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EFFECT OF PROLONGED EXERCISE ON ESTERIFICATION OF BLOOD-BORNE FREE FATTY ACIDS INTO LIPIDS OF THE RAT LIVER

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Abstract. Prolonged exercise affects metabolism of certain lipid fractions in the liver. The most outstanding example is accumulation of triacylglycerols in the tissue. The aim of the present study was to examine the effect of exercise on esterification of the plasma-borne long-chain fatty acids into different lipid fractions in the liver. The experiments were carried out on three groups of male Wistar rats: 1-control, 2-run 3 h (1200 $m \cdot h^{-1}$, $+10^{o}$ incline), and 3-run as above and recovering 3 h thereafter. ^{14}C-palmitic acid suspended in albumin was administered intravenously at a dose of 20 μCi/100g and the liver samples and the blood from the abdominal aorta were taken 20 min later. The liver lipids were separated into the following fractions: phospholipids, mono-, di-, and triacylglycerols, free fatty acids, cholesterol and cholesterol esters and their radioactivity was counted. The content of phospholipids and triacylglycerols in the liver and the concentration of the blood glucose and plasma free fatty acids was also determined. The exercise reduced radioactivity in the fraction of phospholipids and monoacylglycerols but it did not influence the radioactivity of the other lipid fractions. However, the specific activity of the plasma free fatty acids was much lower, both after exercise and recovery, than at rest. When this is taken into account, it is seen that a considerable elevation in the incorporation of the blood- borne fatty acids into each lipid fraction examined occurred. Formation of cholesterol also increased. During recovery, a tendency to normalization is observed. It is concluded that prolonged exercise of moderate intensity affects the activity of the enzymes responsible for esterification of the plasma-borne free fatty acids and synthesis of cholesterol in the liver cells.

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Key words: Exercise - Liver lipids - Plasma-borne fatty acids - Rat

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Introduction

The liver is the principal organ involved in metabolism of fat. It takes up the blood –borne fatty acids and oxidizes or esterifies them into complex lipids. The liver synthesizes fatty acids, triacylgycerols, phospholipids, cholesterol and lipoproteins and produces ketone bodies [29]. There are some data indicating that prolonged exercise influences the metabolism of certain lipid fractions in the liver. The total lipid content in the liver was shown to remain stable after exercise [3,10]. So does the total content of the fraction of phospholipids [3,10,11] and cholesterol [10,11,22]. Production of ketone bodies increases during prolonged exercise [8,19,27]. It is well documented that prolonged exercise reduces the liver potential to synthesize fatty acids [1,15,17]. Concomitantly, the rate of fatty acid oxidation increases [1,2,24]. According to most authors, prolonged exercise results in accumulation of triacylglycerols in the liver. This is a consequence of the increased delivery of plasma-borne long-chain free fatty acids. The presence of glucocorticosteroids is necessary for the triacylglycerols to accumulate [14]. There are no data available on the effect of exercise on esterification of the plasma-borne free fatty acids into other than triacylglycerol lipid fractions in the liver and it was the aim of the present study to examine this question.

Material and Methods

The experimental protocol has been approved by the Ethical Committee in the Medical Academy of Białystok. The experiments were carried out on male Wistar rats 250-280 grams of body weight. A 12 h light/dark cycle was maintained in the animal quarters. The rats had free access to a commercial pellet diet for rodents and tap water. All rats were familiarized with running on an electrically driven treadmill. This was done by forcing them to run for 10 min daily, for 6 days prior to the final experiment. The running speed was 1200 $m \cdot h^{-1}$ and the treadmill incline $+10^{o}$. Thereafter, the rats were divided into three groups: 1-control, 2-run 3 h on the treadmill (conditions as above), and 3-run 3 h (as above) and then allowed to recover 3 h. The food was allowed during the recovery period. The animals were anaesthetized with pentobarbital sodium administered intraperitoneally. 14C-palmitic acid (DuPont; S.A. 57 $mCi \cdot mmol^{-1}$) suspended in albumin was administered in a tail vein in a dose of 20 μCi/100g and the rats were put on a heating pad. 20 min after administration of the label, two samples of the liver were taken and a sample of blood from the abdominal aorta was withdrawn. In one sample the content of glycogen was determined [7]. Another sample was

