Research Article

Effect of Exercise on the Distribution of Triglycerides among Various Tissues of Swiss Albino Mice

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Abstract

Triglyceride (TG) is an important source of fuel during endurance exercise. Lipolysis of adipose tissue and intramuscular triacylglycerol oxidation increases during exercise. In compare with moderate-intensity exercise, high-intensity exercise decreases fat oxidation. For many years it has been debated whether triacylglycerols located in the muscle are utilized during exercise because conflicting results have been appeared. To conduct this research, 8 (eight) male mouse were taken as samples and four mouse were divided into two groups as control (sedentary life) and experimental (routine exercise in treadmill). After one month of close supervision, the mouse were seizured and collect the sample (liver, epidedymal adipose tissue, skeletal muscle) for further experiment. We analyzed the sample and estimate the TG (triglycerides) content from the sample with standard GPO-POD method. In this study, we found that compare with sedentary mouse, exercise group slightly increased liver weight and epididymal adipose tissue TG whereas gastrocnemius muscle weight increased significantly because of exercise induced muscle storage. On the other hand, in compare with body weight, epididymal adipose tissue weight and gastrocnemius muscle TG decreased significantly. There is no alteration in liver weight of both group. Finally, we demonstrate that exercise increased the skeletal muscle weight of trained mice and decreased the TG accumulation in gastrocnemius skeletal muscle and this findings is inversely correlated with insulin resistance.

Keywords: Exercise, Triglycerides, Adipose Tissue, Skeletal muscle.

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Introduction

Exercise is one type of organized physical activity [(1)]. During exercise, energy is mainly supplied from the utilization of carbohydrate and lipid fuels, with a much smaller contribution from protein [(2-3)]. Fat is an important metabolic substrate during prolonged exercise [(4)]. During aerobic exercise, as intensity increases and the contribution of fat to energy production decreases [(5)]. Once exercise stops, fat oxidation is increased during the post-exercise period [(6-7)]. Little information is available on daily rates of fat oxidation with aerobic exercise, although Goldberg *et al.* (1990) observed no difference in 24h RQ with three different levels of aerobic activity [(8)]. Nevertheless, it appears that aerobic exercise can increase total fat oxidation. It has been strongly suggested that low-intensity aerobic activity is best for promoting fat oxidation. Physical exercise stimulates lipolysis in adipose tissue by increasing adipose tissue LPL activity [(9-15)]. M. Snel et.al proposed, exercise has little effect on body weight and depletion of intracellular lipid stores but does improve skeletal muscle insulin sensitivity [(16)]. When exercise intensity increases, alternative sources of fat are used (e.g, other adipose sites or intramuscular fat) [(17)]. Insulin resistance and type 2 diabetes mellitus (T2DM) are positively correlated with the accumulation of triglycerides (TG) in non-adipose tissue especially in liver, skeletal muscle, heart and pancreas [(18-21,16)]. M. Snel et. al. (2012) suggest that diet and exercise can improve the deposition of TG in non-adipose tissue [(16)]. Some studies have shown a less consistency or no effect in IMCLs accumulation [(22-24)], whereas other studies have shown a decreased [(25-26)], or even an increased [(27)] effect. Depending on TG accumulation, the effect of exercise varies with different organs. During exercise intramyocellular lipids (IMCLs) in muscle can either increase [(28-30)] or decrease [(25)] but insulin sensitivity is improved. Endurance-trained athletes have increased IMCLs stores which are used as a readily available energy source during exercise [(18)]. Skeletal muscle cell contains a considerable amount of triglycerides. An increased skeletal muscle TG content is a strong marker of insulin resistance [(31-34)]. Lipoprotein lipase (LPL) activity increases with the depletion of muscle TG [(35)]. On the other hand, a balanced diet with exercise reduce hepatic TG content and thus improves hepatic insulin sensitivity [(36)], but it is not clear whether exercise alone can decrease hepatic TG or not [(37-38)]. Previously exercise trained rats are not protected against adipocyte fat accumulation whether they ingest a standard or a high-fat diet [(39)]. But effects of high-fat diet (HFD) without training induced accumulation of fat in the liver and adipose tissue in female rats. In compare with inactive and sedentary rats, previously trained rats that have been inactive for a while deposit a higher TG in response to a HFD [(40)]. However, it remains unclear whether TG content changes in the skeletal muscles of random exercised mice. Therefore, the aim of the present study is to investigate triglycerides content in various tissues especially gastrocnemius muscle in exercise trained mice. The data will be compared to sedentary mice.

