of 96% hexane to
158 measure triglycerides content (Spinreact, S.A., Girona, Spain).

159 **Macroscopic and microscopic liver study**

160 Liver area of the macroscopic image was estimated in all the liver images of the four experimental groups
161 assayed by morphometric study using the software Image Pro Plus 6.0. A portion of liver was fixed in 10%
162 phosphate-buffered formalin, dehydrated in ethanol, embedded in paraffin, and sectioned for histological
163 examination using hematoxylin-eosin (HE), and Masson's trichrome (MT) staining for general microscopy
164 morphology and fibrosis development, respectively. Four different preparations of each staining were
165 analyzed for each animal, and 10 animals were evaluated in each experimental group (n=40). Histological
166 alterations were evaluated according to the following grading score: -, non-existent; +, mild; ++,
167 mild/moderate; +++, moderate; +++++, abundant; ++++++, severe.

168 **Antioxidant activity assays**

169 Liver was homogenized (1:10 w/v) in 50 mM phosphate buffer (pH 7.8) containing 0.1% Triton X-100
170 and 1.34 mM of DETAPAC using a Micra D-1 homogenizer (ART moderne labortechnik) at 18,000 rpm for
171 30 sec followed by treatment with Sonoplus HD 2070 ultrasonic homogenizer (Bandelin) at 50% power three
172 times for 10 sec. Liver homogenates were centrifuged at $13\,000 \times g$, 4°C for 45 min and the supernatant was
173 used to determine the activity of antioxidant enzymes. Catalase activity was measured by the method of Aebi
174 (1984) and the enzyme unit was defined as μmol of H_2O_2 consumption per min. Total cellular GPX activity
175 was determined by the coupled assay of NADPH oxidation (Lawrence et al. 1974) using cumene
176 hydroperoxyde as substrate. The enzyme unit was defined as nmol of GSH oxidized per min. Total SOD
177 activity was measured as described by Ukeda et al. (1997). Mn-SOD activity was determined by the same
178 method after treating the samples with 4mM KCN for 30 min. Cu,Zn-SOD activity resulted from subtracting
179 the Mn-SOD activity from the total SOD activity. One unit of SOD activity was defined as the enzyme
180 needed to inhibit 50% XTT reduction. Protein concentration was assayed by the method of Lowry et al.
181 (1951).

182 **Liver protein expression analyses**

183 Liver samples were homogenized in 20mM Tris HCl buffer containing 0.1% Igepal, 100mM EGTA, and
184 a cocktail of protease inhibitors (Sigma, St Louis, MO) that provided a final concentration of 100mM
185 dichloro diphenyl trichloroethane (DDT), 100mM orthovanadate, 1mM EDTA, 2mM AEBSF, 130 μ M
186 Bestatin, 14 μ M E-64, 1 μ M Leupeptin and 1 μ M Apoptin. Samples were homogenized as before. Liver
187 homogenates were centrifuged at 13 000 \times g for 45 min, at 4°C and supernatants were aliquoted and stored at
188 -80°C, until further use for western blot analysis. Protein concentration was measured by the method of
189 Lowry et al. (1951). Equal amounts of total protein for each sample were loaded per lane (two samples from
190 each experimental group were run per gel), subjected to 12% SDS-PAGE, and electrophoretically transferred
191 to nitrocellulose membranes (Schleicher&Schuell, Dassel, Germany) by wet transfer at 90 V for 2 h using a
192 Mini Trans-Blot cell system (Bio-Rad Laboratories, Hercules, CA). Membranes were blocked using 5% non-
193 fat dry powdered milk dissolved in Tris-Buffered saline Tween-20 (TBST) for 90 min at room temperature.
194 The primary antibodies for 5'-AMP-activated protein kinase (AMPK), phosphorylated-AMPK (PAMPK)
195 (Cell Signaling Technology, Inc. Danvers, MA, USA), and proliferator activating receptor- γ (PPAR- γ)
196 (Abcam, Cambridge, UK) were used according to the manufacturer recommended dilutions (1:1000) and
197 were incubated overnight at 4°C. The membranes were then washed three times for 10 min with TBST,
198 before incubation for 2 h at room temperature with secondary peroxidase conjugated goat anti-rabbit
199 antibody (Sigma, St Louis, MO) diluted at 1:2000 in 5% nonfat dry milk-TBST. Membranes were washed as
200 before, and the bound antibodies were visualized by an ECL Pro system (PerkinElmer, Boston, USA) using a
201 Fujifilm Luminescent Analyzer LAS-4000 mini System (Fujifilm, Tokyo, Japan). PAMPK expression was
202 determined in relation to AMPK expression while PPAR- γ was normalized to ponceau reagent. Results were
203 expressed in relative density units.

204 **Liver gene expression analyses**

205 Total RNA was extracted from 10-20 mg of frozen liver tissue using TrizolTM reagent (Invitrogen; UK)
206 and following the manufacturer's instructions. RNA purity was determined by the A=260/A=280 ratio, using
207 a UV/VIS spectrophotometer (Thermo Spectronic, Helios γ). Expression of GPX1 and GPX4 genes was
208 measured by semi-quantitative RT PCR. GAPDH gene expression was used as housekeeping gene. Total
209 RNA (100ng) was reverse transcribed using SuperScript III Reverse Transcriptase (Invitrogen, UK), 10mM
210 of each dNTP (Promega, UK), 10-20U RNaseOUT (Invitrogen), 1mg/mL BSA (BioLabs, UK), and
211 500 μ g/ml of Random Hexamers (Promega, UK) as primers. The amplification of cDNA was performed by

212 adding 10x PCR buffer (w/o MgCl₂), 1.75mM MgCl₂, 1U TaqDNA polymerase and 1 μM of each specific
213 primer for GPX1, GPX4 and GAPDH (Table 2). After a hot start (95°C) and 4 min at 94°C, 25 cycles of 1
214 min at 94°C, 2 min at 59°C and 2 min at 72°C, were performed. Samples were further incubated at 72°C for 8
215 min to complete any elongation reaction. PCR products were then separated by gel electrophoresis on a 1.5%
216 agarose gel containing GelRed™ (1:10,000, Biotium, UK). PCR amplified gene products were visualized
217 under UV light and, images were captured using Fusion Fx7 imaging system (PEQLAB Biotechnologies,
218 UK). Optical density of the obtained products was quantified by Image J software. Expression of GPX1 and
219 GPX4 was related to expression of GAPDH. To test the expression stability of GAPDH, equal amounts of
220 PCR product from liver samples of rats within each experimental group were loaded per lane and the band
221 density of the corresponding samples measured and compared among them. The average band density of LS
222 group was assigned with a value of 1, and relative values for the rest of samples in the three remaining
223 experimental groups were calculated and averaged. After statistical comparisons, no significant differences
224 were found for GAPDH expression among the four groups assayed.

225 **Statistical analyses**

226 Time-repeated measurement analysis was applied to weekly food intake and body weight data as well as
227 to blood glucose content after an oral glucose overload in order to analyze within subject effects (time) or
228 within group effects (phenotype or aerobic interval training protocol) on the above parameters. The effect of
229 phenotype and AIT protocol on final body weight, aerobic capacity and physical performance, plasma and
230 liver biochemical parameters, hepatic antioxidant enzyme activity, protein and gene expression was analyzed
231 by 2 × 2 factorial ANOVA with phenotype and AIT protocol as main treatments. Results are given as mean
232 values and pooled standard error of the mean. Bonferroni's test was used to detect differences between
233 treatment means. The analyses were performed with SAS, version 9.0, and the level of significance was set
234 at $P < 0.05$.

235 **Results**

236 **Food intake and body weight**

237 Changes observed in weekly food intake and body weight during the study are presented in Fig. 1A and
238 1B. Time-repeated measurement analysis revealed a significant time effect, phenotype effect, and exercise
239 effect on food intake that was 20% higher in the obese compared to the lean Zucker rats ($P < 0.0001$) and

240 decreased by 5% as a result of the aerobic interval training ($P < 0.05$). Body weight was significantly
241 affected by phenotype and the aerobic interval training, with lower values being found for lean vs obese ($P <$
242 0.0001), and trained vs sedentary ($P < 0.0001$) rats, respectively. The effect of exercise on body weight was
243 more pronounced in the obese when compared to lean rats.

244 **Aerobic capacity and physical performance**

245 The effects of phenotype and AIT protocol on aerobic capacity and physical performance of Zucker rats
246 during an incremental test are shown in Table 3. Blood lactate content at the end of incremental tests was
247 lower in lean when compared to obese rats ($P < 0.0001$), whereas the opposite was observed for the total
248 running time and maximal speed achieved ($P < 0.0001$). Exercise increased all the above parameters ($P <$
249 0.0001) with the exception of final blood lactate that decreased in the obese rats ($P < 0.001$) and was not
250 affected in the lean ones. The effects of exercise on running time and maximal speed were significantly
251 affected by phenotype, a finding that was reflected in significant phenotype \times exercise interactions.