homogenized in chloroform/methanol and lipids were extracted according to Bligh and Dyer [5]. The chloroform phase was divided into two parts. One part was used for quantitative determination of phospholipids [4] and triacylglycerols [6,12]. Lipids present in another part of the chlorofom phase were separated into different fractions by means of thin layer chromatography. Chloroform was evaporated, the residue was dissolved in chloroform/methanol (2:1), spotted on a silica plate (Merck, 0.25 mm) and developed in a solvent composed of heptane, isopropyl ether and acetic acid (60:40:3 v/v/v) [8]. The standards (Sigma) were run along with the samples. The plates were allowed to dry at room temperature, sprayed with 0.2% solution of 2'7'dichlorofluorescein in absolute methanol and exposed briefly on ammonium vapours. Lipid bands were visualized under ultraviolet light. The following lipid fractions were obtained: phospholipids, mono-, di-, and triacylglycerols, free fatty acids, cholesterol and cholesterol esters. The bands containing particular fractions were scraped off the plate into a scintillation vials, a scintillation cocktail (Ultima Gold, Packard) was added and the radioactivity was counted (in Tri-carb 1900 Packard). The concentration of the blood glucose was measured enzymatically using Cormay kit. The plasma free fatty acid concentration was measured according to Duncombe [9].

The results obtained are expressed as means ± standard deviation. N=10 in each group. The results were compared statistically using the Student t-test for unpaired data.

Results

Table 1
Effect of exercise on the concentration of blood glucose, plasma free fatty acids (FFA) and FFA specific activity

	Rest	Exercise (3 h)	Recovery (3 h)
Glucose (mg %)	120.10±17.29	54.16 ± 11.05^{x}	103.54 ± 15.58^{y}
FFA ($\mu mol \cdot l^{-1}$)	171.94±36.86	394.90 ± 105.40^{x}	$283.89\pm61.44^{x,z}$
FFA S.A. ($dpm \cdot \mu mol^{-1}$)	17.78±2.40	7.30 ± 1.91^{x}	$11.96\pm1.90^{x,z}$

Values are mean ± SD; n=10; x-$p<0.001$, vs. the respective rest value; z-$p<0.02$, y-$p<0.001$, vs. the exercise value

The blood glucose concentration was considerably reduced after exercise and it partially recovered after the 3 h rest period. The plasma free fatty acid concentration was more than doubled after exercise. After recovery, it dropped but still remained elevated compared to the resting value (Table 1).

Table 2
Effect of exercise on the content of phospholipids (PH), triacylglycerols (TG) and glycogen (G) in the liver

	Rest	Exercise (3 h)	Recovery (3 h)
PH (μmol Pi·g^{-1})	26.7±2.72	26.5±4.62	27.0±1.94
TG (μmol·g^{-1})	13.2±2.30	39.1±6.57^{x}	33.8±13.13^{x}
G (mmol·g^{-1})	159.5±27.55	6.3±1.34^{x}	38.4±19.9^{x,y}

Values are mean ± SD; n=10; x-$p<0.001$, vs. the respective rest value; y-$p<0.001$, vs. the exercise value

The content of phospholipids in the liver remained stable during exercise and the subsequent period of recovery. The content of triacylglycerols was tripled after exercise. A tendency to a reduction in their content occurred during recovery but did not differ significantly from the post-exercise value. The liver was almost completely depleted of glycogen after exercise. A certain recovery in the glycogen content appeared during recovery but it still remained about four times lower than the pre-exercise value (Table 2).

The radioactivity of the fraction of phospholipids and monoacylglycerols was reduced and the radioactivity of other lipid fractions remained stable after exercise. After recovery, the radioactivity of both fractions returned to the control value, that of the fraction of triacylglycerols, cholesterol and free fatty acids remained stable, it dropped in the fraction of diacylglycerols and the fraction of cholesterol esters it increased compared to the respective control value (Table 3).