Meterials and Methods

Animals

Swiss Albino mouse (Animal House, Icddr,b, Dhaka, Bangladesh) were obtained at 6 weeks of age and the average initial body weight was approximately 29.3 g. Mice were housed in a cage in a temperature-controlled room at 23ºC with a 12:12-h light-dark cycle. The mice were divided into two groups. One group was taken as control group and the other as experimental group (exercised group). Four male mouse were included in the control group. On the other hand the experimental group consist of another four male mouse. The control group mouse were fed on the balanced diet daily for 4 weeks, whereas the mouse in the experimental group were fed on balance diet for the same amount of time with treadmill exercise. Body weight and food intake were measured at regular intervals throughout the feeding intervention. All experiments conducted in this study were approved by the Animal Care Committee of the Faculty of Science and Technology, Islamic University, Kushtia, Bangladesh.

Diet

The routine diet for both two groups of mouse was normal diet which was purchased from Animal House, Bangladesh Council of Scientific and Industrial Research (BCSIR). The composition of the diet was: Rice polish (20%), Wheat bran (21%), Wheat flour (30%), Protein source (Fish-meal) (10%), Oilseed cake (10%), Molasses (5%), Soybean oil (2%), Common salt (1.5%) and Vitamins (0.5%). From the opening day of the experiment, all of these mouse of these two groups i.e. control and exercised, were fed with normal diet.

Exercise Protocol
Four of the mouse are defined as control group & the mouse of this group led a sedentary life style. And the another four mouse in experimental group exercise everyday with a rolling treadmill in the laboratory. In first week, they do exercise 5min/day, in second week 6min/day, in third week 7min/day and in the last week they do 8min/day.

Anatomical procedure and tissues sampling
After one month of observation, the rats were analyzed. The rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (5 mg/100 g body weight; Abbott, IL, USA). Tissues (Liver, Adipose tissues and skeletal muscles) were sectioned and after wrapping with aluminum foil, tissues were stored at -20°C.

Biochemical measurements
Tissue triglycerides (adipose tissue, liver & skeletal muscle) were measured using standard method at the laboratory of the Applied Nutrition & Food Technology of Islamic University, Kushtia. In this laboratory, it has been done by using commercial kits.

Determination of TG content in tissues
TG contents within tissues were determined following a standard protocol (Chul-Hee et al., 2003). Briefly, certain amount of tissues (~25mg) were extracted with $CHCl_3/CH_3OH$ (1:2 vol/vol) and homogenized. Homogenates were centrifuged at 1500 rpm for 30 minutes at 4^0C, washed with 500 µL ice-cold phosphate buffered saline (PBS) and centrifuged as above. After the addition of 2 ml H_2SO_4, the tubes were vortexed and centrifuged at 1500 rpm for 10 minutes at 4^0C. The upper phase was discarded, and 100 mg $Na_2S_2O_3$ was added to the lower phase. The samples were vortexed and centrifuged at 1500 rpm for 5 minutes at 4^0C. The upper phase was removed, and the lower phase was evaporated under N_2. The samples were dissolved with 70% isopropanol for 10 seconds, and then TG was measured in triplicate using a commercial kit (WAKO TG-1E kit; Wako Pure Chemical Co., Osaka, Japan).

Calculation of GPO-POD method :

$$\frac{\text{(A) Sample} \times 200 \text{ (Standard conc.)}}{\text{(A) Standard}} = \text{mg/dL}$$

Triglyceride in the sample.

Conversion factor: mg/dL x 0.0113 mmol/L

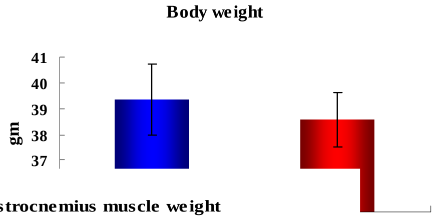
Data analysis
Values represent the mean ± SE. The significance of differences between means was assessed by the *Scheffe* test after analysis of variance had been performed to establish that there were significant differences between the groups.

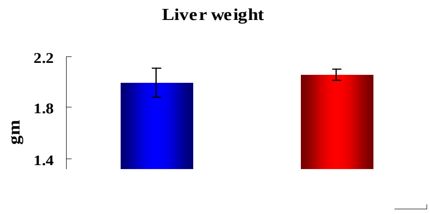
Result:

Body weight, Liver weight, Gastrocnemius muscle weight, Epididymal adipose tissue weight and TG content (100 mg) of liver, gastrocnemius muscle, epididymal adipose tissue from sedentary (control) and exercised (experimental) mouses were analyzed and represents with the bar graphs. Data are expressed as mean ± SEM.*P<0.05 or less vs. Control.