252 **Plasma parameters**

253 The effects of phenotype and AIT protocol on blood and plasma parameters of glucose and lipid
254 metabolism of Zucker rats are presented in Figure 2 and Table 4. With regards to the plasma parameters
255 related to glucose metabolism affected by phenotype (glucose, insulin, and AUC, $P < 0.0001$), the training
256 protocol only had a significant effect on the AUC. Exercise tended to decrease both glucose and insulin
257 concentrations in the obese rats and increase them in the lean ones, although the effects were not significant.
258 AUC was differentially affected by exercise depending on the rat phenotype, a finding that was reflected in
259 phenotype \times exercise interaction ($P < 0.0001$). Exercise caused a 2.7-fold reduction in the AUC of obese rats,
260 returning this index to values similar to those found in lean animals, among which no appreciable effect of
261 this intervention was found. When blood glucose content of lean and obese Zucker rats prior to or at different
262 time points after an oral glucose overload was represented (Fig. 2), the rise in blood glucose during the initial
263 stages after oral administration was more pronounced in obese when compared to lean rats, and higher levels
264 were observed among the former animals for sedentary when compared to trained individuals. Blood glucose
265 levels of obese sedentary animals remained higher than the rest of experimental groups during the 120 min
266 post administration period, whereas those of obese trained animals were not significantly different from the
267 lean ones from 30 min post administration time.

268 The plasma parameters related to lipid metabolism (Total-, LDL-, and HDL-cholesterol, triglycerides)
269 were all significantly affected by phenotype. There was a significant effect of exercise on Total- and LDL-
270 cholesterol contents that were considerably diminished in the obese groups that carried out the training
271 protocol (20 and 41%, respectively) and to a lesser extent in the lean animals, giving rise to a significant
272 phenotype \times exercise interaction. Triglyceride content was considerably reduced by exercise ($P = 0.0845$) in
273 both the obese and lean animals (12 and 74%, respectively).

274 **Liver surface, lipid composition, functionality and antioxidant status**

275 The effects of phenotype and AIT protocol on liver weight and surface, lipid composition, and
276 functionality, are presented in Table 5 and Fig. 3. There was a significant effect of phenotype on liver weight
277 and surface, total fat, and triglyceride content that was higher in obese compared to lean rats. The liver of
278 obese rats showed clear signs of hepatomegalia and steatosis compared to their lean counterparts (Fig. 3).
279 The training protocol caused 8.6 and 9.3% decrease in liver weight and surface, respectively, and significant
280 reductions in hepatic total fat and triglyceride contents in the obese animals (35 and 50%, respectively). Such
281 AIT-induced improvements in the hepatic outcomes of lipid metabolism were associated to a lower body
282 weight exhibited by trained animals (Fig. 1B) and, to a lesser extent, to the lower weight of their abdominal
283 fat pad (6.4 ± 0.2 vs 5.7 ± 0.2 g in OS and OE groups, respectively)

284 Liver function was measured as plasma AST, ALT, ALP, GGT activities. All of these parameters were
285 affected by phenotype, showing a significant increase in the obese animals. The training protocol was
286 effective at reducing the activity of AST in both lean and obese animals, and ALP in obese but not in lean
287 rats, thus giving rise to a significant phenotype \times exercise interaction. However, it did not have any major
288 effect in ALT and GGT activity.

289 With regard to the hepatic antioxidant enzyme activities, there was a significant phenotype effect on SOD
290 activity reflected by lower values for Cu/Zn-SOD and higher values for Mn-SOD in obese compared to lean
291 rats. The training protocol caused a 40% increase in Cu/Zn-SOD activity of obese but no appreciable effect
292 on lean rats, and a 43% increase in Mn-SOD activity of lean rats that in contrast was significantly reduced in
293 their obese counterparts (20%). Such differential effects of exercise depending on rat phenotype gave rise to
294 significant phenotype \times exercise interactions ($P=0.049$ and $P<0.0001$, respectively). GPX activity was
295 significantly affected by phenotype, with lower values in the obese when compared to the lean sedentary

296 rats. The training protocol caused a 17% decrease in GPX activity of lean compared to a 100% increase in
297 obese rats. Such differential effects of the training protocol gave rise to a strong phenotype \times exercise
298 interaction ($P < 0.0001$). Exercise also exhibited a differential effect on catalase activity depending on rat
299 phenotype ($P = 0.0053$), decreasing as a result of the training protocol in the obese rats whereas it increased
300 in their lean controls.

301 The effects of phenotype and AIT protocol on the liver expression of GPX1 and GPX4 genes are shown in
302 Fig. 4. The expression of GPX1 and GPX4 genes, two major redox enzymes that take part in the antioxidant
303 defence system of the liver of Zucker rats, was not affected by phenotype. Exercise had only a significant
304 enhancing effect on the expression of liver GPX4 gene.

305 **Liver histology**

306 Several phenotype-related changes in liver histology were observed under the experimental conditions of the
307 present study (Fig. 5, Table 6). The obese sedentary rats exhibited clear signs of microvesicular steatosis and
308 fatty droplets (Fig. 5C), lipogranulomas and portal inflammation (Table 6) when compared to their lean
309 counterparts. The training protocol improved microvesicular steatosis, reduced the number of fatty droplets
310 (Fig. 5D), and decreased the amount of lipogranulomas and portal inflammation. However, it caused the
311 appearance of multinucleic cells and necrosis (Table 6) followed by the development of fibrosis (Fig. 5H).

312 **Liver protein expression**

313 The effects of phenotype and AIT protocol on the liver expression of AMPK, PAMPK, and PPAR- γ are
314 shown in Fig. 6. Western blot analysis indicated a significantly lower expression and activation of AMPK
315 (shown by the ratio PAMPK/AMPK) (Figure 6A) in the liver of obese compared to lean animals, and the
316 training protocol did not induce major effects on AMPK phosphorylation. No significant differences in
317 PPAR- γ expression were observed between obese and lean rats, whereas the training protocol caused 1.7-
318 fold increment in the obese and lean phenotypes (OE, LE), respectively (Figure 6B).

319 **Discussion**

320 The study of MS and the development of strategies for its prevention and treatment has attracted
321 increasing attention in recent years due to its growing prevalence and associated comorbidities exemplified
322 by cardiovascular disease and NAFLD (Kaur 2014; Marchesini et al. 2003). Changes in lifestyle, i.e. caloric
323 restriction and physical activity, are the primary interventions chosen to improve this condition. However,
324 the type and intensity of exercise are still a matter of debate. In this study, the influence of an aerobic interval

325 training protocol consisting of successive 4 min periods at 65-80% of VO_2 max, followed by 3 min recovery
326 periods at 50-65% of VO_2 max on plasma and liver biochemical parameters, was studied in obese and lean
327 Zucker rats. Obese rats exhibited higher food intake and body weight, and suffered significant alterations in
328 MS-associated parameters such as plasma lipid profile, OGTT and AUC after oral glucose overload, liver
329 histology and functionality, and antioxidant status. Exercise increased the aerobic capacity of both rat
330 phenotypes and diminished the severity of MS alterations, especially those related to glucose and lipid
331 metabolism, affecting the levels and activity of proteins involved in metabolic and antioxidant pathways and
332 the gene expression of GPX4, a key antioxidant enzyme, in the liver. The effects of exercise on glucose and
333 lipid metabolism were independent of hepatic AMPK activation, but matched significant increments in the
334 protein expression of PPAR γ .

335 Zucker obese rats are known to present a genetic defect in leptin receptor that causes the development of
336 hyperphagia and other metabolic disturbances leading to obese phenotype (Galisteo et al. 2008). The
337 anorectic effects of exercise on Zucker rats have been described by (Kibenge and Chan 2002) that related
338 such effects to an increased production of corticotrophin-releasing hormone. Such anorectic effects would in
339 turn lead to a lower weight gain both in obese and lean animals. Decrease in weight gain is among the most
340 widespread recommendations for the treatment of metabolic syndrome and has been associated to significant
341 improvements in cardiovascular health and metabolic disorders intrinsic to that disease.

342 Physical performance was always lower in obese when compared to lean Zucker rats due to the severe
343 metabolic disturbances, impaired skeletal muscle perfusion, and muscular atrophy inherent to this
344 experimental model. Low intrinsic aerobic capacity in rats has been related to lower energy expenditure and
345 reduced whole body and hepatic mitochondrial lipid oxidation, which in turn made the animals more
346 susceptible to dietary-induced hepatic steatosis (Morris et al. 2014). Our results show a clear improvement in
347 the aerobic capacity of lean and obese rats that followed the aerobic interval training protocol although the
348 effect of exercise on VO_2 max did not reach statistical significance. The adaptation changes in blood lactate,
349 maximal speed, and running time were significantly improved in trained rats. The enhancement in aerobic
350 capacity derived from aerobic interval exercise has been reported by other authors (Haram et al. 2009;
351 Tjønnå et al. 2008) that related such changes to amelioration in several risk factors of MS associated
352 cardiovascular disease. Under our experimental conditions, the higher physical performance of trained

353 Zucker rats was related to significant changes in glucose and lipid metabolism as well as to improved hepatic
354 histology and function altered in NAFLD.

355 The experimental model of obese Zucker rat has been described to exhibit impaired lactate transport by
356 the skeletal muscle that can be alleviated by endurance exercise (Metz et al. 2005). The aerobic training
357 protocol tested in our study achieved a consistent reduction in blood lactate after the incremental oxygen
358 consumption test. Since lactate release under exercise conditions is mostly related to skeletal muscle
359 metabolism, our results suggest that the benefits of the AIT protocol on lactate uptake and metabolism are
360 clear. Such improvement represents an important benefit on glucose metabolism in relation to
361 hyperlactatemia and aggravation of insulin resistance (Juraschek et al. 2013; Souto et al. 2011).

