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Exercise Attenuates Ischemia-reperfusion Injury of Nonalcoholic Fatty Liver in OLETF Rat

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Abstract

Background: Steatotic livers are increasingly used for liver transplantation and the strategy to minimize its disadvantage is required. We determined whether exercise could protect liver against ischemia-reperfusion (IR) injury in the anesthetized hyperphagic, Otsuka Long-Evans Tokushima Fatty (OLETF) rat, an animal model of the metabolic syndrome.

Methods: At 16th week of age, male OLETF rats (n=7/group) were randomized to the groups of sedentary (OLETF-SED)-IR, exercise (OLETF-EX)-IR, and OLETF-non IR. Non-hyperphagic, control strain Long-Evans Tokushima Otsuka (LETO) rats were similarly assigned. Rats in the exercise groups were subjected to treadmill running 6 days a week for 5 weeks. Rats were anesthetized with intraperitoneal pentobarbital and subjected to partial liver ischemia (70%) for 30 min and subsequent reperfusion for 120 min. IR injury was evaluated by plasma alanine aminotransferase (ALT)concentration, bile flow rate, liver histopathology, and hepatic microcirculation of the left lateral lobe analyzed by laser Doppler flowmetry.

Results: Increases in body and liver weight were attenuated in the OLETF-EX rats as compared with the OLETF-SED rats. The ALT levels after reperfusion in the OLETF-EX rats were smaller than that in the OLETF-SED rats, and were comparable to those in the LETO-SED and -EX rats. Exercise attenuated the IR-induced reduction of liver blood flow and morphological hepatic damages as observed in the OLETF-SED group. In contrast, exercise provided no influences on the LETO rats.

Conclusion: Exercise attenuated the liver steatosis and IR-induced disturbances of hepatic microcirculation and parenchymal injury in the hyperphagic, OLETF rats.

Key words: nonalcoholic fatty liver disease; OLETF rat; physical activity; steatotic liver.

Introduction

Hepatic ischemia-reperfusion (IR) injury is commonplace in liver transplantation [1] and provides successful therapy for end-stage liver diseases [2-5]. It has been postulated that fatty livers from obesity individuals are less tolerant of IR injury, leading to worse clinical outcome [6,7]. Currently, steatotic livers are increasingly used [4] because of the donor organ shortage [2-5]. The clinical and experimental attempts have been proposed to minimize the disadvantage of steatotic livers. These strategies may comprise donor weight loss through exercise, diet and modified food, employing drug therapy, heat shock preconditioning, ischemia preconditioning, selective anesthesia on donors and the treatment on isolated grafts during preservation [8]. In this respect, exercise training and increasing physical activity have been shown to attenuate fatty liver of animal models [9, 10]. Furthermore, physical exercise combined with an anti-hyperlipidaemia drug successfully reduced macrosteatosis of obese human living donors [11].

However, there is no animal experimental study, which determines whether exercise per se improves the IR injury of steatotic livers of obesity. Therefore, we determined the effects of exercise on IR injury of fatty livers from hyperphagic Otsuka Long-Evans Tokushima Fatty (OLETF) rats, an established animal model of obesity and non-insulin-dependent diabetes mellitus (NIDDM) [12]. The OLETF rats have a mutated and functionally inoperative cholecystokinin-1 receptor [13], which leads to hyperphagia, resulting in obesity and non-alcoholic fatty liver [14].

Materials and Methods

Animals

Male OLETF rats (105±2 g, n=21) and non-hyperphagic, Long-Evans Tokushima Otsuka (LETO) rats, as control group rats (91±1 g, n=21) of 5 week old were purchased from Japan SLC (Shizuoka, Japan). OLETF rats are models of metabolic syndrome and obesity due to hyperphagia and are deficient in the cholecystokinin (CCK-A) receptor gene [12]. All rats were maintained at 23°C and under pathogen-free conditions on a 12:12-hour dark/light cycle and allowed food and water ad libitum. The present experiments were approved by the Animal Research Committee of Kanazawa Medical University.

Exercise training

Treadmill training began at the age of 16 week old in OLETF (n=7) and LETO (n=7) rats, following familiarization of the rats with the apparatus for 4 days by placing them on the motor-driven treadmill (KN-73, Natsume, Tokyo, Japan). Exercise

training was given for 6 days/week for 5 weeks. In the first week, rats were exercised on the treadmill at a speed of 15 m/min for 20 min/day. In the 2nd week, the running speed and time were increased to 20 m/min and 30 min/day, respectively. From the 3rd to 5th week, the running speed and time were 23 m/min and 40 min/day, respectively.

