Eur J Appl Physiol (1996) 74: 470–474

ORIGINAL ARTICLE

A. Frankiewicz-Jóźko · J. Faff
B. Sieradzan-Gabelska

Changes in concentrations of tissue free radical marker and serum creatine kinase during the post-exercise period in rats

Accepted: 22 May 1996

Abstract Changes in the concentrations of thiobarbituric acid-reactive substances (TBARS), an index of lipid peroxidation in liver, heart and soleus muscle, were studied in trained (T) and untrained (U) rats throughout a period of 48–72 h following running until exhaustion. Creatine kinase (CK) concentration in serum was also determined. The running time till exhaustion in group T was significantly longer than in group U [174.5 (SEM 9.8) vs 92.7 (SEM 8.3) min, $P < 0.01$]. In group U TBARS concentration in investigated tissues increased significantly ($P < 0.01$) after exercise with the peak values observed 3 h after running. The postexercise increase in the TBARS concentration persisted longer in the soleus muscle (48 h) than in the liver or heart (3 h). A postexercise increase of TBARS was observed in group T only in the liver. The influence of training on the TBARS content depended on the kind of tissue. The TBARS concentrations in the liver at rest and immediately after the exercise were lower in group U than in group T. In contrast, TBARS concentrations in the heart and soleus muscle were higher in group U than in group T. The exercise resulted, in both groups, in a rise of serum CK concentration, peak values being observed 3 h following the exercise. Postexercise concentrations of CK were considerably lower in group T than in group U [3 h postexercise: 1740 (SEM 170) vs 2750 (SEM 231) $U \cdot l^{-1} P < 0.01$]. A positive correlation ($r = 0.66, P < 0.05$) between TBARS content in muscle and serum CK concentration was found only in group U. The results obtained indicated that the generation of lipid peroxidation products in the soleus muscle was intensified for a relatively long time after the exercise. Endurance training decreased the susceptibility of tissues to the action of free radicals. However, this influence of training was more pronounced in the heart and soleus muscle than in the liver.

Key words Exercise creatine kinase · Lipid peroxidation · Thiobarbituric acid-reactive substances

A. Frankiewicz-Jóźko · J. Faff (✉) · B. Sieradzan-Gabelska
Department of Applied Physiology, Military Institute of Hygiene and Epidemiology, PL-01-163 Warsaw, Kozielska 4, Poland

J. Faff
Department of Physiology, Institute of Sport, Warsaw, Poland

Introduction

Numerous investigations have shown that if free oxygen radicals appear in excess in relation to the protective capacity of the body damage occurs to various cellular components (Davies et al. 1982; Rajguru et al. 1994; Singal et al. 1982; Smith et al. 1989). Intense physical exercise, accompanied by a manyfold increase of oxygen utilization in relation to resting conditions, has been shown to elevate the probability of the appearance of free radicals (Packer 1986). This phenomenon has been found to be promoted by an exercise-related increase in the concentration of hydrogen ions (Singh 1982), by enhanced metabolism of catecholamines (Singal et al. 1982), and by periodic ischaemia of certain tissues with subsequent reoxygenation and muscle damage with infiltration of neutrophils (Smith et al. 1989).

The existing reports on the formation of free oxygen radicals during physical exercise show contradictory results. Numerous investigations have documented increased postexercise concentrations of the markers of lipid peroxidation, such as malondialdehyde or total thiobarbituric acid-reactive substances (TBARS; Davies et al. 1982; Kanter et al. 1988; Maughan et al. 1989; Packer 1986). However, no changes in the levels of these parameters, or even their decrease, have been observed in other studies (Panczenko-Kresowska et al. 1991; Sahlin et al. 1991; Viinka et al. 1984). These contradictions may have been related to differences in

the type, intensity and/or duration of the exercise applied (Lovlin et al. 1987) and to the degree of adaptation to exercise (Kihlstrom 1990, 1992; Salminen and Vihko 1983). On the other hand, it should be stressed that there is no method of choice for measuring exercise-induced lipid peroxidation. All commonly used methods have certain limitations. For instance it has been shown that TBARS, the most commonly used though a nonspecific marker of lipid peroxidation, may sometimes overestimate the extent of the measured process (Janero 1990).

