Kawasaki Journal of Medical Welfare Vol.1 No.1 1995 137～143

Original Paper

Influence of Exercise Training and 48-hour Fasting on the Liver Triglyceride Content in Young Rats

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(Accepted October 30, 1995)

Key words : swimming, fasting, triglyceride(TG) secretion, free fatty acid(FFA).

Abstract

The effect of exercise training and 48-hour fasting on the liver triglyceride(TG) content in male Wistar rats (n=56) was investigated. The rats were divided into four groups: 1) trained for six weeks and fasted for 48 hours , 2) trained for six weeks and fed ad libitum, 3) untrained and fasted for 48 hours, and 4) untrained and fed ad libitum. In addition, the rats in groups 1 and 3 were exercised to an acute swimming state for 120 minutes. Although the training did not affect plasma free fatty acid (FFA) and TG concentrations, it induced a significant decrease in the TG content in the liver ($p<0.01$). Fasting affected the liver TG content ($p<0.05$) and plasma FFA ($p<0.01$) and TG ($p<0.05$) concentrations in rats. In the 48h-fasting state, plasma TG values in the trained and untrained animals were unchanged by acute swimming, but the plasma FFA concentration was increased by acute exercise ($p<0.01$). Acute exercise caused a significant elevation in the liver TG content in the fasted untrained group ($p<0.05$), but the liver TG in the fasted trained rats did not change. In addition, the TG secretion rate in the fasting state was significantly higher in the trained group than in the untrained group after acute swimming ($p<0.01$). These results suggest that exercise training decreases liver TG content, and that the TG content in the liver of trained rats does not increase during exercise, regardless of fasting treatment.

Introduction

It is well known that hepatic glycogenolysis and lipolysis in adipose tissue become elevated in a fasting state[1,2]. MacKay[3] reported that liver triglyceride (TG) content in the rat was increased by fasting. One of the systems of TG storage in the liver during fasting regulates excess plasma free fatty acid (FFA). Accumulation of TG in the liver with chronic starvation, however, causes damage resulting in a fatty liver[4]. Additionally, in a fasting state, endurance exercise requires lipids for energy to heart and skeletal muscle[5]. We and others have reported that liver TG content is increased by prolonged exercise in the fasting state[6,7]. In both in vivo and in vitro experiments, we and others have also demonstrated that the increase in liver TG content is accelerated by an increase in the supply of FFA[6,7,8,9]. However, Gorski and Kiryluk[10] reported that during recovery liver TG content returned promptly to its pre-exercise value despite the fact that the level of plasma FFA was still elevated.

The effects of exercise training on fat metabolism in the liver have been reviewed[11]. Endurance training appears to influence the TG output from the liver and the removal of TG from the circulation[12]. Exercise training[13] and/or fasting[5] also increase the removal of plasma TG by lipoprotein lipase (LPL) activity to heart and skeletal muscle. In the fasting state, however, it is unclear whether accumulation of liver TG occurs in exercise-trained rats. The hypothesis was that the secretion of TG as very low density lipoprotein (VLDL) from the liver into circulation increases without augmentation of liver TG content and the plasma TG concentration during exercise in the fasting state. To investigate the effect of training and fasting, exercise-trained rats were fasted for 48h and then were required to swim.

Materials and Methods

Animals.

Three-week-old male Wistar rats (n=49) were maintained in climate-controlled quarters (20-22°C), and provided with pellet rat chow and water ad libitum. The experimental procedures were carried out according to the guiding principles for the Care and Use of Animals in the Field of Physiological Sciences approved by the council of the Physiological Society of Japan.

Swim training.

After seven days, the rats were divided into trained and untrained groups. With the trained rats, a swimming exercise in water maintained at 32-35°C for 75 min per a day was carried out five days per week for six weeks. A weight equivalent to 2% of the rat's body weight was attached to the tail of each rat. Our experience has been that rats swim more vigorously, when three of them swim together in a pool (27×38×50cm), as they were trained to do in this study. To acclimate the untrained rats to water, they were allowed to swim for 5-15 min, two days per week, until they were well accustomed to swimming. After each training session, the rats were dried with towels.

Acute swimming.

After three days, the trained and untrained animals were randomly divided into two groups, with a fed group (n=10), which was fasted for only 6h, and a fasted group (n=15), which was fasted for 48h. The fasted rats swam for 120 min in water maintained at 33-34°C.

Analysis.