Surgical procedure

Rats were anesthetized with pentobarbital sodium (50 mg/kg, ip) and placed supinely on a heating pad (ATC-101B; Unique Medical, Japan) that maintained body temperature at 37°C throughout the experiment. The adequacy of anesthesia was monitored by the stability of blood pressure and respiration under control conditions and during a pinch of the hindpaw. Supplemental doses of anesthetic (10% of the initial dose) were given intraperitoneally as necessary. The trachea was intubated to facilitate spontaneous breathing. The right jugular vein was catheterized with a polyethylene tube (ID 0.4mm, OD 0.6mm) for measurement of the central venous pressure (CVP) at expiration. The right femoral artery and vein were also catheterized with polyethylene tubes (ID 0.3mm, OD 0.5mm) for measurement of the mean systemic arterial pressure (MAP) and continuous infusion of saline (10 ml/kg/h), respectively. Following a midline incision of the abdominal wall, the common bile duct was cannulated with a polyethylene catheter (OD 0.4 mm) for the collection of bile. Bile was continuously collected in a plastic tube and the bile weight was measured at 15 min intervals for determination of the bile flow rate, as a parameter of hepatocyte function. After dissection of the falciform ligament, the afferent vessels to the median and left lateral lobes were exposed. Liver microvascular perfusion was measured using the laser Doppler flowmetry (LBF-16, Biomedical Science, Kanazawa, Japan), the probe of which was placed on the surface of the left lateral lobe.

The MAP and CVP were continuously measured with pressure transducers (TP-400T, Nihon-Kohden, Japan), and the reference level was set at the level of right atrium. Heart rate (HR) was measured by triggering the systemic arterial pressure. These hemodynamic variables along with bile weight were digitally displayed and recorded at 40 Hz by PowerLab (AD Instruments, Castle Hill, Australia). The signals from the laser Doppler probe were recorded and converted to the digital data with the laser Doppler flowmetry.

Experimental protocol

At the age of 16 week old, male OLETF rats (n=7/group) were randomized to 3 groups of the sedentary rats with IR (OLETF-SED-IR) and without IR (OLETF-SED-nonIR), as well as the exercise rats with IR (OLETF-EX-IR). The LETO rats were similarly assigned to 3 groups of LETO-EX-IR, LETO-SED-IR, and LETO-SED-nonIR.

After the baseline measurement, an atraumatic vascular clip was applied to the afferent vessels to the median and left lateral lobes to induce partial hepatic ischemia (70%) for 30 min in the IR groups, and no clip in the nonIR groups. At the end of the ischemic period, the clip was removed, and subsequent reperfusion was initiated. After 120 min of reperfusion, animals were euthanized by exsanguination. The blood was immediately centrifuged, while the left hepatic lobe was excised, fixed in 4% buffered formaldehyde, and routinely processed for hematoxylin and eosin staining of paraffin sections (4 μm). The severity of IR injury was graded using Suzuki's criteria [15]. In this classification, sinusoidal congestion, hepatocyte necrosis and ballooning degeneration are graded on a scale of 0-4. No necrosis, congestion, or centrilobular ballooning is given a score of 0, whereas severe congestion/ballooning and >60% lobular necrosis are given a value of 4 each.

Liver injuries were evaluated by the following four procedures: 1) blood concentration of alanine aminotransferase (ALT) was determined with a commercially available kit (Wako Pure Chemical Industries, Osaka, Japan) by spectrophotometric method, 2) bile flow rate, which could indicate hepatocellular activity, 3) liver microvascular perfusion, and 4) histology of the liver.

Data analysis

All results are expressed as the means ± SE. For laser Doppler flowmetry signals, results are expressed as percentages of baseline values the mean value over 10 min around the time point being reported. Statistical analyses were performed with the analysis of variance and a P value less than 0.05 was considered significant. When a significant difference was obtained, the Fisher *post hoc* test was performed.

Results

The body weight of rats at 16th week before the start of exercise protocols was significantly greater in the OLETF rats (497±6 g, n=21) than in the LETO rats (403±3 g, n=21). However, there were no significant differences in the body weight among 3 groups of OLETF or LETO rats. Exercise reduced both body and liver weight in the OLETF rats compared with those of the sedentary OLETF rats, while it did not affect those of the LETO rats (Figure 1).