362 The beneficial effects of different types of aerobic exercise on glucose and lipid metabolism have been
363 extensively reported in the literature (Johnson et al. 2009; Rosety-Rodriguez et al. 2012). Our results confirm
364 such positive actions of AIT protocol, and point out to training-induced enhanced insulin sensitivity in the
365 obese animals as seen by changes in blood glucose levels and AUC after an oral glucose load, rather than to
366 changes in insulin secretion. Moreover, the specific action of the training protocol at decreasing total- and
367 LDL-cholesterol, while leaving HDL-cholesterol unchanged, suggests a direct protection against well known
368 cardio-metabolic risk factors. Such effects on the plasma lipid profile could be explained by a lower free
369 fatty acid uptake and lipogenesis in the adipose tissue (Haram et al. 2009). In addition, it has been reported
370 that physical exercise can elicit a significant improvement in the content and functionality of mitochondria
371 measured by increased citrate synthase activity, and palmitate oxidation (Linden et al. 2015). Moreover,
372 physical exercise is a successful strategy to prevent and mitigate NASH-induced mitochondrial bioenergetics
373 impairment, thus improving lipid metabolism in liver (Gonçalves et al. 2014).

374 The aerobic interval training triggered a clear improvement in liver lipid composition (lower total fat and
375 triglyceride content) as described by other authors in different human and animal models (Johnson et al.
376 2009; Linden et al. 2015). AIT can lead to such improvements in lipid composition through increases in
377 mitochondrial content and oxidative phosphorylation, or greater lipid and carbohydrate oxidation (Barker et
378 al. 2014; Larsen et al. 2015). Indeed, a long term aerobic training, for 3 months, at 60-75% of VO_2 max has
379 been shown to induce a decrease in intrahepatic lipids in obese female adolescents (Lee et al. 2013), whereas
380 a 7-day aerobic training protocol during 1 h at 80-85% of maximum heart rate in obese individuals with
381 hepatic steatosis resulted in increased resting fat oxidation and favourable effects in hepatic lipid

382 composition by increasing polyunsaturated lipid index (Haus et al. 2013). Furthermore, the beneficial effect
383 of a 12 week interval training on lipid oxidation was also proven in healthy, sedentary subjects (Astorino et
384 al. 2013).

385 Fatty liver has been associated to high plasma AST and ALT activities, resulting from hepatic damage
386 mediated by inflammation and oxidative stress reflected in higher levels of hepatic nitrate and
387 malondialdehyde (Jung and Kim 2013; Linden et al. 2015). Significant improvements of hepatic plasma
388 parameters have been observed under our experimental conditions related to the fat composition changes in
389 the obese Zucker rats. It is worth mentioning that our exercise training protocol has been beneficial both in
390 acute and chronic hepatic markers (AST and ALP activities, respectively) of altered functional status.

391 Oxidative stress is one of the main factors involved in the development of NAFLD (Rolo et al. 2012;
392 Tariq et al. 2014). Indeed, the “two-hit” hypothesis on NASH development points out to oxidative stress as
393 one of the factors directly promoting the progress from steatosis to the advanced stages of the pathology. A
394 decrease in antioxidant defence system has been described as a major promoting factor in the development of
395 oxidative stress in patients with NASH (Videla et al. 2004), whereas obese Zucker rats with fatty liver have
396 been described to exhibit an altered antioxidant status as shown by the decrease in liver content of GSH,
397 tocopherol, and catalase activity (Soltys et al. 2001). Exercise is a useful lifestyle intervention strategy to
398 improve oxidative stress in the muscle of type 2 diabetic rats (Qi et al. 2011; Rosety-Rodriguez et al. 2012)
399 and plasma of obese middle-age women (Shin et al. 2008). Furthermore, in obese individuals with hepatic
400 steatosis, short-term aerobic exercise has proved to favourably alter hepatic lipid composition, insulin
401 resistance and oxidative stress, risk factors that influence the severity of NAFLD (Haus et al. 2013).
402 However, the effects of exercise on oxidative stress may vary depending on parameters such as age, health
403 status, severity of pathology of the individual, and/or type and intensity of the exercise protocol applied.
404 Although, the effect of exercise on SOD, GPX, and catalase activities differed between obese and lean rats
405 under our experimental conditions, a finding that can be attributed to the compromised antioxidant status of
406 the obese animals associated to their fatty liver condition, exercise was in general terms an effective strategy
407 to lower oxidative stress and balance SOD and GPX activities that were altered in obese sedentary rats,
408 returning them to levels closer or even higher than those of lean animals. Of particular interest was the
409 increment in GPX activity attained by trained obese rats that nearly doubled that of their sedentary
410 counterparts and led us to conduct further experiments to confirm if such increments were related to the

411 induced expression of two relevant genes belonging to the GPX group of selenoenzymes such as GPX1 and
412 GPX4. Nevertheless, neither exercise nor phenotype had a significant effect on the hepatic GPX1 gene
413 expression under our experimental conditions. Similar findings have been observed in pediatric patients with
414 NASH that underwent liver biopsy (Desai et al. 2014) or mononuclear cells isolated from peripheral blood
415 samples of active or inactive healthy participants after completing a 30-min treadmill run at 75–80%
416 VO_2 max (Jenkins et al. 2009). A possible explanation for this lack of coincidence between enzymatic
417 activity and gene expression pattern is the existence of different members within the group of GPX
418 selenoenzymes that are not taken into account when the total GPX activity is measured. Furthermore,
419 Bermano et al. (1995) reported that both the activity of the selenoenzymes and the abundance of their
420 respective mRNAs are not regulated in a similar manner in the liver of rats with different Se status.

421 While GPX4 deficiency has been linked to disorders associated with reactive oxygen species and lipid
422 peroxides generated in mitochondria (Imai and Nakagawa 2003), its overexpression is associated with the
423 inhibition of atherosclerosis development in *ApoE*^{-/-} mice (Guo et al. 2008) and lipid peroxidation in
424 endothelial cells (Sneddon et al. 2003). In our study, hepatic GPX4 gene expression was up-regulated by
425 exercise in both lean and obese groups. Similar results were obtained by (Daussin et al. 2012) in the
426 expression of GPX4 after endurance training for 10 days.

427 The improvement in liver histological features associated to changes in lipid composition and function
428 exerted by the training protocol in the obese Zucker rat shows the prospective benefits of this type of
429 exercise in ameliorating hepatic morphological and histological alterations present in the early stages of
430 NAFLD characteristic of the experimental animal model selected for this study. Nevertheless, although the
431 training protocol has shown interesting results on glucose and lipid parameters in plasma, as well as lower
432 lipid content and decreased fatty droplet accumulation and microvesicular steatosis in liver of obese rats, the
433 potentially deleterious effects of that intensive type of exercise on individuals prone to liver damage (e.g.
434 suffering from MS), should be considered, since necrosis and fibrosis were detected in the liver of trained
435 rats, especially in the obese animals. It has been described that the obese Zucker rat does not spontaneously
436 progress from steatosis to steatohepatitis but needs an additional intervention (Kucera and Cervinkova 2014).
437 It seems that the experimental training protocol assayed was protective against steatosis but triggered some
438 distinctive features of NASH. In fact, exhaustive or strenuous exercise has been shown to increase certain
439 biomarkers of liver damage like AST and ALT and cause oxidative damage to nuclear DNA (Ogonovszky et

440 al. 2005; Ramos et al. 2013). Moreover, some authors have used exhaustive acute exercise to induce liver
441 injury in experimental animal models (Huang et al. 2013; Praphatsorn et al. 2010). Histopathological lesions
442 described in such models were mediated by pro-inflammatory cytokines and consisted on extensive nuclear
443 pyknosis, severe necrosis with haemorrhage and neutrophil infiltration, edema and necroinflammation, as
444 well as accelerated apoptosis. Under conditions of demanding physical exercise blood flow is preferentially
445 derived to skeletal muscle at the expense of other tissues like the liver in which decreased blood flow may
446 induce ischemic hypoxia-reperfusion of hepatocytes and lead to necrosis.

447 Activation of AMPK depends on the ADP:ATP ratio, and it is reduced in the liver of obese Zucker rat
448 due to an excess of energetic substrates entering this tissue (Galisteo et al. 2010). Furthermore, AMPK
449 activity has been shown to be inhibited by insulin and glucose in several tissues, a finding that would be
450 supported by the hyperinsulinemia characteristic of this experimental animal model. Although exercise can
451 activate AMPK in the skeletal muscle, and subsequently up-regulate PPAR- γ expression (Sasaki et al. 2014),
452 the aerobic interval protocol tested under our experimental conditions was not able to ameliorate high plasma
453 insulin levels of obese rats or show any effect on liver PAMPK expression. In contrast, liver AMPK activity
454 in obese Zucker rats has been described to be activated by different nutritional and pharmacological
455 strategies like diet supplementation with *Plantago ovata* or chronic treatment with polyphenols (Galisteo et
456 al. 2010; Rivera et al. 2009). On the other hand, it has been reported that activation of liver PPAR γ improves
457 insulin sensitivity and NASH in human patients (Neuschwander-Tetri et al. 2003) and this correlates with the
458 significant increase of liver PPAR- γ protein expression by our exercise protocol. Therefore PPAR- γ
459 activation under our experimental conditions appeared to be independent of the AMPK pathway.

