



Prevention of muscle soreness by pretreatment with antioxidants

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A free radical-scavenging preparation (pollen extract) or the corresponding placebo was given to 50 volunteers over a period of 4 weeks to test the hypothesis that muscle soreness is associated with the generation of free radicals. The increase in malonyldialdehyde and lactate immediately after exercise both in blood and in muscle tissue was significantly lower after treatment with the scavenging preparation. The same was true for the prolonged post-exercise increase in creatine kinase over a 5-day period. The post-exercise glycogen content of muscle was higher in the pollen extract group, as were the subjective feelings of pain, oedema, discomfort and tension in the working muscle. We conclude that the beneficial preventive effect of pollen extract on post-exercise muscle soreness and lowering of the concentration of lipid peroxides indicate that free radicals are probably involved in the development of muscle soreness.

Key words: free radicals; muscle soreness; MDA; exercise; creatine kinase; antioxidant

Free radicals are ubiquitous in biological systems and have been implicated as factors in cellular differentiation (1), aging (2), mutagenesis (3) and carcinogenesis (4). Furthermore, the actions of free radicals have been implicated in the pathophysiology of many diseases, including ischaemia reperfusion injury involving the brain (5), heart (6), skin (7), intestines (5, 7), pancreas (4, 5, 7), liver (4, 5, 7), muscles (8), kidneys (9) and lungs (4).

The realization that free radicals might be injurious to organisms has been slow, perhaps because of the difficulties encountered in detecting these short-lived moieties in biological tissues. Nevertheless, the many animal and human studies claiming that modification of free radical production attenuates or eliminates tissue destruction have forced the medical community, and particularly cardiologists, to consider a specific therapy against free radicals as a realistic approach to disease after unaccustomed physical exercise. Muscle soreness is common and is observed to reach peak intensity 1-5 days later (10,11). The subjective sensation of soreness is accompanied by evidence of damage

to the affected muscles. Ultrastructural examination of muscle samples has shown extensive degenerative changes affecting a large part of muscle (10, 12). Evidence of an inflammatory response is also present, necrotic tissue being invaded by macrophages (13-15) and increased local levels of lysosomal enzymes (16). Related to these changes is the release into the lymph, and subsequently into plasma, of cytoplasmic enzymes, which are normally unable to cross the cell membrane. Large increases in the serum levels of creatine kinase, lactate dehydrogenase (LDH) and other enzymes of muscular origin occur, peak activities being observed at varying times from a few hours to several days after exercise (17). This enzyme efflux is considered to reflect a change in normal membrane structure, such that permeability to large protein molecules is increased. Although the mechanisms underlying these changes in muscle after exercise are not clear at present, there are striking similarities between the observed response and the tissue changes induced by an increased production of free radicals (15).

If the post-training syndrome and muscle soreness with enzyme leakage, pain, discomfort, oedema and tenderness of working muscles are due to the generation of free radicals and the reperfusion injury of skeletal muscles, supplementation with free radical scavengers should prevent or ameliorate the condition.

The aim of this study was to test the effect of a pollen extract preparation, known to be rich in SOD (superoxide dismutase) mimics, on the biochemical, morphological and clinical signs of muscle soreness.

Pollen-pistil extract with antioxidative activity

The preparation used in this investigation is unique as to its composition, method of production, source and high SOD activity. The source is freshly harvested pollen grains and pistils from the family *Gramineae* spp. The pollen grains and the pistils are collected separately by machines specially designed for this purpose. After collection, they are thoroughly analysed for purity and specificity (18). The SOD active base material is produced in a reactor where pollen grains and pistils are allowed to react under very specific and well-defined conditions. The reactant solution is partly evaporated to concentrate the solution and increase the activity. The hypothesis regarding what substances are obtained in this reaction is still under investigation (19), but the following substances are probably present in the reactant solution:

1. SOD mimics, such as flavonoids, tannins and polyphenols. These are low-molecular-weight substances and are therefore absorbed through the intestinal wall (20, 21).
2. Maillard reactants, formed mainly from the reaction between certain amino acids and sugars via the Maillard reaction. This yields small amounts of antioxidative substances. These phenomena have been studied for many years and are not yet fully understood (22, 23).
3. SOD enzyme, released from disrupted mitochondria and pollen tubes.

