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Are indices of free radical damage related to exercise intensity

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Summary. The possibility that plasma levels of malonaldehyde (MDA) are altered by exercise has been examined. The presence of MDA has been recognized to reflect peroxidation of lipids resulting from reactions with free radicals. Maximal exercise, eliciting 100% of maximal oxygen consumption ($\dot{V}_{O_{2max}}$) resulted in a 26% increase in plasma MDA ($P<0.005$). Short periods of intermittent exercise, the intensity of which was varied, indicated a correlation between lactate and MDA ($r^2=0.51$) ($p<0.001$). Blood lactate concentrations increased throughout this exercise regimen. A significant decrease (10.3%) in plasma MDA occurred at 40% $\dot{V}_{O_{2max}}$. At 70% $\dot{V}_{O_{2max}}$ plasma MDA was still below resting values, however the trend to an increase in MDA with exercise intensity was evident. At exhaustion, plasma MDA and lactate were significantly greater than at rest. These results suggest, that exhaustive maximal exercise induces free radical generation while short periods of submaximal exercise (i.e. $<70\%$ $\dot{V}_{O_{2max}}$) may inhibit it and lipid peroxidation.

Key words: Fatigue — Free radicals — Lipid peroxidation

Introduction

Membrane peroxidation by free radicals may be potentially detrimental. Ronquist (1982) suggested that peroxidation of membrane lipids, particularly during re-oxygenation of hypoxic tissues, leads to a number of changes in cell functions. These include increased membrane permeability, decreased Ca^+ transport in the sarcoplasmic reticulum, altered mitochondrial function, formation of other toxic metabolites, and alteration of cellular glutathione metabolism. During exercise, with the redistribution of blood, some tissues may become hypoxic and thus, on reperfusion, may become more susceptible to peroxidation. Since mitochondrial swelling, edema formation, proteinuria, and hemolysis have been observed following exercise, perhaps, exercise induced free radical generation and it's subsequent events contribute to the altered function(s).

Information on the relation of exercise to free radical generation is limited and in some instances contradictory. Davies et al. (1982) found a significant increase in free radical generation and lipid peroxidation in rats due to exercise whereas in a more recent study by Viinkka et al. (1984) on humans, no change in plasma peroxide levels were found. Dillard et al. (1978) used the amount of pentane in expired air as an index of lipid peroxidation and found an increase during exercise.

Factors that may be implicated in the generation of free radicals and lipid peroxidation include substrate depletion (e.g. glucose), and disturbances in the oxidation/reduction status of the cell. Evidence has indicated that a number of the free radical scavenging enzymes require NADH and/or NADPH as essential co-factors for activity. Thus, the stress of exercise, by causing substrate depletion and modification of the NADH/NADPH ratio by effecting lactate production, uptake and removal, may also directly effect free radical generation and lipid peroxidation. In the present study a possible relationship between exercise intensity, lactate metabolism and lipid peroxidation was investigated.

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Materials and methods

Six male subjects gave informed consent and completed five exercise sessions. Two sessions were initially conducted to determine the subjects' maximum oxygen consumption ($\dot{V}_{O_{2max}}$), while the remaining three sessions, based upon the results of the two maximal tests, were intermittent-incremental exercise tests to voluntary exhaustion. All sessions were conducted on a calibrated Monark ergometer, 1 week apart. Oxygen uptake ($\dot{V}_{O_2}$) and heart rates (HR) were recorded every 30 s (Beckman Metabolic Cart). The $\dot{V}_{O_{2max}}$ protocol included a 5 min warm-up at 88 W, then increased by 30 W each minute until exhaustion. The termination point was voluntary, however, objective criteria of HR 180 bt · min^{-1} and lactate levels 9 mmol L^{-1} were employed. In the three remaining sessions, the subjects first rested 15 min on the ergometer, with a blood sample drawn after 7 min. The subjects then exercised at 40% $\dot{V}_{O_{2max}}$ for 5 min and rested for 5 min. This was immediately followed by 5 min of exercise at 70% $\dot{V}_{O_{2max}}$ and 5 min rest. The subjects then completed the intermittent exercise regime by pedaling (60 rev · min^{-1}) to voluntary exhaustion a an increasing load (30 W each minute). Blood samples were drawn into 7 ml heparinized vacutainers at the midpoint of the rest periods and after voluntary exhaustion. The samples were immediately placed on ice and held until completion of the exercise test when they were centrifuged to separate plasma from packed cells. The plasma was stored in the refrigerator and analyzed the next day. Three plasma aliquots (300 µl) were analyzed for evidence of lipid peroxidation using the method of Buege and Aust (1978). In this, the plasma aliquot was added to 700 µl distilled water before addition of the HCL-THIOBARBITURIC (TBA) reagent solution. After mixing, the samples were digested at 95°C for 8 min then removed from the hot water bath and placed in a water bath at room temperature to cool. After centrifugation for 10 min, the optical density of aliquots of the supernatant were determined at 535 nm. Tetramethylacetal (TMA) was used as external standard. Lipid peroxides were expressed as mmol · L^{-1}.