Hemodynamic variables of MAP, CVP and HR at baseline did not differ among any groups studied. Any of these variables did not significantly change during ischemia or after reperfusion.

Figure 2 shows the results of plasma ALT levels. In the sedentary rats, IR induced a significantly greater increase in plasma ALT level of OLETF rats than that of LETO rats (997±178 vs. 557±101 IU/ml, 60 min; 1740±443 vs. 939±197 IU/ml, 120 min), although the basal levels were not significantly different (48±3 vs. 52±2 IU/ml). Exercise attenuated significantly the IR-induced increase in the ALT level of OLETF rats, but not that of LETO rats (466±105 vs. 598±112, 60 min; 725±159 vs. 847±191, 120 min), as compared with the sedentary rats, and these levels of exercise rats were apparently similar between OLETF and LETO rats. The nonIR rats of the OLETF-SED-nonIR or LETO-SED-nonIR group showed no significant changes in ALT levels throughout the experimental period.

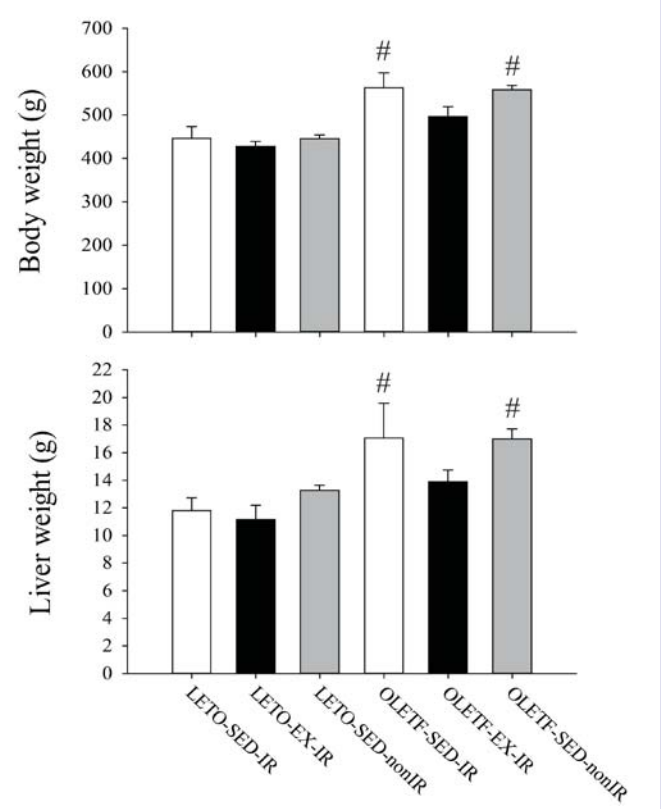

Figure 1: The body weight and liver weight of the LETO and OLETF rats in each group. Values are means±SE. #P < 0.05 vs. all groups except the OLETF-SED-IR and the OLETF-SED-nonIR groups.

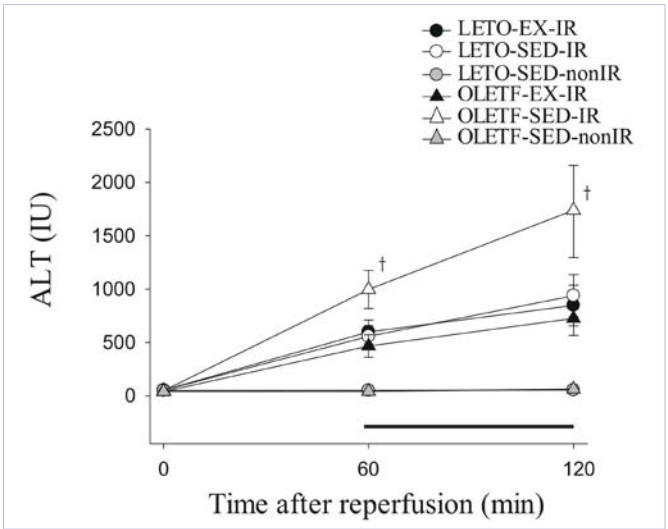

Figure 2: The plasma ALT concentrations in the LETO-EX-IR (closed circle, n=7), LETO-SED-IR (open circle, n=7), LETO-SED-nonIR (gray circle, n=7), OLETF-EX-IR (closed triangle, n=7), OLETF-SED-IR (open triangle, n=7), and OLETF-SED-nonIR (gray triangle, n=7) groups. Values are means±SE. Each point of variables during the time periods indicated by the black bar is significantly different from the corresponding baseline values except for the non-IR groups. †P < 0.05 vs. all the other groups.