This pollen-pistil extract exhibits an SOD activity of approximately 30,000 units^a per gram of

substance (24). When adsorbed and complexed to a defined mixture of proteins, it gives an SOD activity of 4000-6000 F per gram of extract (19). The test preparation, pollen extract (Polbax[®]) (Allergon, Sweden), is manufactured from this extract (20). The contribution of SOD activity from the 3 possible sources mentioned above has not yet been fully established.

With reference to the results achieved in clinical trials, it seems likely that low-molecular-weight substances, which are easily absorbed from the gastrointestinal tract, contribute a major part of the antioxidative activity. Only a minor part can theoretically come from native SOD enzyme, which might be absorbed via the endocytosis-exocytosis mechanism (25). This mechanism has been shown in rats (26, 27). However, direct absorption of SOD – a rather large protein molecule – has not been verified in humans and should be considered a rather unlikely possibility. On the contrary, anthocyanidins, pyknogenols, other polyphenols and tannins present in pollen extract have been found to be fully absorbable, and their antioxidant activity is several times higher than that of vitamin E (28-30).

Material and methods

Fifty male volunteers were recruited to the study via a local daily newspaper. Before admission to the study, all participants were informed about the aims, methods, anticipated benefits and potential hazards of the study, and verbal consent to inclusion was obtained from each of them. The study protocol was approved by the Ethics Committee of the University of Göteborg. The inclusion criteria were absence of hypertension, diabetes, cardiovascular disease, organic brain disease, alcohol or drug dependence and any other deviation from good health, no physical training on a permanent or intensive basis and the lack of any ongoing medication. All volunteers were explicitly asked to follow their habitual style of life, particularly with regard to diet and level of physical activity. All patients were allocated to one of two groups (36 to the pollen extract and 14 to the placebo group). The analysis of variance did not reveal any significant difference between

the groups with respect to any variable shown in Table 1.

General design

After the first selection, each participant was allocated to the first pretreatment period. During this period, participants were asked to report their daily food intake while keeping their body weight stable to within ± 200 g. At the end of this period (1 week), blood samples were drawn from the antecubital vein after an overnight fast. Body weight and anthropometric variables (waist to hip circumferences) were measured on the same day. The next day, participants arrived at the laboratory at 0800. After the initial blood sampling, a short venous catheter was inserted into an antecubital vein and the volunteer started to perform the exercise programme.

Acute exercise test

After 10 min of rest, participants started to perform the following exercise:

- 10 min on the step test (stepping up and down a 45-cm foot-stool 15 times per min);
- 30 min cycling at 70% of $V_{O_{2max}}$ followed by 10 min on the step test;

Determination of $V_{O_{2max}}$

Participants started cycling on the electrically braked ergometer bicycle (Monark, Varberg, Sweden). Blood pressure and heart rate were measured during the last minute of the 6-min steady-state period at the submaximal working capacity (i.e., oxygen uptake) was then calculated according to Astrand (31).

Immediately after the acute exercise test, muscle biopsies were taken with an alligator forceps (32) from the lateral vastus. The muscle specimens were divided into 2 parts: one was frozen immediately in liquid nitrogen and used for analysis of enzymatic activities; the other part was trimmed, mounted and frozen in cooled isopentane (-160°C) and used to histochemical analysis. Both parts were stored at -80°C until analysed. In the histochemical analysis, the myofibrillar adenosine triphosphatase (ATPase) method was used for muscle fibre classification (33, 34). Amylase-periodic acid-Schiff staining was used to visualize capillaries (35), and the number of capillaries per fibre and the fibre area

per capillary were calculated for the different fibre types. Glycogen synthase activity in the lateral vastus muscle was measured according to previous methods (36, 37), malonyldialdehyde (MDA) by the thiobarbituric acid method (38),

Table 1. General characteristics of participants (body composition, anthropometry and maximum oxygen uptake) (means \pm SEM)