Lactate concentrations were determined enzymatically by a method involving conversion of lactate to pyruvate in the presence of lactate dehydrogenase (LDH) and NAD^+. Values obtained were compared to those obtained when a stock solution of lactate was diluted to form a series of standards in the range of 0—0.6 mmol · L^{-1}. All samples were analyzed in triplicate after adding 0.1 ml of the deproteinized sample to 2.9 ml of the reagent solution. After incubating the samples for 30 min at 37°C, optical density was read at 340 nm. Lactate concentrations were calculated from a linear, least squares regression equation based on the standards for a given assay. The same procedure was followed for determination of MDA based on MDA standards for a given assay. Statistical analysis was performed with Student's *t*-test, paired data.

Results

The male subjects used in this study were physical education students with a mean age of 21 years and a mean maximal oxygen consumption of 47 ml · kg^{-1} · min^{-1}. The maximal ergometer test to elicit $\dot{V}_{O_{2max}}$ had a mean time of 13.5 ± 4.2 min. The reproducibility (Pearson Product) of the maximum oxygen consumption tests was 0.92.

Maximal exercise to exhaustion resulted in a 26% increase in plasma lipid peroxides; from 2.26 mmol · L^{-1} at rest, to 2.88 mmol · L^{-1} following the maximal tests. The mean result from the 3 incremental exercise sessions to exhaustion can be seen in Table 1. Lactate concentrations increased throughout the exercise regimen. Plasma MDA was found to decrease at 40% $\dot{V}_{O_{2max}}$ (Table 1). At 70% $\dot{V}_{O_{2max}}$, plasma MDA (2.13 mmol · L^{-1}) was still lower than the pre-exercise value (2.26 mmol · L^{-1}). As the work load was increased and the subject reached exhaustion, lactates (18.77 mmol · L^{-1}) and MDA (2.88 mmol · L^{-1}) exceeded all other values (Table 1). Lactate concentration was found to correlate with that of MDA (correlation coefficient = 0.51, $p < 0.001$). No significant differences in hematocrit were observed.

Discussion

The findings in this study agree with Dillard et al. (1978) who indicated that exhaustive exercise induces lipid peroxidation. However, they differ from those of Viinkka et al. (1984) who did not find a change in plasma peroxides following exhaustive exercise. We suggest, that the differences observed may be due, in part, to differences in the sensitivity of the method used for determination of lipid peroxides, the intensity of exercise, and/or the fitness level of the subject.

According to Stanley et al. (1985), the more intense the exercise, the more lactate is produced and taken up by the working muscle. Since the

Table 1. Mean blood lactates (mmol · l^{-1}) and plasma lipid peroxides (mmol · l^{-1}) in male subjects during intermittent-graded bicycle exercise to exhaustion. Results are mean ± standard deviation of the mean

	Pre Ex.	40%[a]	70%	100%
Lactates (mmol · l^{-1})	0.832 ± 0.53	1.89 ± 1.63*	6.07 ± 2.90*	18.77 ± 4.30*
MDA (mmol · l^{-1})	2.26 ± 0.10	2.00 ± 0.11*	2.13 ± 0.18	2.88 ± 0.25*

[a] % $\dot{V}_{O_{2max}}$
* $p \leq 0.005$ pre vs. exercise loads
Lactate/MDA correl. coeff. = 0.51 ($p < 0.001$)