The post-exercise specific activity of both the fraction of phospholipids and triacylglycerols was markedly lower than at rest. It only partially recovered in the 3 h after exercise (Table 4).

Table 3
Effect of exercise on incorporation of the blood-borne ^{14}C-palmitic acid into different lipid fraction of the rat liver

	Rest	Exercise (3 h)	Recovery (3 h)
PH	169108.1±35509.07	99888.5±7242.62^{x}	147350.4±39571.73^{y}
MG	3430.2±613.11	2861.2±500.97^{z}	3354.5±890.25
1,2-DG	6278.4±930.28	6023.5±697.65	4984.7±866.16^{w,v}
1,3-DG +Ch	3963.7±832.6	4679.0±754.59	3628.8±730.10
FFA	4797.6±1086.53	4848.6±1613.91	5608.6±1891.13
TG	123376.1±24324.36	84260.2±18170.97	115593.3±46303.88
ECh	2725.1±338.17	2567.3±768.08	3325.4±628.42^{z,v}

The numbers are dpm·g^{-1}; Values are mean ± SD; n=10; PH – phospholipids, MG – monoacylglycerols, 1,2-DG – 1,2 diacyloglycerols, 1,3-DG – 1,3 diacylglycerols, Ch – cholesterol, FFA – free fatty acids, TG – triacyloglycerols, ECh – cholesterol esters; z-$p<0.05$, w-$p<0.01$, x-$p<0.001$, vs. the respective rest value; v-$p<0.05$, y-$p<0.001$, vs. the exercise value

Table 4
Specific activity of the fraction of phospholipids (PH) and triacyglycerols (TG)

	Rest	Exercise (3 h)	Recovery (3 h)
PH (dpm/μmol Pi)	8544.51±1748.08	3553.74±430.12^{x}	4775.07±744.50^{x,y}
TG (dpm/μmol)	9346.67±1842.68	2154.99±482.63^{x}	3556.72±1424.73^{x,w}

Values are mean ± SD; n=10; x-$p<0.001$, vs. the respective rest value; w-$p<0.02$, y-$p<0.001$, vs. the exercise value

Discussion

The exercise did not affect the content of phospholipids and it resulted in a considerable accumulation of triacylglycerols in the liver which is in agreement with the data obtained so far and presented in the "Introduction". We have further shown that the post - exercise normalization in the content of triacylglycerols in the liver is rather a slow process. There is no reason to presume that secretion of very low density lipoproteins is impaired after exercise. However, the plasma concentration of free fatty acids remains markedly elevated during the recovery period. This suggests that increased synthesis of triacylglycerols continued during this period of time. The rate of synthesis must nearly have matched the rate of secretion so that their content decreased only insignificantly compared to the content directly after cessation of exercise.

The radioactivity of the fraction of phospholipids and monoacylglycerols was reduced and radioactivity of the other fractions remained stable after exercise. This cannot, however, be interpreted as a reduction or stability in the incorporation of the plasma-borne fatty acids into the examined lipid fractions. It should be taken into account that the same amount of radioactivity was given before and after exercise whereas the plasma concentration of free fatty acids increased 2.3 fold. As a result the specific activity of the plasma free fatty acids decreased respectively. The real entry of the plasma free fatty acids into the lipids was, therefore, about 2.3 times higher than that indicated by the radioactivity of particular lipids. Bearing this in mind, one can conclude that prolonged exercise considerably increased esterification of the blood-borne fatty acids into the liver lipids. Increased radioactivity of the fraction of mono-, di-, and triacylglycerols could simply be interpreted as a reflection of increased synthesis of triacylglycerols. The situation is much more complex in the case of phospholipids. The content of this fraction remains stable whereas esterification of fatty acids into the phospholipid moieties increased after exercise. Phospholipids contain two long chain fatty acid residues, a saturated one esterified at the sn1 position and an unsaturated residue esterified at the sn2 position of the glycerol backbone. Since radioactive palmitic (i.e. saturated) acid was used, one may conclude that exercise increases the turnover of fatty acids at the sn1 position of the phospholipid moiety. Phospholipase A1 and glycerol-3-phosphate acyltransferase catalyse hydrolysis and esterification of the acyl residue at this position [26]. It is, therefore, obvious that the activity of these enzymes must be elevated during prolonged exercise. Nothing can be said about the turnover of unsaturated fatty acids at the sn2 position. However, there is no reason to exclude this as a possibility.