	Pollen extract group		Placebo group	
	Before treatment	After treatment	Before treatment	After treatment
Age (years)	35.1 \pm 2.62		35.1 \pm 2.62	
Height (cm)	183.6 \pm 2.26		180.7 \pm 1.73	
Waist (cm)	85.9 \pm 1.35	85.8 \pm 1.40	85.0 \pm 1.13	85.2 \pm 1.30
Hip (cm)	98.5 \pm 1.33	98.7 \pm 1.37	97.1 \pm 0.84	98.9 \pm 1.10
Waist-to-hip ratio	0.87 \pm 0.01	0.87 \pm 0.01	0.88 \pm 0.01	0.88 \pm 0.01
Weight (kg)	80.6 \pm 1.98	80.6 \pm 2.10	76.5 \pm 2.15	76.7 \pm 2.13
Body K^{+} (mmol)	4493 \pm 136	4525 \pm 132	4296 \pm 146	4291 \pm 155
Body fat (kg)	14.6 \pm 1.93	14.2 \pm 1.97	13.5 \pm 1.96	13.7 \pm 1.91
Fat free mass (kg)	66.0 \pm 2.01	66.4 \pm 1.94	63.1 \pm 2.15	63.0 \pm 2.27
$V_{O_{2max}}$ (l/min)	3.35 \pm 0.19	3.45 \pm 0.19	3.36 \pm 0.11	3.41 \pm 0.15

- 30 min cycling at 60% of $V_{O_{2max}}$ followed by 10 min on the step test;
- 30 min cycling at 60% $V_{O_{2max}}$;
- 10 min on the step test;
- 30 min cycling at 60% $V_{O_{2max}}$.

vitamin E and ceruloplasmin according to Storer et al. (39) and body composition by calculating naturally occurring 40 K (40). Other enzymes (ALAT, ASAT, alkaline phosphatase and creatine kinase) and other metabolic variables were

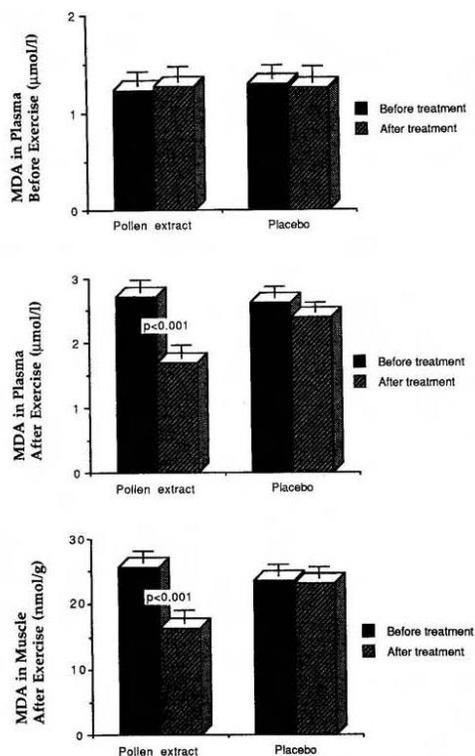
determined according to routine automated hospital methods. Muscles enzyme activities were measured according to Lowry et al. (41, 42) and lactate concentration according to Karlsson (43).

One hour after the exercise test, and then at the same time on the second, third, fourth and fifth days, the participants rated their pain, oedema and discomfort in the working muscles on a 10-cm visual analogue scale ranging from no pain

As mentioned before, all participants were allocated either to the placebo group or to treatment with the pollen extract with antioxidant effect. The pollen extract group received 2 tablets 3 times a day, and the placebo group received the same number of identical placebo tablets. The tablets were given in a double-blind manner. All tests and measurements were performed in an identical manner before and 4 weeks after treatment. Results were calculated by 2-way analysis of variance by means of Macintosh StartView statistical program.

Results

Four weeks of treatment with pollen extract or placebo did not induce any changes in



and

Fig. 1. The concentration of malonyldialdehyde (MDA) in plasma before and immediately after strenuous exercise and in the lateral vastus muscle immediately after strenuous exercise (mean+SEM) and before and after 4 weeks of treatment with either pollen extract (n=36) or placebo (n=14).