The baseline bile flow rates did not differ significantly among any groups studied and it was 0.34±0.01 g/15 min (n=42). The bile flow rate in any IR groups decreased similarly after reperfusion, followed by a return towards the baseline level at 120 min after reperfusion. The recovery of the bile flow after reperfusion in the OLETF-SED-IR group tended to be attenuated compared with that in the OLETF-EX-IR group, but there were no significant differences among IR groups studied (Figure 3A).

Figure 3B shows the summarized data of liver blood flow, expressed as % of baseline. In any IR groups, liver blood flow reduced to almost zero after the start of ischemia. The liver blood flow began to return towards baseline after reperfusion, but it remained below the baseline at the end of the experimental period. The liver blood flow during post-ischemia reperfusion

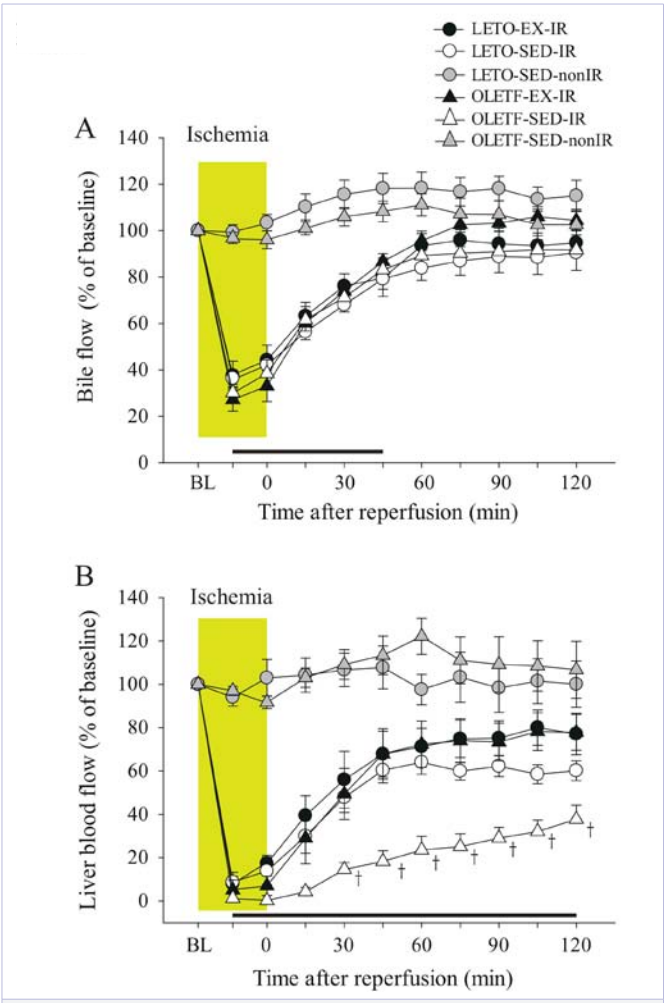

Figure 3: Time course changes in bile flow (A) and liver blood flow (B) in the LETO-EX-IR (closed circle, n=7), LETO-SED-IR (open circle, n=7), LETO-SED-nonIR (gray circle, n=7), OLETF-EX-IR (closed triangle, n=7), OLETF-SED-IR (open triangle, n=7), and OLETF-SED-nonIR (gray triangle, n=7) groups. Values are means±SE. Each point of variables during the time periods indicated by the black bar is significantly different from the corresponding value in the control group except for the non-IR groups. †P < 0.05 vs. all the other groups.

was significantly lower in the OLETF-SED-IR group than in other IR groups (Fig, 3B). Actually, exercise attenuated the IR induced decrease in liver tissue blood flow of the OLETF rats to the extent similar to that of the LETO rats.

The livers in the OLTF-SED-IR showed severe disruption of lobular architecture with ballooning change, hemorrhage, and hepatocyte necrosis (Figure 4a). In contrast, the livers in the OLETF-EX-IR group revealed only necrotic foci, but no lobular ballooning changes (Figure 4b). Similar findings were obtained in the LETO-SED-IR and LETO-EX-IR groups (Figures 4d and 4e). IR-related changes were not observed in either the OLETF-SED- nonIR or LETO-SED-nonIR group (Figure 4c and 4f). The total Suzuki scores of the OLETF-SED-IR, OLETF-EX-IR, LETO-SED-IR and LETO-EX-IR groups were 3.8±0.8, 1.4±0.4, 1.8±0.2 and 1.5±0.4, respectively. Exercise therefore, led to a significant reduction in Suzuki scores of OLETF rats but not LETO rats.