The incorporation of the label into the cholesterol pool was stable after exercise. However, since the specific activity of the plasma free fatty acids was reduced, a real incorporation of carbon from fatty acids into the cholesterol molecule was elevated, accordingly. It may, thus, be concluded that synthesis of cholesterol increases during this type of exercise. It is a new finding. As already mentioned, the content of cholesterol in the liver remains stable after acute exercise. Thus, the newly synthesized cholesterol must either be secreted along with VLDL, or with bile. Another alternative is that the turnover of cholesterol in the liver increases during prolonged exercise. The latter possibility is very likely since the expiratory excretion of $14CO_2$ has been shown, both in man [21] and in rats [20], to increase during exercise. Cholesterol excretion into the bile increases during exercise in humans [23] but it is reduced in rats [22]. The latter would thus rather add to accumulation in the newly synthesized cholesterol in the liver.

Exercise has previously been shown [28] to increase the content of the fraction of free fatty acids in the heart muscle. There are no respective data in the liver. The present results, taking into account the above considerations would indicate that the fraction of free fatty acids in the liver also increases after prolonged exercise. Obviously, it also indicates that the rate of delivery of the acids into the cells exceeded the rate of their disposal. Accumulation of the free acids in the cell is regarded to be harmful [26]. However, it remains to be established whether this extent of accumulation is great enough to exert adverse effects. Moreover, it is uncertain what length of exposure is needed for the acids to be toxic for the cell.

The changes in radioactivity after 3h of recovery are neither particularly pronounced nor characteristic when compared to the respective values directly after exercise. When a reduction in the specific activity of the plasma free fatty acids is taken into account, a tendency to normalization can generally be observed. However, this also indicates that changes in the activity of the enzymes involved in incorporation of the label into the examined lipid fractions are long lasting. Factors maintaining their activity remain, however, to be explored.

Finally, one more question should be addressed, namely what is the mechanism responsible for the increased transport of fatty acids from plasma to the places of destination in the liver cells during exercise. There is a great debate over the mechanism of transport of free fatty acids across the plasma membrane. For now, it seems very likely that they cross the membrane both diffusing passively according to the transmembrane concentration gradient as well as with the assistance of a carrier protein [16,25]. This is also a good reason to presume that they are transported by cytosolic fatty acid binding protein inside the aqueous environment of the cytosol [13]. It is claimed that in contracting skeletal muscles, both types

ways of fatty acid transport across the plasma membrane contribute to increased delivery of the acids into the cytosol [25]. Most likely, this is also true for the liver.

Conclusion:

Prolonged exercise of moderate intensity affects the activity of the enzymes responsible for esterification of the plasma-borne free fatty acids and synthesis of cholesterol in the liver cells.

References

1. Barakat H.A., G.J.Kasperek, G.L.Dohm (1983) Progressive changes in fatty acid metabolism in rat liver and muscle during exercise. *Biochem.Med.* 29:298-306

2. Barakat H.A., G.J.Kasperek, G.L.Dohm, E.B.Tapscott, R.D.Snider (1982) Fatty acid oxidation by liver and muscle preparations of exhaustively exercised rats. *Biochem.J.* 208:419-424

3. Barakat H.A., S.Overton (1985) Changes in rat liver lipid during exercise and recovery. *IRCS Med.Sci.*13:991-992

4. Bartlett R.G. (1959) Phosphorus assay in column chromatography. *J.Biol.Chem.* 234:466-478

5. Bligh E.G., W.J.Dryer (1959) A rapid method for total lipid extraction and purification. *Can.J.Biochem.* 37:911-917

6. Carlson L.A. (1963) Determination of serum triglycerides. *J.Atheroscler.Res.* 3:334-336

7. Carroll N., R.W.Longley, J.H.Roe (1956) The determination of glycogen in liver and muscle by use of anthrone reagent. *J.Biol.Chem.* 220:583-587