460 In conclusion, the AIT protocol used in this study is a feasible intervention strategy to improve plasma and
461 hepatic biochemical parameters as well as hepatic histological alterations inherent to early stages of NAFLD
462 in obese Zucker rats, although it caused the development of fibrosis. The training protocol was especially
463 efficient to improve insulin sensitivity and decrease the hepatic lipid content, as well as ameliorating the
464 oxidative stress conditions in this organ. Such effects run in parallel to an increased expression of liver
465 PPAR- γ .

466 **Acknowledgements**

467 We want to thank Encarnación Rebollo and Lucía Bustos for skillful technical assistance. This study was
468 funded by Grant P09-AGR-4658 from Junta de Andalucía, Spain, and is part of the PhD Thesis of Garyfallia
469 Kapravelou, “Effects of legume protein hydrolyzates on lipid metabolism in an obese rat experimental
470 model. Interaction with aerobic physical exercise”. Gene expression work was carried out at Robert Gordon
471 University and was funded by the Institute for Health and Wellbeing Research. The authors also want to
472 acknowledge the Ministry of Economy and Competitiveness (MINECO, Spain) and the European Union
473 through project number AGL2013-43247-R and FEDER program, respectively.

474 **References**

- 475 Aebi, H. 1984. Catalase in vitro. *Methods Enzym.* **105**: 121–126. doi: 10.1016/S0076-6879(84)05016-3.
- 476 Al Rifai, M., Silverman, M.G., Nasir, K., Budoff, M.J., Blankstein, R., Szklo, M., et al. 2015. The
477 association of nonalcoholic fatty liver disease, obesity, and metabolic syndrome, with systemic
478 inflammation and subclinical atherosclerosis: the Multi-Ethnic Study of Atherosclerosis (MESA).
479 *Atherosclerosis.* **239**(2): 629-633. doi: 10.1016/j.atherosclerosis.2015.02.011
- 480 Angelico, F., Del Ben, M., Conti, R., Francioso, S., Feole, K., Fiorello, S., et al. 2005. Insulin resistance, the
481 metabolic syndrome, and nonalcoholic fatty liver disease. *J. Clin. Endocrinol. Metab.* **90**(3): 1578–1582.
482 doi: 10.1210/jc.2004-1024.
- 483 Astorino, T.A., Schubert, M.M., Palumbo, E., Stirling, D., and McMillan, D.W. 2013. Effect of two doses of
484 interval training on maximal fat oxidation in sedentary women. *Med Sci Sports Exerc* **45**(10): 1878–1886.
485 doi: 10.1249/MSS.0b013e3182936261.
- 486 Barker, A.R., Day, J., Smith, A., Bond, B., and Williams, C.A. 2014. The influence of 2 weeks of low-
487 volume high-intensity interval training on health outcomes in adolescent boys. *J. Sports Sci.* **32**(8): 757–
488 765. doi: 10.1080/02640414.2013.853132..
- 489 Bermano, G., Nicol, F., Dyer, J., Sunde, R., Beckett, G., Arthur, J., et al. 1995. Tissue-specific regulation of
490 selenoenzyme gene expression during selenium deficiency in rats. *Biochem. J.* **311**: 425-430. PMID:
491 7487877
- 492 Brunt, E.M., and Tiniakos, D.G. 2010. Histopathology of nonalcoholic fatty liver disease. *World J.*
493 *Gastroenterol.* **16**(42): 5286–5296. doi: 10.3748/wjg.v16.i42.5286.

- 494 Clemente, V.J.C., Martín, S., Porres, J., Fuentes, S., and Aranda, P. 2011. Efecto de la suplementación de
495 vitamina en el rendimiento de una prueba incremental de consumo máximo de oxígeno en ratas wistar.
496 Arch. Med. Dep. XXVIII: 168–173.
- 497 Daussin, F.N., Rasseneur, L., Bouitbir, J., Charles, A.-L., Dufour, S.P., Geny, B., et al. 2012. Different
498 timing of changes in mitochondrial functions following endurance training. Med. Sci. Sports Exerc.
499 **44**(2): 217–224. doi: 10.1249/MSS.0b013e31822b0bd4.
- 500 Desai, S., Baker, S.S., Liu, W., Moya, D.A., Browne, R.W., Mastrandrea, L., et al. 2014. Paraoxonase 1 and
501 oxidative stress in paediatric non-alcoholic steatohepatitis. Liver. Int. **34**(1): 110–117. doi:
502 10.1111/liv.12308.
- 503 Donnelly, J.E., Blair, S.N., Jakicic, J.M., Manore, M.M., Rankin, J.W., and Smith, B.K. 2009. Appropriate
504 Physical Activity Intervention Strategies for Weight Loss and Prevention of Weight Regain for Adults.
505 Med. Sci. Sports Exerc. **41**(2): 459–471. doi: 10.1249/MSS.0b013e3181949333.
- 506 European Union Council. Directional on the protection of animals used for scientific purposes. 2010,
507 October 20. Official Journal of the European Union.
508 http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm.
- 509 Folch, J., Lees, M., and Sloane Stanley, G.H. 1957. A simple method for the isolation and purification of
510 total lipids from animal tissues. J Biol Chem **226**(1): 497–509. PMID: 13428781
- 511 Galisteo, M., Duarte, J., and Zarzuelo, A. 2008. Effects of dietary fibers on disturbances clustered in the
512 metabolic syndrome. J. Nutr. Biochem. **19**(2): 71–84. doi: 10.1016/j.jnutbio.2007.02.009.
- 513 Galisteo, M., Morón, R., Rivera, L., Romero, R., Anguera, A., and Zarzuelo, A. 2010. Plantago ovata husks-
514 supplemented diet ameliorates metabolic alterations in obese Zucker rats through activation of AMP-
515 activated protein kinase. Comparative study with other dietary fibers. Clin. Nutr. **29**(2): 261–267. doi:
516 10.1016/j.clnu.2009.08.011.
- 517 Gonçalves, I.O., Passos, E., Rocha-Rodrigues, S., Diogo, C.V., Torrella, J.R., Rizo, D., et al. 2014. Physical
518 exercise prevents and mitigates non-alcoholic steatohepatitis-induced liver mitochondrial structural and
519 bioenergetics impairments. Mitochondrion **15**: 40–51. doi: 10.1016/j.mito.2014.03.012.
- 520 Grundy, S.M. 2005. Metabolic Syndrome Scientific Statement by the American Heart Association and the
521 National Heart, Lung, and Blood Institute. Arterioscler. Thromb. Vasc. Biol. **25**(11): 2243–2244. doi:
522 10.1161/01.ATV.0000189155.75833.c7.

- 523 Guo, Z., Ran, Q., Roberts, L.J., Zhou, L., Richardson, A., Sharan, C., et al. 2008. Suppression of
524 atherogenesis by overexpression of glutathione peroxidase-4 in apolipoprotein E-deficient mice. *Free*
525 *Radic. Biol. Med.* **44**(3): 343–352. doi: 10.1016/j.freeradbiomed.2007.09.009.
- 526 Haram, P.M., Kemi, O.J., Lee, S.J., Bendheim, M., Al-Share, Q.Y., Waldum, H.L., et al. 2009. Aerobic
527 interval training vs. continuous moderate exercise in the metabolic syndrome of rats artificially selected
528 for low aerobic capacity. *Cardiovasc. Res.* **81**(4): 723–732. doi: 10.1093/cvr/cvn332.
- 529 Haus, J.M., Solomon, T.P.J., Kelly, K.R., Fealy, C.E., Kullman, E.L., Scelsi, A.R., et al. 2013. Improved
530 hepatic lipid composition following short-term exercise in nonalcoholic fatty liver disease. *J. Clin.*
531 *Endocrinol. Metab.* **98**(7): E1181–E1188. doi: 10.1210/jc.2013-1229.
- 532 Hey-Mogensen, M., Jeppesen, J., Madsen, K., Kiens, B., and Franch, J. 2012. Obesity augments the age-
533 induced increase in mitochondrial capacity for H₂O₂ release in Zucker fatty rats. *Acta Physiol.* **204**(3):
534 354–361. doi: 10.1111/j.1748-1716.2011.02347.x.
- 535 Huang, C.-C., Huang, W.-C., Yang, S.-C., Chan, C.-C., and Lin, W.-T. 2013. *Ganoderma tsugae*
536 hepatoprotection against exhaustive exercise-induced liver injury in rats. *Molecules.* **18**(2): 1741–1754.
537 doi: 10.3390/molecules18021741.
- 538 Imai, H., and Nakagawa, Y. 2003. Biological significance of phospholipid hydroperoxide glutathione
539 peroxidase (PHGPx, GPx4) in mammalian cells. *Free Radic. Biol. Med.* **34**(2): 145–169. PMID:
540 12521597
- 541 Jenkins, N.T., Witkowski, S., Spangenburg, E.E., and Hagberg, J.M. 2009. Effects of acute and chronic
542 endurance exercise on intracellular nitric oxide in putative endothelial progenitor cells: role of NADPH
543 oxidase. *Am. J. Physiol. Heart Circ. Physiol.* **297**(5): H1798–1805. doi: 10.1152/ajpheart.00347.2009.
- 544 Jiang, H.K., Wang, Y.H., Sun, L., He, X., Zhao, M., Feng, Z.H., et al. 2014. Aerobic Interval Training
545 Attenuates Mitochondrial Dysfunction in Rats Post-Myocardial Infarction: Roles of Mitochondrial
546 Network Dynamics. *Int. J. Mol. Sci.* **15**(4): 5304–5322. doi: 10.3390/ijms15045304.
- 547 Johnson, N.A., Sachinwalla, T., Walton, D.W., Smith, K., Armstrong, A., Thompson, M.W., et al. 2009.
548 Aerobic exercise training reduces hepatic and visceral lipids in obese individuals without weight loss.
549 *Hepatology.* **50**(4): 1105–1112. doi: 10.1002/hep.23129.