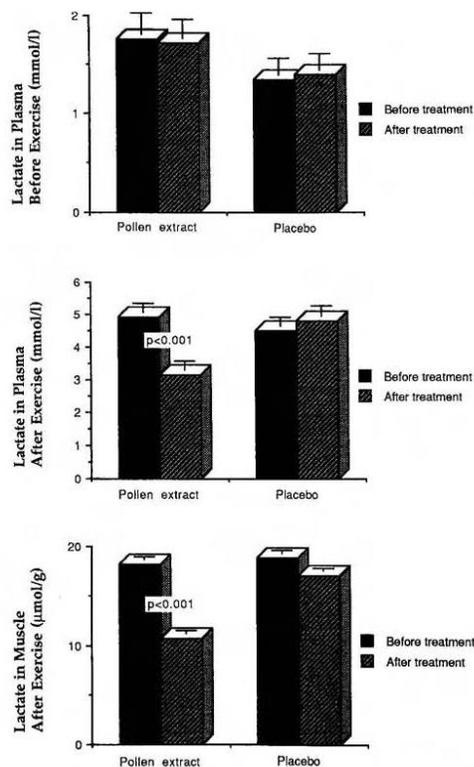


Fig. 2. The concentration of lactate in plasma before and after strenuous exercise and in muscle immediately after strenuous exercise (mean+SEM), and before and after treatment with either pollen extract (n=36) or placebo (n=14).

discomfort at the far left to intense pain and discomfort at the far right. Visual analogue scales were used in a similar way for the questions related to oedema and tenderness and the feeling of muscle tension.

body weight or body composition (Table 1). There was no difference in the concentration of MDA in plasma before exercise between the pollen extract and placebo groups. Immediately after exercise, the concentration of MDA increase significantly ($P<0.001$) in both groups. However, after pollen extract treatment increase in MDA level (Fig. 1) immediately after exercise was significantly lower than in the placebo group, in which placebo had no effect on the post-exercise MDA levels. The concentration of MDA in muscle, measured immediately after exercise, decreased significantly after treatment with pollen extract but remained unchanged in the placebo group (Fig. 1). The decreases in plasma and muscle MDA levels at the end of the acute exercise test in the pollen extract group were 30% and 50%, respectively. The concentration of MDA 5 min and 24 and 48 h after the termination of exercise did not change after the pollen extract treatment (data not shown). The concentration of lactate in plasma before exercise was very similar in the placebo and the pollen extract groups and did not change after treatment (Fig. 2). In contrast, plasma lactate concentration immediately after exercise increased significantly. Treatment with pollen extract resulted in a significant diminution

of this increase, whereas the placebo treatment appeared to have to detectable effect on the exercise-induced increase in lactate concentration. When measured immediately after exercise, muscle lactate content showed a significant decrease after 4 weeks of treatment with pollen extract. There was no difference in muscle lactate content after the corresponding placebo treatment (Fig.2). The decrease in the post-exercise lactate concentration in plasma was found to be 28% and 39% in muscle. In the group treated with pollen extract, the percentage decrease in lactate concentration in muscle after exercise was positively correlated with the percentage decrease in muscle MDA concentrations (Fig. 3). In comparison with

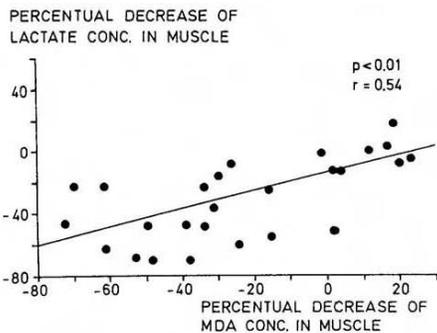


Fig. 3. The relationship between the percent age decrease of the concentration of lactate and the percentage decrease of the malonyldialdehyde (MDA) concentration in the lateral vastus muscle after 4 weeks of treatment with pollen extract. $P<0.01$, $r=0.54$.

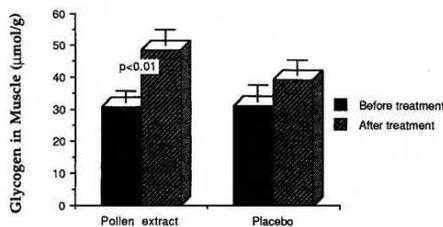


Fig. 4. The concentration of glycogen (mean+SEM) in the lateral vastus muscle before and after treatment with pollen extract or placebo.