Exercise-induced generation of free oxygen radicals has aroused considerable interest. Nonetheless, very few publications have appeared in which the markers of lipid peroxidation have been determined, and only in serum, in the course of the postexercise recovery (Maughan et al. 1989). No reports on changes in the levels of lipid peroxidation markers in animal tissues, such as heart, liver or muscle during the first days following exercise have been found in the literature and measuring them in human beings would hardly be justifiable.

One of the results brought about by free oxygen radicals appears to be the peroxidation of cell membrane lipids. Numerous investigators have postulated that this phenomenon is responsible for elevated activities of cellular enzymes in blood serum during exercise and in the postexercise period (Davies et al. 1982; Kanter et al. 1988; Maughan et al. 1989). Kanter et al. (1988) and Panczenko- Kresowska et al. (1991) have observed a relationship between the concentrations of TBARS and creatine kinase (CK) in serum. The CK concentration is the most commonly used marker of skeletal muscle damage. In fact, this enzyme is located mainly in skeletal muscles and heart. However, the quantitatively similar ratios of total CK and its Cardiospecific iso-enzyme (CK – MB) in the muscle and of that in serum following exercise have suggested that the postexercise elevation of CK concentration in serum is largely of skeletal muscle origin (Kanter et al. 1988; Kosano et al. 1986; Miles and Schneider 1993). On the other hand, the assessment of lipid peroxidation in skeletal muscles by the serum TBARS concentration is rather difficult. It has been shown that the TBARS may diffuse into serum from various sources and then may be rapidly cleared by the liver (Jenkins et al. 1993). However, in the literature available no reports have been found on the relationships between concentrations of lipid peroxidation markers in skeletal muscles and serum concentrations of CK.

The principal aim of the present study was to examine the influence of exhausting exercise on the concentration of TBARS in liver, heart and *soleus* muscle at different times after completion of exercise. The second aim was to determine the relationship between the TBARS content in the *soleus* muscle and CK concentration in blood serum. In addition, the effect of endurance training on these parameters was studied.

Methods

Subjects and procedure

Male Wistar rats [mean body mass in untrained (U) group 275.0 (SEM 6.0) g and in trained (T) group 271.7 (SEM 7.3) g] were used in the experiments. The animals were fed a pelleted standard diet for rodents supplemented with micro-elements and vitamins (MURIGRAN) ad libitum. The U rats were only briefly (2×10 min) accustomed to working on the treadmill, while the T rats were exercised for 4 weeks (1 h daily, 5 day a week). After having completed the training, and following a 2-day rest all the animals (groups U and T) were forced to run on the treadmill with electric stimulation until exhaustion. Average running speed was 28 $m \cdot min^{-1}$ (range 27–29 $m \cdot min^{-1}$), at a gradient of 15%. Randomly selected animals from both groups (15 rats at each time) were sacrificed by cervical dislocation immediately before, and following the exercise, as well as 3 and 24 h there-after. Rats from group U were also sacrificed 48 and 72 h after the exercise. Samples of liver, heart and soleus muscle were collected from every animal. The TBARS were determined using the method of Ohkawa et al. (1979). In the blood serum collected by heart puncture, the concentration of CK was determined spectrophotometrically by using commercial diagnostic kits (Boehringer-Mannheim).

Statistics

For selected variables Pearson's coefficient of correlation was calculated. Two-way analysis of variance was used to analyse the effect of training and of exercise on various dependent variables. Dunnet's test was applied to detect significant differences between rest and post-exercise states (Steel and Torrie 1960). Student's *t*-test was applied to detect differences between the U and T groups.