Four or five animals in each group were sacrificed before the acute exercise to obtain pre-exercise data, and an additional four or

five were sacrificed after the exercise. All animals were sacrificed under pentobarbital anesthesia (60mg·kg^{-1}) and blood was collected from the abdominal aorta. The liver was washed by injection of physiological saline (4ml·min^{-1}) into the portal vein with a peristaltic pump. Then the liver was removed and immediately frozen and stored at −40°C until it was assayed.

In five animals in both the trained and untrained fasted groups, the TG secretion into the rat's plasma was assayed using Triton WR-1339 (300mg·ml^{-1})[14]. Triton inhibits the removal of TG from circulation by interrupting the contact of lipoprotein-TG with LPL activity. Triton (600mg·kg^{-1}) was injected into the external iliac vein. Blood samples were taken from the tail vein at 0, 30, and 60 min after the animals, which had finished swimming, were anesthetized with pentobarbital (30mg·kg^{-1}). The samples and sample times were used to determine TG secretion by the following equation:

$$\text{TG secretion} = \frac{\frac{TG_{30}-TG_0}{30}+\frac{TG_{60}-TG_0}{60}}{2}\times 60$$

TG_0, TG_{30} and TG_{60} indicate TG concentrations in plasma collected at 0, 30 and 60 min after the Triton injection.

Assay.

Plasma FFA and TG concentrations were determined using an analysis kit (Wako Pure Chemical Ind., Ltd., Osaka, Japan). TG in the liver was analyzed by the chromatropic sulfuric acid method[15] after total lipid was extracted by the Folch method[16].

Statistics.

All results were presented as the means ± SEM. The differences were analyzed using a two-way analysis of variance (ANOVA) to test the effects of training and 48h fasting as well as their interaction. The differences in

Table 1 Characteristics of rats in the four groups.

	trained fed	trained fast	untrained fed	untrained fast
n	10	9	10	10
body mass (g)**††	329.7±6.7	293.7±7.6	364.4±10.5	320.2±8.1
liver wet wt (g)**††	14.5±0.8	9.3±0.3	16.8± 1.0	11.1±0.4
liver/body mass (%)††	4.4±0.2	3.2±0.0	4.6± 0.2	3.5±0.1

Values are means ± SEM.
* Statistically significant training effect: **p<0.01
† Statistically significant fasting effect: ††p<0.01

Table 2 Effect of exercise training and fasting on liver TG content and plasma FFA and TG concentrations in rats.

	trained fed	trained fast	untrained fed	untrained fast
n	10	9	10	10
liver TG**†	7.76±0.75	12.57±1.55	17.62±0.75	19.26±1.80
plasma FFA††	0.44±0.06	1.06±0.08	0.59±0.09	1.02±0.09
plasma TG†	75.95±10.35	39.67±3.66	64.40±8.66	61.20±6.37

Values are means ± SEM.; liver TG-μmol·g^{-1}, FFA-mEq·l^{-1}, plasma TG-mmol·l^{-1}
* Statistically significant training effect: **p<0.01
†Statistically significant fasting effect: † p<0.05 and †† p<0.01

Table 3 Effect of acute exercise on liver TG and plasma FFA and TG in trained and untrained rats in the fasting state.

	trained		untrained	
	pre	post	pre	post
n	4	5	5	5
liver TG	12.60±3.30	12.54±1.42	15.27±0.97	21.26±2.85†
plasma FFA	0.86±0.10	1.22±0.06††	0.76±0.04	1.29±0.04††
plasma TG	33.25±2.11	44.80±5.54	60.10±7.41	62.30±11.27

Values are means ± SEM.
† Statistically significant acute exercising effect (versus the pre-exercise values in each group): † $p<0.05$ and †† $p<0.01$

the effect of the acute exercise in the fasting state and the differences in TG secretion (n= 5) were analyzed using both parametric (two sample T test) and nonparametric (Mann-Whitney U test) approaches, and the results were consistent.

Results

Training of the rats affected body mass, but not relative liver wet wt. Fasting affected both body mass and liver wet wt (Table 1). The plasma FFA and TG concentrations were unchanged by training. However, the liver TG content was significantly lower in the trained rats than in the untrained group ($p<0.01$). Fasting produced significant elevations in the liver TG and the plasma FFA, and a significant reduction in the plasma TG (Table 2).

In the 48h-fasting state, the acute swimming exercise increased the plasma FFA in both the trained and untrained rats ($p<0.01$). The liver TG in the fasted trained group was not increased by the acute exercise, but that in the untrained group was ($p<0.05$). The plasma TG was unchanged in both of these groups (Table 3). However, the TG secretion from the liver was significantly higher in the fasted trained animals than in the fasted untrained group following the acute swimming ($p<0.01$, Fig. 1).