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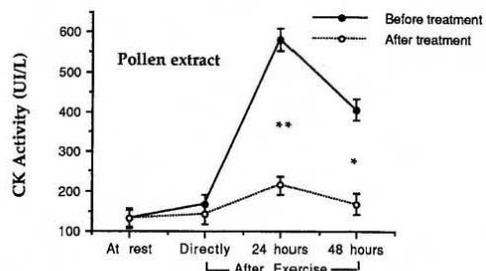
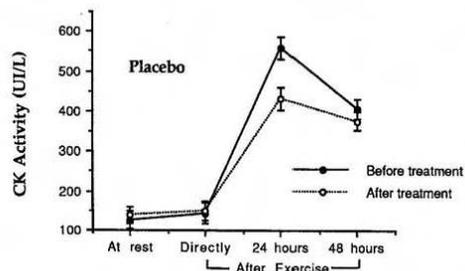


Fig. 5. The activity of creatine kinase (CK) (mean+SEM) before strenuous exercise and immediately and 24 and 48 h after exercise, before and after 4 weeks treatment with either pollen extract or placebo. * $P<0.05$; ** $P<0.01$.

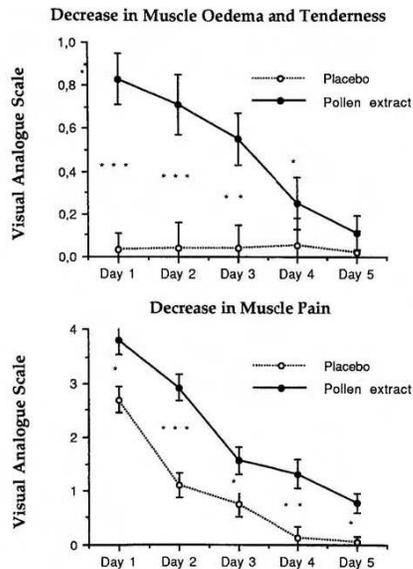


Fig. 6. Decrease in muscle oedema, tenderness and pain as evaluated by the participants on visual analogue scales on 5 consecutive days after strenuous exercise, after the treatment with either pollen extract or placebo. The decrease is calculated as a difference between the starting values (i.e., rated after strenuous exercise before the treatment) and the values rated after the treatment. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

the pretreatment values, there was a significant increase in the concentration of glycogen in the lateral vastus muscle after the treatment with pollen extract, whereas no significant change in muscle glycogen concentration was found after placebo treatment (Fig. 4). Creatine kinase activity increased significantly 24 and 48 h after strenuous exercise. The increase was found to be almost completely eliminated in the pollen extract-treated group but not in the placebo-treated group (Fig. 5). When using the visual analogue scales, the participants reported a significant decrease in muscle oedema and tenderness (Fig. 6), muscle pain (Fig. 6) and feeling of tension in muscles after the treatment with pollen extract. These decreased remained significant for all the 5 days, during which the participants were asked to record their subjective feeling on visual analogue scales. The differences between the two groups decreased gradually from day 1 to day 5 (Fig. 6).

Heart rate at rest and the different work loads on the ergometer bicycle, as well as blood pressure,

remained uninfluenced by both pollen extract and placebo treatment (Table 2).

No difference in either the peak isokinetic strength or the average value for the whole range of movement after the treatment with pollen extract or placebo was observed. Strength was tested on the KinCom dynamometer for knee extension at 60°/s angular velocity. The same (no change after treatment) was true of isometric strength measured by knee extension at a 60° knee angle and for the eccentric strength at the 60°/s angular velocity.

Neither pollen extract and placebo treatment nor the exercise itself has been shown to have any significant effect on the activities of aminotransferases (ASAT, ALAT) and alkaline phosphatase on the bilirubin concentration.

The vitamin E concentration in plasma increased slightly in the group treated with pollen extract 5 min after the exercise but remained unchanged 24 and 48 h after the exercise.

The 4-week course of treatment with pollen extract did not evoke any significant effect on the concentration of ceruloplasmin in plasma.

No differences in the concentration of plasma triglycerides 5 min or 24 and 48 h after exercise were noted after the pollen extract treatment. The same was true for the plasma cholesterol level.

The activities of different enzymes in the lateral vastus muscle (oxidative: hydroxy-acyl dehydrogenase, citrate synthase, glycolytic: hexokinase and LDH), expressed per gram of muscle wet weight or per gram of protein, did not change after either pollen extract or placebo treatment. The activity of muscle glycogen synthase, both its V_{max} and its fractional velocity, did not change significantly after either form of treatment.