Results

The time of running until exhaustion in the U group was 92.7 (SEM 8.3) min and was significantly ($P < 0.01$) shorter than that in T group [174.5 (SEM 9.8) min]. Figure 1 gives the concentrations of TBARS in the tissues examined. The analysis of variance for TBARS showed a significant ($P < 0.05$) main effect of training and of exercise and also a significant training-exercise interaction. In liver, higher values of TBARS during rest ($P < 0.05$) and immediately after exercise ($P < 0.01$) were found in group T, while in the remaining periods, the concentration of TBARS appeared to be alike in both groups. In contrast, in heart muscle the concentrations of TBARS were lower in group T both at rest ($P < 0.05$) and after the exercise ($P < 0.01$). Similarly in the *soleus* muscle. TBARS concentrations at rest as well as at 3 and 24 h after the completion of exercise were lower in group T ($P < 0.01$). The exercise resulted in a significant increase ($P < 0.01$) in the TBARS concentration in all tissues examined in the animals from group U. It is to be emphasized the this postexercise increase in TBARS in the U group persisted longer in the soleus muscle (48 h), than in the liver or heart (3 h). In the T group, the postexercise increase

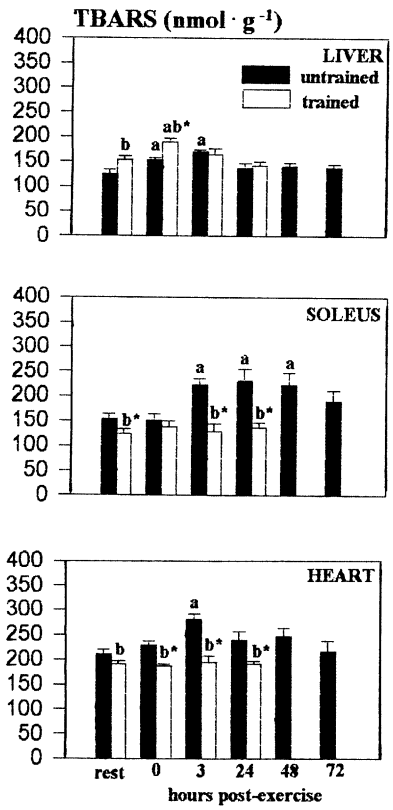

Fig. 1 Thiobarbituric acid-reactive substances (*TBARS*) concentrations in the liver, soleus muscle and heart before and after exercise in untrained and trained animals (means and SEM). Significantly different from the respective value for untrained group: *b* $P < 0.05$, *b** $P < 0.01$. Significantly different from the respective rest value: *a* $P < 0.01$

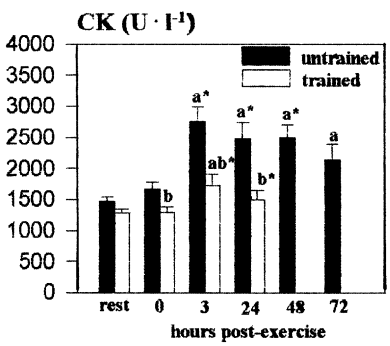

Fig. 2 Serum creatine kinase (*CK*) concentration before and after exercise in untrained and trained animals. Significantly different from the respective value for untrained group: *b* $P < 0.05$, *b** $P < 0.01$. Significantly different from the respective rest value: *a* $P < 0.05$, *a** $P < 0.01$

in TBARS was seen only in the liver immediately after exercise ($P < 0.01$).

A significant ($P < 0.05$) main effect of training, of exercise and the training-exercise interaction was also demonstrated for CK concentration. As presented in Fig. 2, both the rest and postexercise values of serum CK concentration in the animals of group T were significantly lower than in those of group U. In both groups an exercise-induced increase in CK concentration was observed, which peaked 3 h after the completion of the trial. A positive correlation between TBARS content in the *soleus* muscle and CK concentration was found in the U rats ($r = 0.66$, $P < 0.05$), while no significant correlation was found in the T rats ($r = 0.35$).

Discussion

The results obtained showed that an exhausting exercise led to a considerable increase in TBARS concentrations in the tissues of the U rats. This confirms the earlier studies of other authors who have observed the effects of intense exercise on the generation of free oxygen radicals (Davies et al. 1982; Kanter et al. 1988; Maughan et al. 1989; Packer 1986). However, some authors have not found elevation of lipid peroxidation markers during an intense physical effort (Panczenko-Kresowska et al. 1991; Sahlin et al. 1991; Viinka et al. 1984). One of the possible reasons for these discrepancies may be the degree of adaptation to physical effort. This is supported by the differences shown in this study between the T and U animals. It is worth pointing out that lower TBARS concentrations in the T animals were observed in spite of a correspondingly higher work output (longer running time) compared with the U animals. The results obtained confirmed earlier observations of Kihlstrom (1990, 1992) and Salminen and Vihko (1983) on the influence of endurance training on the susceptibility of tissues to the action of free radicals.