- 550 Jung, J.H., and Kim, H.S. 2013. The inhibitory effect of black soybean on hepatic cholesterol accumulation
551 in high cholesterol and high fat diet-induced non-alcoholic fatty liver disease. *Food Chem. Toxicol.* **60**:
552 404–412. doi: 10.1016/j.fct.2013.07.048.
- 553 Juraschek, S.P., Selvin, E., Miller, E.R., Brancati, F.L., and Young, J.H. 2013. Plasma lactate and diabetes
554 risk in 8,045 participants of the Atherosclerosis Risk in Communities (ARIC) study. *Ann Epidemiol.*
555 **23**(12): 791–796. doi:10.1016/j.annepidem.2013.09.005
- 556 Kapravelou, G., Martínez, R., Andrade, A.M., Sánchez, C., Chaves, C.L., López-Jurado, M., et al. 2013.
557 Health promoting effects of Lupin (*Lupinus albus* var. *multolupa*) protein hydrolyzate and insoluble fiber
558 in a diet-induced animal experimental model of hypercholesterolemia. *Food Res. Int.* **54**(2): 1471–1481.
559 doi: 10.1016/j.foodres.2013.10.019.
- 560 Kaur, J. 2014. A Comprehensive Review on Metabolic Syndrome. *Cardiol. Res. Pract.* **2014**: e943162. doi:
561 10.1155/2014/943162.
- 562 Kibenge, M.T., and Chan, C.B. 2002. The effects of high-fat diet on exercise-induced changes in metabolic
563 parameters in Zucker fa/fa rats. *Metabolism* **51**(6): 708–715. doi: 10.1053/meta.2002.32727.
- 564 Kistler, K.D., Brunt, E.M., Clark, J.M., Diehl, A.M., Sallis, J.F., and Schwimmer, J.B. 2011. Physical
565 Activity Recommendations, Exercise Intensity, and Histological Severity of Nonalcoholic Fatty Liver
566 Disease. *Am. J. Gastroenterol.* **106**(3): 460–468. doi: 10.1038/ajg.2010.488.
- 567 Kucera, O., and Cervinkova, Z. 2014. Experimental models of non-alcoholic fatty liver disease in rats.
568 *World. J. Gastroenterol.* **20**(26): 8364-8376. doi: 10.3748/wjg.v20.i26.8364.
- 569 Larsen, S., Danielsen, J.H., Søndergaard, S.D., Søgaard, D., Vigelse, A., Dybboe, R., et al. 2015. The effect
570 of high-intensity training on mitochondrial fat oxidation in skeletal muscle and subcutaneous adipose
571 tissue. *Scand. J. Med. Sci. Sports.* **25**(1):e59-69. doi: 10.1111/sms.12252.
- 572 Lawrence, R., Sunde, R., Schwartz, G., and Hoekstra, W. 1974. Glutathione peroxidase activity in rat lens
573 and other tissues in relation to dietary selenium intake. *Exp Eye Res.* **18**: 563–569. PMID: 4852169
- 574 Lee, S., Deldin, A.R., White, D., Kim, Y., Libman, I., Rivera-Vega, M., et al. 2013. Aerobic exercise but not
575 resistance exercise reduces intrahepatic lipid content and visceral fat and improves insulin sensitivity in
576 obese adolescent girls: a randomized controlled trial. *Am. J. Physiol. - Endocrinol. Metab.* **305**(10):
577 E1222–E1229. doi: 10.1152/ajpendo.00285.2013.

- 578 Linden, M.A., Fletcher, J.A., Morris, E.M., Meers, G.M., Laughlin, M.H., Booth, F.W., et al. 2015. Treating
579 NAFLD in OLETF Rats with Vigorous-Intensity Interval Exercise Training. *Med. Sci. Sports Exerc.*
580 **47**(3): 556–567. doi: 10.1249/MSS.0000000000000430.
- 581 Lochhead, P.A., Salt, I.P., Walker, K.S., Hardie, D.G., and Sutherland, C. 2000. 5-aminoimidazole-4-
582 carboxamide riboside mimics the effects of insulin on the expression of the 2 key gluconeogenic genes
583 PEPCK and glucose-6-phosphatase. *Diabetes*. **49**(6):896-903. PMID: 10866040
- 584 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. 1951. Protein Measurement with the Folin
585 Phenol Reagent. *J. Biol. Chem.* **193**(1): 265–275. PMID: 14907713
- 586 Marchesini, G., Bugianesi, E., Forlani, G., Cerrelli, F., Lenzi, M., Manini, R., et al. 2003. Nonalcoholic fatty
587 liver, steatohepatitis, and the metabolic syndrome. *Hepatology* **37**(4): 917–923.
588 doi: 10.1053/jhep.2003.50161.
- 589 Metz, L., Vermaelen, M., Lambert, K., Broca, C., Sirvent, P., Raynaud, E., et al. 2005. Endurance training
590 increases lactate transport in male Zucker fa/fa rats. *Biochem. Biophys. Res. Commun.* **331**(4): 1338–
591 1345. doi: 10.1016/j.bbrc.2005.04.054.
- 592 Morris, E.M., Jackman, M.R., Johnson, G.C., Liu, T.-W., Lopez, J.L., Kearney, M.L., et al. 2014. Intrinsic
593 aerobic capacity impacts susceptibility to acute high-fat diet-induced hepatic steatosis. *Am. J. Physiol.*
594 *Endocrinol. Metab.* **307**(4): E355–E364. doi: 10.1152/ajpendo.00093.2014.
- 595 Morris, R.T., Fine, D.M., Lees, S.J., Booth, F.W., Link, C.D., Ferrario, C.M., et al. 2007. Exercise training
596 prevents development of cardiac contractile dysfunction in hypertensive TG(mREN2)27 rats. *J. Am. Soc.*
597 *Hypertens.* **1**(6): 393–399. doi: 10.1016/j.jash.2007.09.001.
- 598 Neuschwander-Tetri, B.A., Brunt, E.M., Wehmeier, K.R., Oliver, D., and Bacon, B.R. 2003. Improved
599 nonalcoholic steatohepatitis after 48 weeks of treatment with the PPAR-gamma ligand rosiglitazone.
600 *Hepatology*. **38**(4): 1008–1017. doi: 10.1053/jhep.2003.50420.
- 601 *Nutrient Requirements of Laboratory Animals, Fourth Revised Edition, 1995.*
- 602 Ogonovszky, H., Sasvári, M., Dosek, A., Berkes, I., Kaneko, T., Tahara, S., et al. 2005. The effects of
603 moderate, strenuous, and overtraining on oxidative stress markers and DNA repair in rat liver. *Can. J.*
604 *Appl. Physiol.* **30**(2): 186–195. doi: 10.1139/h05-114.

- 605 Polyzos, S.A., Kountouras, J., and Zavos, C. 2009. Nonalcoholic Fatty Liver Disease: The Pathogenetic
606 Roles of Insulin Resistance and Adipocytokines. *Curr. Mol. Med.* **9**(3): 299–314. doi:
607 10.2174/156652409787847191.
- 608 Praphatsorn, P., Thong-Ngam, D., Kulaputana, O., and Klaikeaw, N. 2010. Effects of intense exercise on
609 biochemical and histological changes in rat liver and pancreas. *Asian Biomedicine.* **4**(4): 619–625.
- 610 Prieto, P.G., Cancelas, J., Villanueva-Peñacarrillo, M.L., Valverde, I., and Malaisse, W.J. 2004. Plasma D-
611 glucose, D-fructose and insulin responses after oral administration of D-glucose, D-fructose and sucrose
612 to normal rats. *J. Am. Coll. Nutr.* **23**(5): 414–419. doi: 10.1080/07315724.2004.10719386
- 613 Qi, Z., He, J., Zhang, Y., Shao, Y., and Ding, S. 2011. Exercise training attenuates oxidative stress and
614 decreases p53 protein content in skeletal muscle of type 2 diabetic Goto-Kakizaki rats. *Free Radic. Biol.*
615 *Med.* **50**(7): 794–800. doi: 10.1016/j.freeradbiomed.2010.12.022.
- 616 Ramos, D., Martins, E.G., Viana-Gomes, D., Casimiro-Lopes, G., and Salerno, V.P. 2013. Biomarkers of
617 oxidative stress and tissue damage released by muscle and liver after a single bout of swimming exercise.
618 *Appl. Physiol. Nutr. Metab.* **38**(5): 507–511. doi: 10.1139/apnm-2012-0302.
- 619 Reaven, G. 2004. The metabolic syndrome or the insulin resistance syndrome? Different names, different
620 concepts, and different goals. *Endocrinol. Metab. Clin. North Am.* **33**(2): 283–303. doi:
621 10.1016/j.ecl.2004.03.002.
- 622 Reeves, P.G., Nielsen, F.H., and Fahey Jr, G.C. 1993. AIN-93 purified diets for laboratory rodents: final
623 report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-
624 76A rodent diet. *J Nutr* **123**(11): 1939–1951. PMID: 8229312
- 625 Ristow, M., Zarse, K., Oberbach, A., Klötting, N., Birringer, M., Kiehntopf, M., et al. 2009. Antioxidants
626 prevent healthpromoting effects of physical exercise in humans. *Proc Natl Acad Sci USA.* **106**(21): 8665–
627 8670. doi: 10.1073/pnas.0903485106.
- 628 Rivera, L., Morón, R., Zarzuelo, A., and Galisteo, M. 2009. Long-term resveratrol administration reduces
629 metabolic disturbances and lowers blood pressure in obese Zucker rats. *Biochem Pharmacol.* **77**(6):1053-
630 1063. doi: 10.1016/j.bcp.2008.11.027.
- 631 Rolo, A.P., Teodoro, J.S., and Palmeira, C.M. 2012. Role of oxidative stress in the pathogenesis of
632 nonalcoholic steatohepatitis. *Free Radic. Biol. Med.* **52**(1): 59–69. doi:
633 10.1016/j.freeradbiomed.2011.10.003.