Discussion

In the present study, we found that the treadmill-running exercise attenuated development of steatotic liver, as well as excessive body weight gain in the metabolic syndrome model rats of OLETF rats, and thereby attenuated the IR-induced hepatic damage, as assessed by blood ALT levels, hepatic microcirculation and liver histology. These results suggest that exercise is a good strategy to obtain a hepatic graft of better quality for liver transplantation from the obesity persons with fatty livers.

Liver transplantation is a crucial therapy for end-stage liver diseases, but it is restricted by a huge donor deficit [2-5]. The shortage of donor livers has resulted in an increased use of livers with steatosis, a metabolic abnormality that increases the likelihood of primary non-function (PNF) in the allograft and poorer outcome [16]. The method development to rescue steatotic donor livers before transplantation has been required. Physical exercise combined with an anti-hyperlipidaemia drug successfully reduced macrosteatosis on obese human living donors [11]. In this respect, the present study is the first animal experimental investigation to demonstrate the efficacy of exercise per se to attenuate the IR injury of the steatotic livers from OLETF rats, although exercise in the form of running wheel activity significantly reduced the degrees of body weight gain and adiposity, and prevented the development of NIDDM in OLETF rats [17, 18].

The beneficial effects of exercise for the prevention of steatotic liver using obesity model rats were reported [17-19]. Several mechanisms for the exercise-induced prevention of obesity and steatotic liver in the OLETF rats could be provided as follows. Firstly, Rector et al. [19] reported that daily physical activity attenuated hepatic steatosis and nonalcoholic fatty liver disease via alteration of fatty acid synthesis in OLETF rats. Biochemical study revealed that exercise increased the percent of palmitate which oxidized completely to CO_2 but did not alter AMP-activated protein kinase (AMPK)-alpha or AMPK phosphorylation status, and that it reduced fatty acid synthase and acetyl-coenzyme A carboxylase (ACC) content, resulting in the elevation of ACC phosphorylation and cytochrome c content. They concluded that the effect of exercise is likely mediated, in part, by enhancement of hepatic fatty acid oxidation and reductions in key protein intermediates of fatty acid synthesis

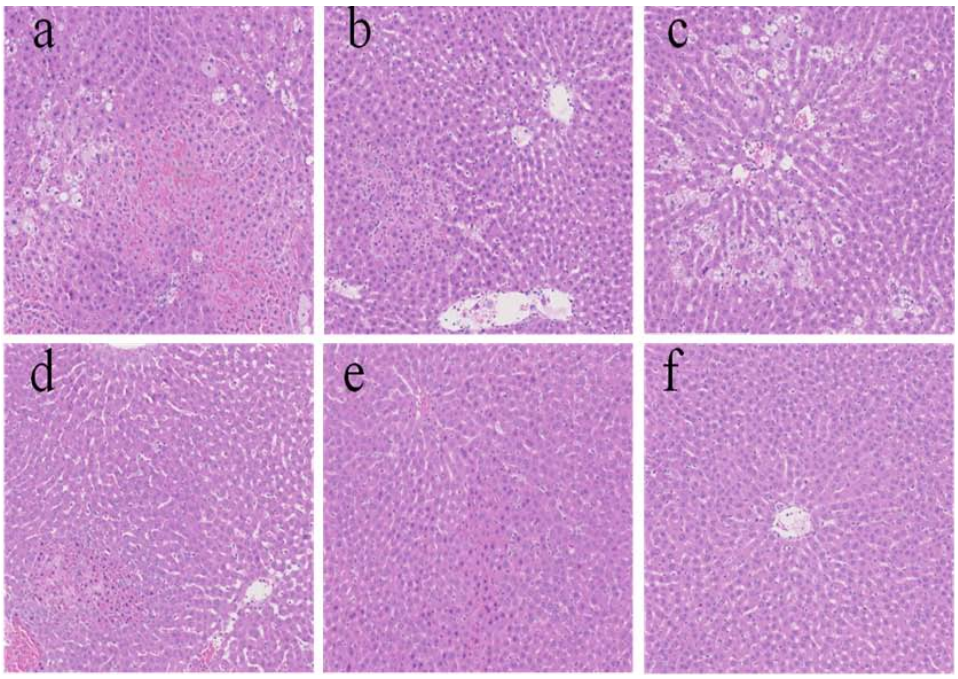

Figure 4: Liver histology of the OLETF-SED-IR (a), OLETF-EX-IR (b), OLETF-SED-nonIR (c), LETO-SED-IR (d), LETO-EX-IR (e) and, LETO-SED-nonIR (f) groups.