The results obtained showed that TBARS concentrations in group U rose during the postexercise period. Peak values tended to appear after 3 h, the increased values persisting for a period of 3–48 h. Similar results have been obtained by Maughan et al. (1989), who have investigated TBARS concentrations in human serum. These authors have found increased concentrations of TBARS after exercise with peak values at 6 h postexercise. Serum TBARS concentrations returned to normal values after 72 h, similar to our investigations in the soleus muscle.

It should be mentioned that TBARS formed as an effect of lipid peroxidation has been reported to undergo further metabolism. For instance in the study performed by Siu and Draper (1982), 60%–70% of malondialdehyde administered orally, was found to have been metabolized to carbon dioxide within 12 h. Moreover, as has been demonstrated by Jenkins et al. (1993), TBARS may rapidly diffuse from exercising muscle and pass into urine. Our results would thus indicate that the process of formation of lipid peroxidation products in the soleus muscle is intensified for a relatively long time after the completion of exercise. It has been suggested that this phenomenon may be

related, to some extent, to a prolonged, exercise-induced inflammatory response, reflected by the accumulation of neutrophils and macrophages within the capillary bed of damaged muscle tissue (Armstrong et al. 1983; Jones et al. 1986; Kuipers et al. 1983). It is likely that those inflammatory cells, which are capable of producing free oxygen radicals, may contribute to lipid peroxidation (Romson et al. 1983; Smith et al. 1989).

An interesting phenomenon observed by us was the diversified effect of training on TBARS concentration in individual tissues. In group T the postexercise increase in TBARS content occurred only in the liver (immediately after the exercise) and TBARS concentrations at rest and immediately after exercise were higher than those in group U. Higher concentrations of TBARS in the liver of the T animals might be explained by increased generation of free radicals under the influence of repeated exercise during the training and by accumulation of their metabolic products. Moreover, the higher value of TBARS found in the T group might have been related to the work time until exhaustion being almost doubled, compared with the U group. However, in contrast, significantly lower TBARS concentrations were found in the heart muscle in group T throughout the study period. Also in soleus muscle, TBARS concentrations in group T were lower than those found in group U.

The observed phenomena may have been, to some degree, connected with differences in the influence of training on anti-oxidative enzymes in various tissues. As has been shown by Ji (1993), endurance training results in an increase in catalase and glutathione peroxidase activities in muscle tissue. Also other authors (Higuchi et al. 1985; Quintanilha 1984) have shown increases in the muscle catalase and superoxide dismutase during the period of training. An increase in glutathione peroxidase activity in rat muscles under the influence of training has been demonstrated by Quintanilha (1984) and Ji et al. (1988). However, according to Criswell et al. (1993), training-induced increases in superoxide dismutase and in glutathione peroxidase activities are limited to skeletal muscles composed predominantly of type I fibres (soleus).

Contrary to skeletal muscles, in the liver no influences of training on the activities of anti-oxidative enzymes have been observed (Ji 1993; Ji et al. 1988) and in the case of cytosolic and mitochondrial glutathione peroxidase even a lowering of their activities has been found (Ji 1993). The lower concentrations of free radical products in the heart under the influence of training, as found in our investigations, remains consistent with earlier observations of Kihlstrom (1990, 1992). It is of interest that in heart muscle, as in liver, no effect of endurance training on the activity of anti-oxidative enzymes has been observed (Kihlstrom 1990). However, as has been pointed out by Kihlstrom (1992), the lowered susceptibility of heart muscle to free radicals, visible in trained animals, may be related to an increase in the concentration of a reduced form of glutathione.