- 634 Rosety-Rodriguez, M., Rosety, I., Fornieles-Gonzalez, G., Diaz-Ordenez, A.J., Camacho, A., Rosety, M.A.,
635 et al. 2012. A 6-week training program increased muscle antioxidant system in elderly diabetic fatty rats.
636 *Med. Sci. Monit.* **18**(9): BR346–BR350. doi: 10.12659/MSM.883343.
- 637 Sasaki, T., Nakata, R., Inoue, H., Shimizu, M., Inoue, J., and Sato, R. 2014. Role of AMPK and PPAR γ 1 in
638 exercise-induced lipoprotein lipase in skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* **306**(9):
639 E1085–1092. doi: 10.1152/ajpendo.00691.2013.
- 640 Shin, Y.-A., Lee, J.-H., Song, W., and Jun, T.-W. 2008. Exercise training improves the antioxidant enzyme
641 activity with no changes of telomere length. *Mech. Ageing Dev.* **129**(5): 254–260. doi:
642 10.1016/j.mad.2008.01.001.
- 643 Sneddon, A.A., Wu, H.-C., Farquharson, A., Grant, I., Arthur, J.R., Rotondo, D., et al. 2003. Regulation of
644 selenoprotein GPx4 expression and activity in human endothelial cells by fatty acids, cytokines and
645 antioxidants. *Atherosclerosis.* **171**(1): 57–65. doi: 10.1016/j.atherosclerosis.2003.08.008
- 646 Soltys, K., Dikdan, G., and Koneru, B. 2001. Oxidative stress in fatty livers of obese Zucker rats: Rapid
647 amelioration and improved tolerance to warm ischemia with tocopherol. *Hepatology* **34**(1): 13–18. doi:
648 10.1053/jhep.2001.25452.
- 649 Souto, G., Donapetry, C., Calviño, J., and Adeva, M.M. 2011. Metabolic acidosis-induced insulin resistance
650 and cardiovascular risk. *Metab Syndr Relat Disord.* **9**(4):247-253. doi: 10.1089/met.2010.0108.
- 651 Souza-Mello, V. 2015. Peroxisome proliferator-activated receptors as targets to treat non-alcoholic fatty liver
652 disease. *World J Hepatol.* **7**(8):1012-1019. doi: 10.4254/wjh.v7.i8.1012.
- 653 Sullivan, S., Kirk, E.P., Mittendorfer, B., Patterson, B.W., and Klein, S. 2012. Randomized trial of exercise
654 effect on intrahepatic triglyceride content and lipid kinetics in nonalcoholic fatty liver disease.
655 *Hepatology.* **55**(6): 1738–1745. doi: 10.1002/hep.25548.
- 656 Tariq, Z., Green, C.J., and Hodson, L. 2014. Are oxidative stress mechanisms the common denominator in
657 the progression from hepatic steatosis towards non-alcoholic steatohepatitis (NASH)? *Liver Int.*
658 **34**(7):e180-90. doi: 10.1111/liv.12523.
- 659 Tjønnå, A.E., Lee, S.J., Rognmo, Ø., Stølen, T.O., Bye, A., Haram, P.M., et al. 2008. Aerobic interval
660 training versus continuous moderate exercise as a treatment for the metabolic syndrome. A pilot study.
661 *Circulation* **118**(4): 346–354. doi: 10.1161/CIRCULATIONAHA.108.772822.

- 662 Ukeda, H., Maeda, S., Ishii, T., and Sawamura, M. 1997. Spectrophotometric assay for superoxide dismutase
663 based on tetrazolium Salt 3'-{1-[(Phenylamino)-carbonyl]-3,4-tetrazolium}-bis(4-methoxy-6-
664 nitro)benzenesulfonic acid hydrate reduction by xanthine-xanthine oxidase. *Anal. Biochem.* **251**(2): 206-
665 209. doi: 10.1006/abio.1997.2273.
- 666 Videla, L.A., Rodrigo, R., Orellana, M., Fernandez, V., Tapia, G., Quiñones, L., et al. 2004. Oxidative stress-
667 related parameters in the liver of non-alcoholic fatty liver disease patients. *Clin. Sci.* **106**(3): 261-268. doi:
668 10.1042/CS20030285.
- 669 Wisløff, U., Helgerud, J., Kemi, O.J., and Ellingsen, Ø. 2001. Intensity-controlled treadmill running in rats:
670 VO_{2max} and cardiac hypertrophy. *Am. J. Physiol. Heart Circ. Physiol.* **280**(3): H1301-H1310. PMID:
671 11179077
- 672

673 **Table 1.** Details of the AIT protocol.

| Week (5 days/week) | Work Time (min/day) | % VO ₂ max |
|-----------------------|------------------------|-----------------------|
| 1 | 45' | 50%→3 min |
| | | 65%→4 min |
| 2 | 50' | 55%→3 min |
| | | 70%→4 min |
| 3 | 50' | 60%→3 min |
| | | 75%→4 min |
| 4 | 55' | 60%→3 min |
| | | 75%→4 min |
| 5-8 | 60' | 65%→3 min |
| | | 80%→4 min |

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675

676 **Table 2.** Sequence of primers used in RT-PCR

| Gene | Primer's 5'-3' sequence | |
|-------|-------------------------|----------------------|
| GPX1 | Forward | CACCGAAATGAATGATCTGC |
| | Reverse | TGTATCTGCGCACTGGAACA |
| GPX4 | Forward | CCGGCTACAATGTCAGGTTT |
| | Reverse | CGGCAGGTCCTTCTCTATCA |
| GAPDH | Forward | ATGGGAAGCTGGTCATCAAC |
| | Reverse | GTGGTTCACACCCATCACAA |

677 GPX1: Glutathione peroxidase 1; GPX4: Glutathione peroxidase 4.

678 **Table 3.** Effect of AIT protocol on aerobic capacity and physical performance of lean and obese Zucker rats.

| | Lean | | Obese | | SEM | R ² | Phenotype Effect | Exercise Effect | Phenotype × Exercise |
|---|-------------------|-------------------|-------------------|-------------------|------|----------------|---------------------|--------------------|----------------------------|
| | Sedentary | Exercise | Sedentary | Exercise | | | | | |
| Exercise Lactate (mmol/L) | 6.8 ^A | 6.2 ^A | 15.7 ^C | 10.4 ^B | 0.79 | 0.7822 | P < 0.0001 | P = 0.0003 | P = 0.0076 |
| VO₂max (mL/min/kg^{0.75}) | 18.7 ^A | 19.9 ^A | 17.7 ^A | 19.5 ^A | 1.01 | 0.1049 | P = 0.3318 | P = 0.1380 | P = 0.7525 |
| Running Time (min) | 13.3 ^B | 23.6 ^C | 7.6 ^A | 11.2 ^B | 0.72 | 0.9204 | P < 0.0001 | P < 0.0001 | P < 0.0001 |
| Maximal Speed (cm/sc) | 55.8 ^B | 85.6 ^C | 38.9 ^A | 49.8 ^B | 2.2 | 0.9161 | P < 0.0001 | P < 0.0001 | P = 0.0002 |

679 ^{A,B,C} Results are mean of 8-10 rats. Means within the same row with different superscripts differ significantly (P < 0.05). SEM, pooled standard error of the mean.

Table 4. Effect of AIT protocol on plasma parameters of lean and obese Zucker rats.

| | Lean | | Obese | | SEM | R ² | Phenotype Effect | Exercise Effect | Phenotype × Exercise |
|--------------------------------|--------------------|--------------------|--------------------|--------------------|-------|----------------|------------------|-----------------|----------------------|
| | Sedentary | Exercise | Sedentary | Exercise | | | | | |
| Glucose (mg/dL) | 177.6 ^A | 238.3 ^A | 400.2 ^B | 341.9 ^B | 30.5 | 0.5114 | P<0.0001 | P=0.9679 | P=0.0607 |
| Insulin (ng/mL) | 0.062 ^A | 0.126 ^A | 0.685 ^B | 0.558 ^B | 0.081 | 0.6929 | P < 0.0001 | P = 0.6687 | P = 0.1992 |
| AUC (arbitrary units) | 2417 ^A | 2516 ^A | 7054 ^B | 2599 ^A | 301 | 0.8470 | P < 0.0001 | P < 0.0001 | P < 0.0001 |
| T-Cholesterol (mg/dL) | 74.9 ^A | 74.2 ^A | 209.2 ^C | 167.5 ^B | 6.1 | 0.9224 | P<0.0001 | P=0.0016 | P=0.0021 |
| LDL-Cholesterol (mg/dL) | 4.9 ^A | 3.4 ^A | 19.9 ^C | 11.8 ^B | 1.27 | 0.7772 | P<0.0001 | P=0.0007 | P=0.0148 |
| HDL-Cholesterol (mg/dL) | 27.5 ^A | 29.4 ^A | 51.0 ^B | 45.3 ^B | 3.88 | 0.4619 | P<0.0001 | P=0.6331 | P=0.3395 |
| Triglycerides (mg/dL) | 100.2 ^A | 26.5 ^A | 279.3 ^B | 246.2 ^B | 29.9 | 0.6025 | P<0.0001 | P=0.0845 | P=0.5051 |

^{A,B,C} Results are mean of 8-10 rats. Means within the same row with different superscripts differ significantly (P < 0.05). AUC, Area under the curve. SEM, pooled standard error of the mean.