Figure-1 shows that body weight decreased non-significantly due to acute effects of exercise in mice where figure-2 represents that liver weight increased slightly but non-significantly in exercise trained mice. In the figure-3, the weight of gastrocnemius muscle increased significantly in exercised trained muscle but figure-4 shows that epididymal adipose tissue weight decreased significantly in exercise trained mice.

Figure-5 also depicts that acute exercise showed no alteration in TG content in liver of mice. In compare with sedentary mice, figure-6 represents that TG content in gastrocnemius muscle of exercise trained mice decreased significantly where figure-7 shows that TG content in epididymal adipose tissue of exercise trained mice increased non-significantly.





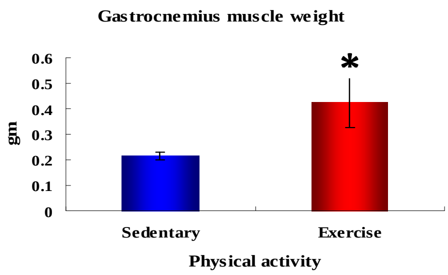


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Fig-3: *Effects of exercise on Gastrocnemius muscle weight of mouses.*

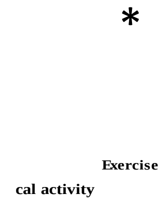
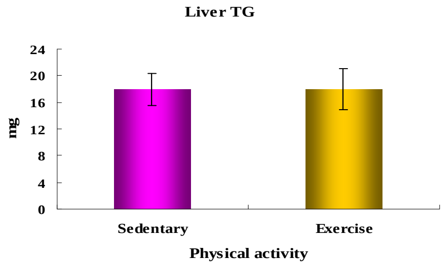


Fig. 4: *Effects of exercise on Epididymal adipose tissue weight of mouses.*



Fia.5: *Effects of exercise on liver TG content in mouses.*

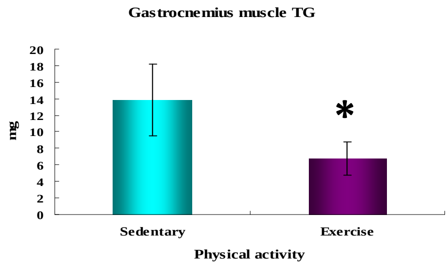


Fig. 6: *Effects of exercise on gastrocnemius muscle TG content in mouses.*

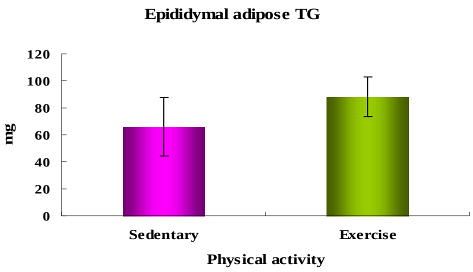


Fig. 7: *Effects of exercise on epididymal adipose tissue TG content in mouses.*

Discussion:

For many years a conflicting results have been debated that intramyocellular triglyceride (IMTG) are used during exercise [41]. A single session of exercise can protect against fatty acid–induced insulin resistance by increasing lipogenic capacity of muscle and a resultant increase in partitioning of excess fatty acids toward triglyceride synthesis in muscle [42]. Whereas prolonged exercise accumulates TG in the liver [43]. It is well established that exercise enhance insulin action in the liver but the benefits of exercise on hepatic insulin action may relate to the potential effects of exercise on regulating/preventing hepatic lipid accumulation [44]. In our research, it has been investigated to find out any relation between the effect of exercise on the bodily system and the distribution of triglycerides in different tissues such as liver, skeletal muscle, adipose tissues etc. In this study, two groups of mouse were taken as control and experimental (exercised) group. After one month of observation, mouse of two groups were analyzed. From the analysis of these data, we found that the body weight of exercised mouse decreased due to acute effect of exercise. The liver weight and gastrocnemius muscle weight of exercised mouse were increased in compared with sedentary mouse. This study also found that exercise has an effect on the deposition of adipose tissues. The epidedymal adipose tissue deposition was increased significantly in sedentary mice but continuous exercise decreases the epidedymal adipose tissue content. This study also conducted the estimation of the triglycerides (TG) content in liver, epidedymal adipose tissue and gastrocnemius muscle of the Swiss Albino mice. The TG content of liver showed no alteration in mice but gastrocnemius muscle TG decreased significantly and apidedymal adipose tissue TG increased slightly in exercised mice compared with sedentary mice. From the overall data analysis, it has been found that there is a significant effect of exercise on the distribution of triglycerides in various tissues.

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