Increases in CK concentration, similar to those obtained by us, have frequently been described in the literature (Faff et al. 1988; Kanter et al. 1988; Maughan et al. 1989; Panczenko-Kresowska et al. 1991). It has been suggested that the release of cellular enzymes into the extracellular environment may have been related to mechanical injury of the cells, increase of body temperature (Young 1974), lower content of high energy substrates in the cells (Thomson et al. 1975) or an exercise-induced increased influx of lymph and soluble muscle cell proteins present in the interstitium into the bloodstream (Komulainen and Vihko 1994). As has been postulated by Davies et al. (1982), exercise-induced damages of cell membranes may be associated with the peroxidation of lipids. This suggestion is supported by the correlation between the TBARS concentration in the soleus muscle and serum CK concentration, as shown in our present study. A positive correlation between TBARS and CK concentrations has also been found in the serum of human beings by Kanter et al. (1988) following a 80-km run and by Panczenko-Kresowska et al. (1991) in exercised skaters. In studies on the isolated rat heart following hypoxic perfusion and reoxygenation, Gauduel et al. (1989) have shown the concentration of TBARS in heart muscle and CK release to be correlated with one another. However, Van der Heide et al. (1987) have not found such a correlation in the isolated rat heart following a chemically induced lipid peroxidation.

No increases in the TBARS concentrations in the heart and the soleus muscle were observed by us in the T animals. Nevertheless, a significant rise in serum CK concentration took place. This rise reached a maximum 3 h after the exercise, as in the U animals. The increase in CK concentration in group T was considerably lower than in group U, which is in accordance with reports of other authors on the influence of training on postexercise concentrations of cellular enzymes in serum (Schwane and Armstrong 1983). In group T however, we did not observe a significant correlation between TBARS content in soleus muscle and serum CK concentration. It may be suggested that lipid peroxidation may, to some extent, enhance the permeability of cell membranes leading to an increased leakage of CK into the serum. However, the postexercise increases in concentration of cellular enzymes in serum may have resulted from factors other than free radical generation.

References

Armstrong RB, Ogilvie RW, Schwane JA (1983) Eccentric exercise-induced injury to rat skeletal muscle. J Appl Physiol 54: 80–93

Criswell D, Powers S, Dodd S, Lawler J, Edwards W, Renshler K, Grinton S (1993) High intensity training-induced changes in skeletal muscle antioxidant enzyme activity. Med Sci Sports Exerc 25:1135–1140

Davies KJA, Quintanilha AT, Brooks GA, Packer L (1982) Free radicals and tissue damage produced by exercise. Biochem Biophys Res Commun 107:1198–1204

Faff J, Dudkiewicz J, Józefczak E (1988) Effect of $NaHCO_3$ treatment on exercise induced rise of some serum enzymes levels. Biol Sport 5:5–9

Gauduel Y, Menasche P, Duvelleroy M (1989) Enzyme release and mitochondrial activity in reoxygenated cardiac muscle: relationship with oxygen-induced lipid peroxydation. Gen Physiol Biophys 8:327–340

Higuchi ML, Cartier J, Chen M, Holloszy JO (1985) Superoxide dismutase and catalase in skeletal muscle: adaptive response to exercise. J Gerontol 40:281–286

Janero DR (1990) Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative injury. Free Rad Biol Med 9:515–540

Jenkins RR, Krause K, Schofield LS (1993) Influence of exercise on clearance of oxidant stress products and loosely bound iron. Med Sci Sports Exerc 25:213–217

Ji LL (1993) Antioxidant enzyme response to exercise and aging. Med Sci Sports Exerc 25:225–231

Ji LL, Stratman FW, Lardy HA (1988) Antioxidant enzyme system in rat liver and skeletal muscle: influences of selenium deficiency acute exercise and chronic training. Arch Biochem Biophys 263:150–160

Jones DA, Newham DJ, Round JM, Tolfree SEJ (1986) Experimental human muscle damage: morphological changes in relation to other indices of damage. J Physiol (Lond) 375:435–448

Kanter MM, Lesmes GR, Kaminski LA, Ham-Saeger JL, Nequin ND (1988) Serum creatine kinase and lactate dehydrogenase changes following an eighty kilometer race. Eur J Appl Physiol 57:60–63

Kihlstrom MT (1990) Protection effect of endurance training against reoxygenation-induced injuries in rat heart. J Appl Physiol 68:1627–1678

Kihlstrom MT (1992) lipid peroxidation capacities in the myocardium of endurance-trained rats and mice in vitro. Acta Physiol Scand 146:177–183