Table 5. Effect of AIT protocol on liver weight, composition, and function of lean and obese Zucker rats.

| | Lean | | Obese | | SEM | R ² | Phenotype Effect | Exercise Effect | Phenotype × Exercise |
|--|---------------------|--------------------|---------------------|--------------------|------|----------------|---------------------|--------------------|-------------------------|
| | Sedentary | Exercise | Sedentary | Exercise | | | | | |
| Weight (g FW) | 8.2 ^A | 9.3 ^A | 17.5 ^B | 16.0 ^B | 0.5 | 0.8738 | P < 0.0001 | P = 0.7508 | P = 0.0255 |
| Surface (cm²) | 12.5 ^A | 13.2 ^A | 22.6 ^B | 20.5 ^B | 0.5 | 0.8450 | P < 0.0001 | P = 0.293 | P = 0.048 |
| Fat (g/100 g DM) | 7.1 ^A | 4.3 ^A | 19.6 ^C | 12.7 ^B | 1.2 | 0.7412 | P < 0.0001 | P = 0.0005 | P = 0.1040 |
| Triglycerides (mg/g DM) | 5.3 ^A | 4.1 ^A | 26.9 ^B | 13.4 ^A | 2.5 | 0.6139 | P < 0.0001 | P = 0.0096 | P = 0.0204 |
| Liver function plasma markers | | | | | | | | | |
| AST (U/L) | 98.4 ^A | 66.9 ^A | 182.3 ^B | 107.3 ^A | 14.7 | 0.5226 | P < 0.0001 | P = 0.0010 | P = 0.1494 |
| ALT (U/L) | 25.7 ^A | 31.8 ^A | 61.0 ^B | 59.7 ^B | 4.7 | 0.5917 | P < 0.0001 | P = 0.6134 | P = 0.4287 |
| ALP (U/L) | 98.2 ^A | 100.7 ^A | 202.6 ^B | 137.6 ^A | 10.5 | 0.6823 | P < 0.0001 | P = 0.0055 | P = 0.0030 |
| GGT (U/L) | 0.10 ^A | 0.70 ^A | 13.9 ^B | 9.3 ^B | 1.56 | 0.6440 | P < 0.0001 | P = 0.2162 | P = 0.1098 |
| Antioxidant enzymes | | | | | | | | | |
| Cu/Zn-SOD (Units/mg protein) | 223.8 ^C | 233.4 ^C | 112.5 ^A | 157.7 ^B | 8.5 | 0.7987 | P < 0.0001 | P = 0.0047 | P = 0.0494 |
| Mn-SOD (Units/mg protein) | 26.8 ^A | 38.2 ^B | 85.8 ^D | 68.9 ^C | 2.6 | 0.9019 | P < 0.0001 | P = 0.4839 | P < 0.0001 |
| Catalase (μmol H₂O₂/min/mg protein) | 487.1 ^{AB} | 551.8 ^B | 503.9 ^{AB} | 461.7 ^A | 19.4 | 0.2803 | P = 0.0630 | P = 0.4260 | P = 0.0053 |
| GPX (nmol NADPH/min/mg protein) | 9.2 ^B | 7.6 ^{AB} | 6.4 ^A | 13.0 ^C | 0.5 | 0.7040 | P = 0.03 | P < 0.0001 | P < 0.0001 |

^{A,B,C,D} Results are mean of 8-10 rats. Means within the same row with different superscripts differ significantly ($P < 0.05$). FW, fresh weight, DM, dry matter, AST, aspartate aminotransferase, ALT, alanine transaminase, ALP, Alkaline Phosphatase, GGT, Gamma-glutamyl transpeptidase, GPX, Glutathione peroxidase. SEM, pooled standard error of the mean.

Table 6. Effect of AIT protocol on liver histology of lean and obese Zucker rats.

| | Microvescicular steatosis | Fatty droplets | Multinucleic cells | Lipogranulomas | Portal inflammation | Necrosis | Fibrosis |
|-----------|----------------------------------|-----------------------|---------------------------|-----------------------|----------------------------|-----------------|-----------------|
| LS | - | - | - | - | -/+ | - | - |
| LE | - | - | - | + | + | -/+ | - |
| OS | ++++ | ++++ | - | +++ | +++ | - | - |
| OE | +++ | +++ | ++ | ++/+++ | ++/+++ | ++ | ++ |

LS, Lean (fa/+) sedentary rats, LE, Lean (fa/+) rats performing a protocol of aerobic interval exercise, OS, Obese (fa/fa) sedentary rats, OE, Obese (fa/fa) rats performing a protocol of aerobic interval exercise. Grading score of the histological alterations: -, non existent; +, mild; ++, mild/moderate; +++, moderate; +++++, abundant; ++++++, severe.

Fig. 1. Effect of AIT protocol on food intake and body weight of lean and obese Zucker rats. (A) Weekly food intake (grams Dry Matter/day). (B) Weekly body weight (grams). Groups: LS, Lean (fa/+) sedentary rats, LE, Lean (fa/+) rats performing aerobic interval exercise, OS, Obese (fa/fa) sedentary rats, OE, Obese (fa/fa) rats performing aerobic interval exercise. Values are means \pm SEM depicted by vertical bars (n = 8-10).

Fig. 2. Effect of AIT protocol on blood glucose levels of lean and obese Zucker rats prior to or at different time points after oral glucose overload. Groups: LS, Lean (fa/+) sedentary rats, LE, Lean (fa/+) rats performing aerobic interval exercise, OS, Obese (fa/fa) sedentary rats, OE, Obese (fa/fa) rats performing aerobic interval exercise. Values are means \pm SEM depicted by vertical bars (n = 8-10). The following notation is used to express significant differences ($P < 0.05$) between groups pointed out by Dunnett's t-test: a, OS vs LS, b, OE vs LS, c, LE vs LS.

Fig. 3. Effect of AIT protocol on liver morphology of lean and obese Zucker rats. Groups: LS, Lean (fa/+) sedentary rats, LE, Lean (fa/+) rats performing aerobic interval exercise, OS, Obese (fa/fa) sedentary rats, OE, Obese (fa/fa) rats performing aerobic interval exercise. Photographs are representative of livers of 8-10 different rats for each experimental group.

Fig. 4. Effect of AIT protocol on GPX1 and GPX4 mRNA levels in liver of lean and obese Zucker rats. Hepatic GPX1 and GPX4 mRNA relative expression. Groups: LS, Lean (fa/+) sedentary rats, LE, Lean (fa/+) rats performing aerobic interval exercise, OS, Obese (fa/fa) sedentary rats, OE, Obese (fa/fa) rats performing aerobic interval exercise. GPX1 and GPX 4 levels are expressed as percentage of the mean value obtained from liver of the LS group (100%). Results represented in the graphs are means \pm SEM depicted by vertical bars (n = 10). Means within the same gene expression without a common letter differ, $P < 0.05$. Image of gel used for determination of GPX1 and GPX4 expression by semiquantitative RT-PCR is representative of RNA samples of 8-10 rats for each experimental group; all samples were derived at the same time and processed in parallel. The samples were analyzed for expression of GAPDH, GPX1, and GPX4. GAPDH expression was not different among the experimental groups.

Fig. 5. Effect of AIT protocol on liver histology of lean and obese Zucker rats. (A) Histological view of control LS liver HE stain, (B) Histological view of LE liver HE stain, (C) Histological view of OS liver HE stain with clear signs of microvesicular steatosis (mv) and fatty droplet accumulation (fd), (D) Histological view of OE liver HE stain with diminished signs of microvesicular steatosis (mv) and fatty droplet accumulation (fd), (E) Histological view of control LS liver MT stain, (F) Histological view of LE liver MT

stain, (G) Histological view of OS liver MT stain, (H) Histological view of OE liver MT stain with signs of fibrosis (fb). Groups: LS, Lean (fa/+) sedentary rats, LE, Lean (fa/+) rats performing aerobic interval exercise, OS, Obese (fa/fa) sedentary rats, OE, Obese (fa/fa) rats performing aerobic interval exercise. Photographs are representative of livers of 8-10 different rats for each experimental group.