[19]. Second, there is a possibility that exercise reduces the increase in food intake which is the characteristics of the OLETF rat. Actually, feeding OLETF rats with the same amount of food as the LETO control rats completely prevented their obesity and increased fat deposition [20]. The exercise-induced decrease in food intake by OLETF rat is evidenced by changes in hypothalamic expression of the genes which is responsible for the food intake [13, 21]. Bi et al. [21] have demonstrated that exercise limits the elevation of expression of neuropeptide Y, a feeding peptide regulating energy balance, in the dorsomedial hypothalamus (DMH), resulting in the suppression of hyperphagia and obesity of OLETF rats. In addition, following running wheel exercise, mRNA expression of DMH corticotrophin releasing factor, which could induce anorexia, was elevated in OLETF rats [21]. Finally, the changes in body weight in OLETF rats might have been simply the result of increased energy expenditure.

Bile flow is an established marker of liver function in IR models [22-25]. In the present study, the bile flow recovery after reperfusion in the OLETF-EX-IR tended to be better as compared with the OLETF-SED-IR group, but statistical significance could not be obtained. This finding was not consistent with the results on hepatic damage parameters such as elevated plasma ALT levels, liver histological scores and hepatic microcirculatory disturbance, all of which were significantly greater in the OLETF-SED-IR group than in the LETO-SED-IR group. One of the reasons for the absence of significant difference in post-reperfusion bile flow rate between the OLETF-SED-IR and the other IR groups may be related to the method to collect the bile. In the present study, the bile catheter was not inserted deep enough to collect the bile selectively from the ischemic lobes of the bilateral median and left lateral lobes [25]: the bile catheter tip was not positioned just at the bile duct bifurcation coming from the bilateral median and left lateral lobes but from the non-ischemic lobes of the right lateral and caudate lobes. Therefore, the bile we collected came from both of ischemic and non-ischemic lobes. This contamination from non-ischemic origin might account for the insensitivity of bile flow to severity of IR damages. Of note, in a similar fatty liver IR injury study, the extent and rate of recovery of bile flow during 60-min reperfusion in steatotic livers were similar to those in lean livers [26]. Thus, the bile flow changes may not be sensitive to IR injury of the fatty livers.

The strength of exercise adopted in this study, 23 m/min, 40 min/day, 6 days/wk for the last 3 week, was smaller than that of a previous study for Sprague Dawley rats, 30 m/min, 30 min/day [27]. This exercise protocol was similar to the exercise training (20 m/min, 60 min/day, 5 days/wk treadmill running) previously used for OLETF rats [28]. The intensity of the present final exercise was considered to be in the range of moderate and approximately 60% of the rat maximal oxygen consumption [29]. This physical activity level was expected to achieve similar benefits to those reported in NIDDM patients [30].

Recently the detrimental effects of exercise have been reported in rat models [31, 32]. Li et al [31] reported that rats which had exercise training only for 5 days showed oxidative stress-induced liver damage when the rats were forced to run in a treadmill for 1 hour at 13 m/min until exhaustion. Ramos et al. [32] also reported that an exposure of rats to single bout of swimming exercise causes elevation of plasma liver enzymes and oxidative stress biomarkers. However, these studies used rats which were not well exercise trained and subjected to acute high intensity exercise. These experimental protocols were different from the present study. Actually, the baseline of plasma ALT levels for the OLETF-EX-IR and LETO-EX-IR were not elevated (Figure 2).

In summary, we examined whether the tread mill exercise confers protection against IR injury of the fatty liver in the animal model of the metabolic syndrome, OLETF rats, as compared with the control LETO rats. Exercise reduced the increase in body and liver weight of the OLETF rats, to the degrees comparable to those of the LETO rats. Consequently in the OLETF rats, exercise attenuated the IR-induced hepatic damages, as evidenced by elevated blood ALT levels, reduced bile flow rate, hepatic microcirculatory disturbance and hepatic morphological changes, to the levels similar to those in LETO rats. These results suggest that exercise could be a strategy to provide liver of better quality for transplantation from the obesity persons with steatotic liver.

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