appearance of lactate exceeds the disappearance at maximal work loads, this indicates a possible drop in the concentration of cytoplasmic NADH and/or NADPH. Consequently, at maximal exercise, the activity of the free radical scavenging enzymes may be compromised and substrates that generate free radicals would accumulate. That free radical accumulation may occur during exhaustive exercise is indicated in a study by Corbucci et al. (1984) of muscle tissue from marathon runners. After a marathon race, they found significant increases in the level of oxidized glutathione (GSSG) in muscle and suggested a deficiency of NADPH as a possible cause. One would expect, that without adequate NADPH, H_2O_2 would accumulate and the potentiality for free radical generation would tend to be enhanced. Of interest was their finding that the activity of glutathione peroxidase was increased three fold following the race suggesting there had been an accumulation of H_2O_2. The activity of superoxide dismutase, another free radical scavenging enzyme, was unaffected by the race. Davies et al. (1982) also found substantial increases in free radicals and MDA (an index of lipid peroxidation) in muscle and liver from rats following exhaustive exercise. As pointed out by Kappus and Sies (1981) any stress on the system, such as hypoxic tissues, which result in depletion of glycolytic substrates, may cause a decrease in the generation of NADH and NADPH. The net result, during maximal rates of exercise, would be an enhanced potential for lipid peroxidation and production of MDA. Our results, which indicate a significant increase in blood lactate and plasma MDA following the maximal exercise tests would support this hypothesis.

During submaximal exercise, on the other hand, there is some evidence that lactate metabolism and thus the NAD/NADPH ratio may be related to tissue adaptive responses. Bonen et al. (1979) has indicated that the rate of lactic acid removal is influenced by the distribution of slow twitch fibers. Ivy et al. (1980), Sjodin et al. (1981) and Rusko et al. (1980) stress the importance of the muscle cells' respiratory capacity as a factor determining lactate uptake. The greater the functional capacity of different enzyme systems in the muscle and the greater the capillary density, the more lactate will be utilized. Since these tissue adaptations usually occur as a result of aerobic training, and our subjects are above average in fitness according to Canadian and Swedish norms, lactate uptake may be enhanced during submaximal exercise in these subjects. McLellan and Skinner (1982) found that if subjects "warmed down" by pedaling at approximately 40% $V_{O_{2max}}$, lactate uptake by the working muscle is enhanced. This suggests, that during submaximal efforts, capillaries may open enhancing blood flow and lactate uptake. With lactate utilization at submax efforts, the generation of cytosolic NADH/NADPH would increase and the activity of the antioxidant enzymes is likely enhanced thus reducing the concentration of substrates that generate free radicals. If this occurs, lipid peroxidation at this intensity of exercise may decrease with a corresponding drop in the production of MDA. Our finding a drop in plasma MDA at this level of exercise supports this view and suggests that the fitness level of the subject may influence free radical generation and lipid peroxidation.

As the intensity of the exercise increased to 70% MVO_2 lactate production increased significantly above the resting and 40% values (Table 1). This suggests that the cytosolic pool of NADH may be decreased and the activity of the antioxidant enzymes may be compromised with the possible accumulation of substrates that generate free radicals. Consequently, lipid peroxidation increased along with the generation of MDA. Continuation of the exercise at maximal V_{O_2} to exhaustion augmented this trend, i.e. a further increase in the concentration of lactate and MDA which were significantly greater than the previous values (Table 1).

Our results support the work of Stanley et al. (1985) who found that exercise intensity influences lactate production and removal. Although we did not measure lactate uptake, our finding a direct correlation between blood lactates and plasma MDA would support such an hypothesis. A drop in plasma MDA levels during short periods of submaximal exercise would suggest that peroxidative processes may have been inhibited. This would indicate that cytosolic NADH may have increased due to lactate uptake and enzymes that inhibit peroxidative processes would demonstrate increased activity. Furthermore, we suggest, that exercise intensity, by regulating the concentration of reducing equivalents (NADH/NADPH) may also influence the process of lipid peroxidation. Several authors (Halliwell and Gutteridge 1984; Repine and Tate 1983; Kontos et al. 1982; DelMaestro et al. 1981; Deneke and Barry 1980; Ohmori et al. 1978) have indicated that lipid peroxidation is the event most likely to be responsible for increased permeability of and damage to the plasma membrane. Some workers have demonstrated, both invitro and in-

vivo, that exposure of red blood cells to H_2O_2, or to enzymatic reactions which produce free radicals, promotes hemolysis suggesting that peroxidation increases the permeability of the red cell membrane (Kellogg and Fridovich 1977; Michelson and Durosay 1977). Peroxidation of the red cell membrane may also be one of the factors contributing to the "sport anemia" syndrome. Pfafferott et al. (1982) found that MDA, by cross linking membrane associated particles in the red cell membranes, decreased the deformability of the cell. A less deformable red cell would probably be sequestered and destroyed more readily than others. Perhaps, exercise induced lipid peroxidation also contributed to the loss of membrane integrity as observed by Davies et al. (1982) in SR, ER, and mitochondrial systems. Our data would support this hypothesis.

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