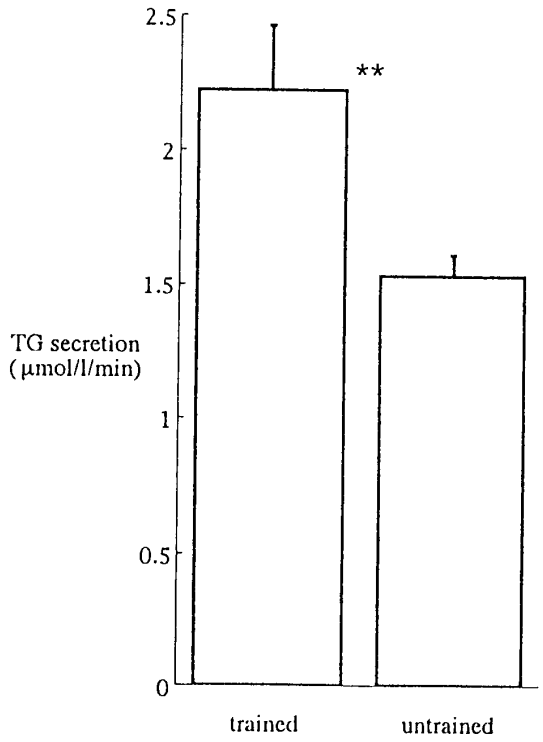

Fig. 1 Effect of exercise training on TG secretion in the fasted rats from immediately to 60 min after acute swimming. Values are the means ± SEM. The significance of the differences between the values in the trained and untrained rats: **$p<0.01$

Discussion

During prolonged exercise in a fasting condition, lipolysis in adipose tissue increases and glycogenolysis in the liver decreases[17]. The level of fatty acid in blood regulates TG

production by the liver[9]. In this study using trained rats, however, despite an increase in both the plasma FFA concentration during exercise and the plasma level in untrained rats, an increase in the TG content in the liver was not observed in the fasting state (Table 3). The result in the trained rats did not agree with the relationship between plasma FFA concentration and liver TG content. Since the review by Brindley[4], it has been considered that the accumulation of TG in the liver increases because the supply of FFA to the liver exceeds its need for energy production via β-oxidation. The excess acids and their acyl-CoA esters are potentially toxic, so their conversion into TG makes it possible for them to be stored temporarily in a safe form. However, if this condition becomes chronic, the result is a fatty liver. Accordingly, our results seemed to indicate that TG was not required to be in the liver of the fasted trained rats during exercise, despite excess fatty acid.

Other investigations suggested that TG production by the liver depends on the availability of fatty acid to the liver and the hepatic capacity to esterify the available fatty acid to glycerol[8,18]. However, the esterification of fatty acid in the liver was reported to be unaffected by training[18]. The absence of change in the liver TG content of the fasted trained rats might have been due to change in the rate of TG secretion from the liver. An increase in the concentration of plasma FFA was reported to cause a rise in TG secretion from the liver into circulation[13]. The liver from fed animals, however, secretes TG as VLDL at a higher rate than that from fasted animals[19]. In addition, it has been demonstrated that fasting decreases total VLDL and apoprotein secretion from the liver[20]. We also reported that TG secretion from the liver into circulation was decreased during prolonged exercise in fasted rats[7]. In the trained rats in this study, however, the TG secretion was higher than that in the untrained rats after acute exercise following 48h fasting state. Thus, the TG secretion of the trained and untrained rats differed following acute swimming in the fasting state. Glyceride synthesis in the liver by the glycerol-3-phosphate pathway has been reported to be unaffected by training[8,18]. If true, the increased TG secretion in our trained rats might have been accelerated by lipoprotein synthesis in the liver, but this was not investigated in the present study.

In the 48h fasting state, the TG secretion of the trained rats increased after acute swimming as compared with that of the untrained rats. However, the plasma TG concentration did not increase. LPL activity, which is enhanced in heart or skeletal muscle by training[13], might explain this finding. Furthermore, muscle contractions also increase the uptake of circulating TG during fasting[5]. Inhibition of an increasing plasma TG level by high LPL activity might lead to greater TG secretion from the liver. Training appears to influence the amount of TG output from the liver and its removal from the circulation[8]. Taken together, the secretion of TG from the liver in the trained rats increases without augmentation of liver TG and plasma TG during exercise in the fasting state.

Summarizing the data obtained, we concluded that exercise training inhibits accumulation of TG in the liver and that liver TG content in trained rats is not increased by a high level of TG secretion from the liver during exercise, regardless of fasting treatment.

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