8. Conquer J., V.G.Mahadevappa (1991) Evidence for the possible involvement of protein kinase C in the activation of non-specific phospholipase A_2 in human neutrophils. *J.Lipid Med.* 3:112-123

9. Duncombe W.G. (1964) The colorimetric micro-determination of non-esterified fatty acids in plasma. *Clin.Chim.Acta* 9:122-125

10. Fröberg S.O. (1971) Effect of acute exercise on tissue lipids in rats. *Metabolism* 20:714-720

11. Fukuda M., K.Hort, M.Sugano (1976) Effect of exercise on plasma and liver lipids of rats. Part 1. Effects of the lapse of time after exercise and of the intensities of exercise on lipids in rats. *J.Agric.Chem.Soc.* (Japan) 50:17-22

12. Galletti F. (1977) An improved colorimetric micromethod for the determination of serum triglycerides. *Clin.Chim.Acta* 15:184-186

13. Glatz J.F.C., J.Storch (2001) Unravelling the significance of cellular fatty acid-binding proteins. *Curr.Opin.Lipidol.* 12:267-274

14. Górski J., L.B.Oscai, W.K.Palmer (1990) Hepatic lipid metabolism in exercise and training. *Med.Sci.Sports Exerc.* 22:213-221

15. Griffiths M.A., R.Fiebig, M.T.Gore, D.H.Baker, K.Esser, L.Oscai, L.L.Ji (1996) Exercise down-regulates hepatic lipogenic enzymes in food-deprived and refed rats. *J.Nutr.* 126:1959-1971

16. Hamilton J.A., F.Kamp (1999) How are free fatty acids transported in membranes? Is it by proteins or by free diffusion through the lipids? *Diabetes* 48:2255-2269

17. Kasperek G.J., G.L.Dohm, H.A.Barakat, P.H.Strausbauch, D.W.Barnes, R.D.Snider (1982) The role of lysosomes in exercise-induced hepatic protein loss. *Biochem.J.* 202:281-288

18. Keul J., E.Doll, D.Keppler (1972) Energy Metabolism of Human Muscle. Univ. Park Press, Baltimore

19. Koeslag J.H. (1982) Post-exercise ketosis and the hormone response to exercise: a review. *Med.Sci.Sports Exerc.* 14:327-334

20. Malinow M.R., P.McLaughlin, A.Perley (1968) Cholesterol: treadmill activity accelerates oxidation in rats. *Science* 160:1239-1240

21. Malinow M.R., A.Perley (1969) The effect of physical exercise on cholesterol degradation in man. *J.Atheroscler.Res.* 10:107-111

22. Simko V., V.Chorvathova (1968) The effect of short-term exercise on the degradation of cholesterol in rats. *Physiol.Bohemoslov.* 17:583-586

23. Simko V., R.E.Kelly, A.M.Connell (1976) The effect of short-term physical exercise on bile composition in human volunteers (Abstract). *Gastroenterology* 70:938

24. Tate C.A., P.E.Wolkowicz, J.McMillin-Wood (1982) Exercise-induced alterations in hepatic mitochondrial function. *Biochem.J.* 208:695-701

25. Turcotte L.P. (2000) Muscle fatty acid uptake during exercise: possible mechanisms. *Exerc. Sport Sci. Rev.* 28: 4-9

26. Van der Vusse G.J, J.F.C.Glatz, H.C.G.Stam, R.S.Reneman (1992) Fatty acid homeostasis in the normoxic and ischemic heart. *Physiol.Rev.* 72:881-940

27. Wahren J., P.Felig, L.Hagenfeldt (1978) Physical exercise and fuel homeostasis in diabetes mellitus. *Diabetologia* 14:213-222

28. Wójcik B., A.Nawrocki, G.Chocian, J.Górski (1999) Effect of exercise on fatty acid content in the rat heart. *Biol.Sport* 16:87-96

29. Zakim D., T.D.Boyer (1982) Hepatology: A Textbook of Liver Disease. W.B. Saunders Co., Philadelphia

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