Fig. 6. Effect of AIT protocol on AMPK α /PAMPK α and PPAR γ protein expression in the liver of lean and obese Zucker rats. Western blot analysis of (A) AMPK α /PAMPK α and (B) PPAR γ expression. Groups: LS, Lean (fa/+) sedentary rats, OS, Obese (fa/fa) sedentary rats, LE, Lean (fa/+) rats performing aerobic interval exercise, OE, Obese (fa/fa) rats performing aerobic interval exercise. Immunoblots are representative of liver homogenates from eight different rats for each experimental group; two samples of each experimental group were loaded per gel and processed in parallel. The amount of sample loaded per lane was 100 μ g of protein for AMPK α /PAMPK α and 80 μ g of protein for PPAR γ . Levels of PAMPK were normalized to the total AMPK. Levels of PPAR γ were normalized to ponceau reagent. Densitometric analysis values represented in the graphs are means \pm SEM depicted by vertical bars (n = 8). Means without a common letter differ, P < 0.05.

Figure 1

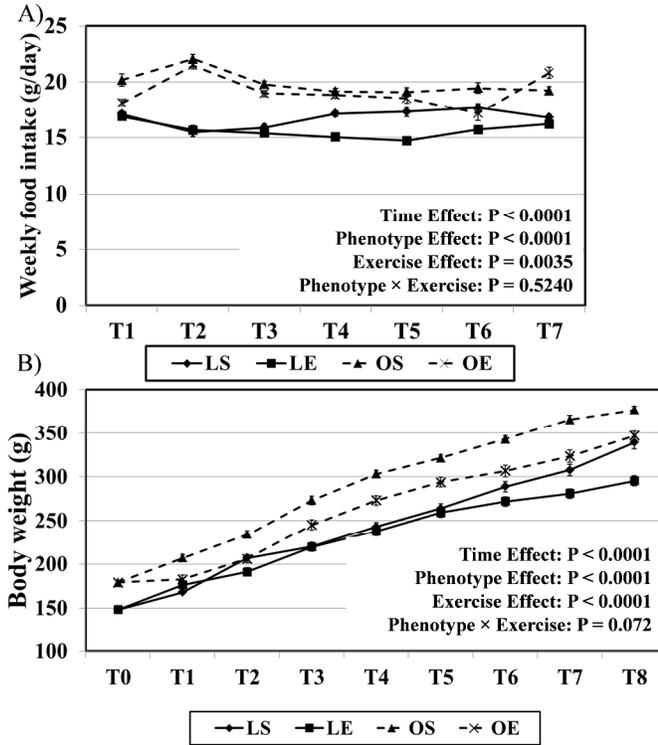


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Figure 2

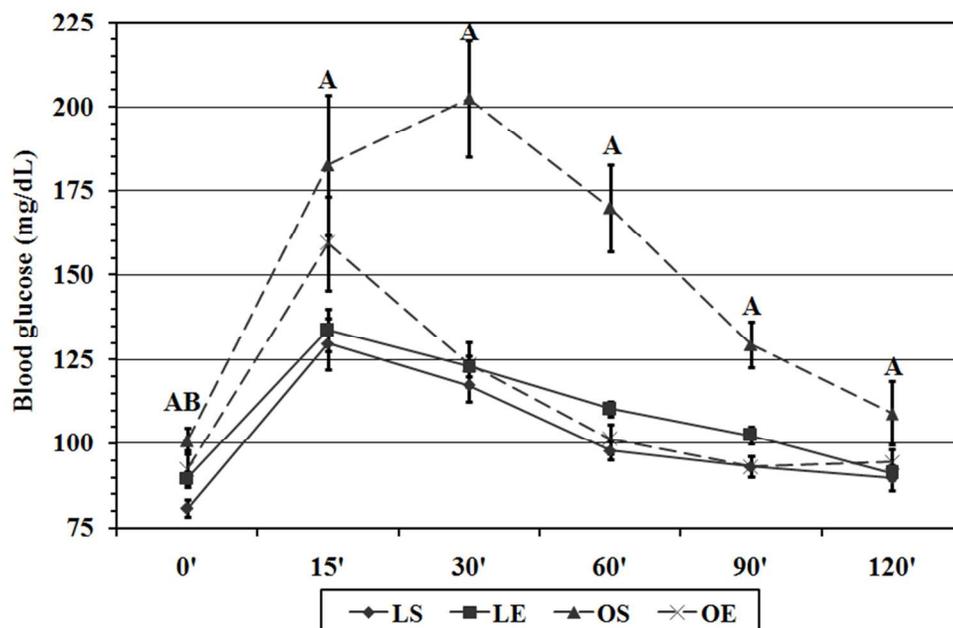


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Figure 3

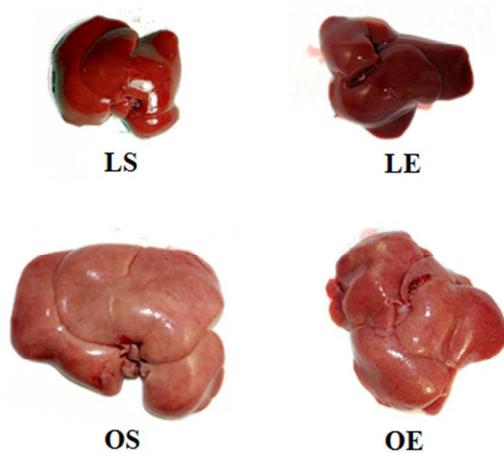


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Figure 4

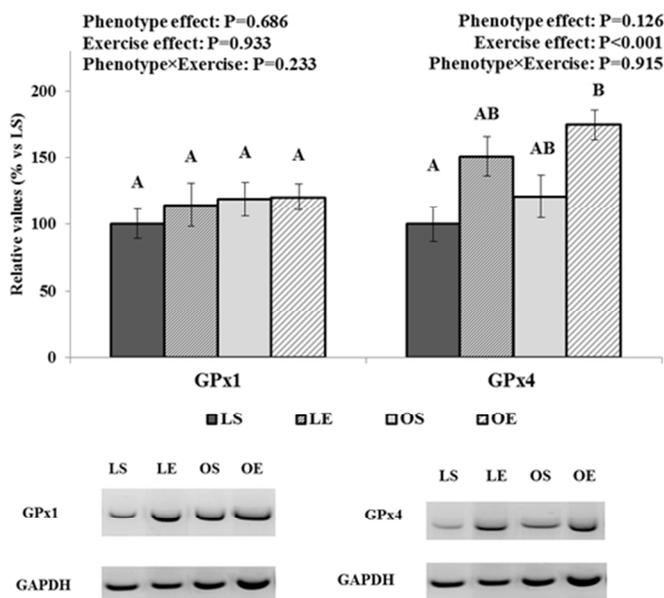


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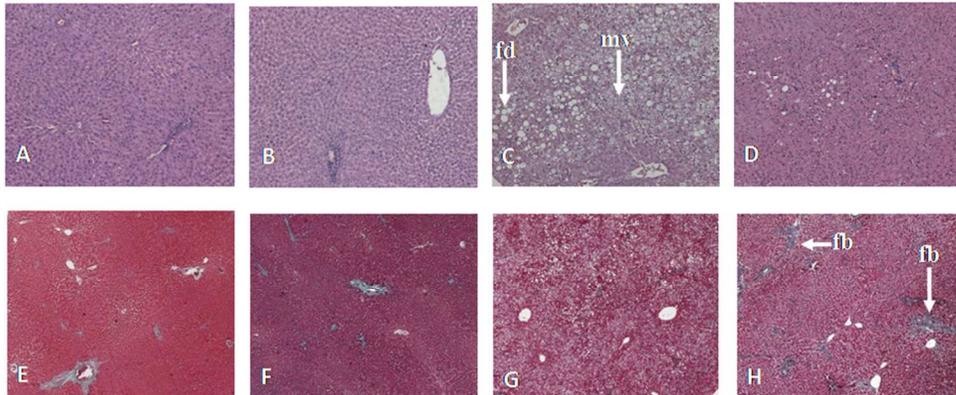
Figure 5

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Figure 6

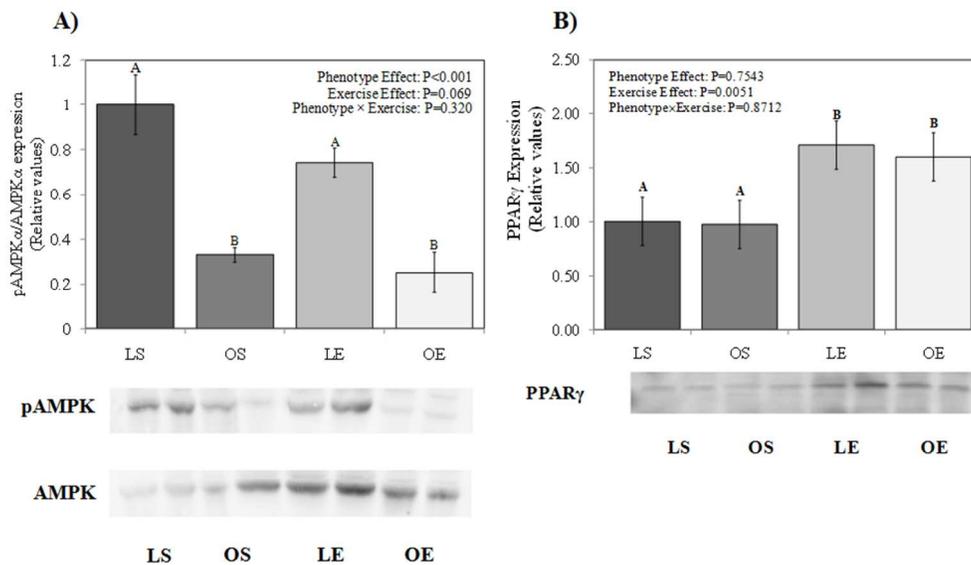


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