Komulainen J, Vihko V (1994) Exercise-induced necrotic muscle damage and enzyme release during a four-day period following prolonged submaximal running in rats. Pflügers Arch 428:346–351

Kosano H, Kinoshita T, Nagata N, Takatani O, Isobe M, Yazaki Y (1986) Changes in concentrations of myogenic components of serum during 93 h of strenuous physical exercise. Clin Chem 32:346–348

Kuipers H, Drukker J, Frederik PM, Geurten P, Van Kranenburg G (1983) Muscle degeneration after exercise in rats. Int J Sports Med 4:45–51

Lovlin R, Cottle W, Pyke I, Kavanagh M, Belcastro AN (1987) Are indices of free radical damage related to exercise intensity. Eur J Appl Physiol 56:313–316

Maughan RJ, Donnelly AE, Gleeson M, Whiting PH, Walker KA, Clough PJ (1989) Delayed onset muscle damage and lipid peroxidation in man after a downhill run. Muscle Nerve 12:332–336

Miles MP, Schneider CM (1993) Creatine kinase isoenzyme MB may be elevated in healthy young women after submaximal eccentric exercise. J Lab Clin Med 122:197–201

Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351–358

Packer L (1986) Oxygen radicals and antioxidants in endurance exercise. In: Benzi L, Packer L, Silipiandi N (eds) Biochemical aspects of physical exercise. Elsevier Science, New York, pp 73–92

Panczenko-Kresowska B, Hubner-Woźniak E, Ziemlański S, Woźny E, Dziedziak W (1991) Effects of physical exercise on the changes in antioxidant levels in speed skaters. Biol Sport 8:19–24

Quintanilha AT (1984) The effect of physical exercise and/or vitamin E on tissue oxidative metabolism. Biochem Soc Trans 12:403–404

Rajguru SU, Yeargans GS, Seidler NW (1994) Exercise caused oxidative damage to rat skeletal muscle mikrosomes while increasing cellular sulfhydryls. Life Sci 54:149–157

Romson JL, Hook BG, Kunkel SL, Abrams GD, Schork A, Lucchesi BR (1983) Reduction of the extent of ischemic myocardial injury by neutrophil depletion in the dog. Circulation 67:1016–1023

Sahlin K, Ekberg K, Cizinsky S (1991) Changes in plasma hypoxanthine and radical markers during exercise in man. Acta Physiol Scand 142:275–281

Salminen A, Vihko V (1983) Endurance training reduces the succeptibility of mouse skeletal muscle to lipid peroxidation in vitro. Acta Physiol Scand 117:109–113

Schwane JA, Armstrong RB (1983) Effects of training on skeletal muscle injury from downhill running in rats. J Appl Physiol 55:969–975

Singal PK, Kapur N, Dhillon KS, Beamish RE, Dhala NS (1982) Role of free radicals in catecholamine-induced cardiomyopathy. Can J Physiol Pharmacol 60:1390–1397

Singh A (1982) Chemical and biochemical aspects of superoxide radicals and related species of activated oxygen. Can J Physiol Pharmacol 60:1330–1345

Siu GM, Draper HH (1982) Metabolism of malondialdehyde in vivo and in vitro. Lipids 17:349–355

Smith JK, Grisham MB, Granger DN, Korthuis RJ (1989) Free radical defense mechanism and neutrophil infiltration in postischemic skeletal muscle. Am J Physiol 25:H 789–H 793

Steel RGD, Torrie JH (1960) Principles and procedures in statistics. McGraw-Hill, New York

Thomson WHS, Sweeting JC, Hamilton IJD (1975) ATP and muscle enzyme eflux after physical exertion. Clin Chim Acta 59:241–245

Van der Heide RS, Sobotka PA, Ganote CE (1987) Effects of the free radical scavenger DMTU and mannitol on the oxygen paradox in perfused rat hearts. J Mol Cell Cardiol 19:615–625

Viinka L, Vuori J, Ylikorkala D (1984) Lipid peroxides, prostacyclin and tromboxane A_2 in runners during acute exercise. Med Sci Sports Exerc 16:275–277

Young D (1974) The origin of serum enzymes and the basis for their variation. In: Blume P, Freier EF (eds) Enzymology in the practise of laboratory medicine. Academic Press, New York, pp 253–269