Table 2. Heart rate during cycling (means±SEM)

	Pollen extract group		Placebo group	
	Before treatment	After treatment	Before treatment	After treatment
Heart rate (beats/min)				
At rest	63.4±2.38	65.6±2.22	65.5±2.51	62.8±3.01
After cycling 50 W	84.7±2.90	87.3±2.29	84.1±2.96	84.7±3.64
After cycling 100 W	110.4±3.77	113.6±2.96	109.0±3.40	109.4±3.32
After cycling 150 W	139.9±5.00	136.1±3.64	137.1±3.42	136.3±4.22
After cycling 200 W	171.1±5.60	168.9±5.31	177.5±6.96	169.8±5.45

There was no difference in the relative percentage distribution of the different types of muscle fibres between the two groups. No changes were found after pollen extract or placebo treatment. The number of capillaries in contact with different types of fibres did not change after either of the treatments. The number of capillaries around type IIB fibres was found to be lower than those supplying type I and type IIA muscle fibres ($P<0.05$). The mean muscle fibre cross-sectional area did not change after either of the treatments.

Discussion

The main findings of the study suggest that muscle soreness is at least to some extent caused by the creation of free radicals and lipid peroxides and secondary damage to muscle membranes followed by the leakage of muscle enzymes. Factors that may be implicated in the generation of free radicals and lipid peroxidation include substrate depletion and disturbances in the oxidation and reduction status of the cell. Lovlin et al. (44) found a similar parallel increase of lactate and MDA in blood as we observed in our study. The authors discuss these findings, quoting the available literature, and argue that exercise intensity influences both lactate production and removal, and secondarily the concentration of reducing equivalents NADH/NADPH and the production of free radicals and lipid peroxidation. Although we did not measure lactate uptake, our findings of a direct correlation between blood lactate and plasma MDA, and muscle lactate and muscle MDA, as well as the correlation between the differences in

MDA and lactate concentrations after treatment, support such a hypothesis. As pointed out by Kappus & Sies (45), any stress on the system, such as hypoxic tissues, that results in depletion of glycolytic substrates, may cause a decrease in the generation of NADH and NADPH.

Although it seems theoretically possible that pollen extract could influence the system and have some glycogen-sparing effect ameliorating the glycogen depletion and lactate production, it can hardly influence the NADPH concentration to a degree directly affecting the production of free radicals. The concentration of NADPH is, on the other hand, involved in the glutathione-dependent oxido-reductive protecting system (46-48).

Submaximal exercise has been reported to deplete the concentration of glutathione, and NADPH may be regarded as a natural reducing agent to restore the concentration of glutathione. Although glutathione depletion is likely to occur, the involvement of NADPH, in association with the lactate production, seems less probable.

On the other hand, it seems probable that pollen extract could influence the generation of ATP. During the exhaustive exercise, regeneration of ATP is insufficient due to the high rate of ATP turnover. The concomitant accumulation of AMP, causing increasing amounts of hypoxanthine and the conversion of xanthine dehydrogenase to xanthine oxidase (as described during ischaemia-reperfusion injury), causes the creation of free radicals (46, 49). The concentration of lactate is an indirect measure of

the relative ischaemia, insufficient regeneration of ATP, build-up of hypoxanthine, and the creation of free radicals and lipid peroxide. This concept explains the correlation between the concentration of lactate and MDA. It is possible that this coincidence (between the lowering of both MDA and lactate post-exercise concentrations) also indicates some other kind of indirect effect on the muscle cell metabolism. Pollen extract can act by accelerating the restoration of ATP-yielding metabolism or by some mechanisms preventing or delaying the shift from aerobic to anaerobic metabolism. Similarly, any influence on the phosphofructokinase or any other mechanism preventing the depletion of glycolytic substrate can prevent or delay the decrease in the generation of NADH and NADPH. As discussed above, the decreased concentration of these reducing compounds can lead to higher generation of free radicals and lipid peroxides. Thus, the mechanism of action of pollen extract could depend on the effect on the metabolic pathways determining both the generation and regeneration of ATP and the production of lactate. No data are so far available explaining the exact influence of pollen extract on the intracellular muscle metabolism, and further studies are required to elucidate the parallel changes in MDA and lactate concentration both in blood and in muscle tissue.

An increase of hypoxanthine 10 min after termination of exercise, together with a significant arteriovenous difference, and together with the concomitant increase of the blood and plasma concentration of glutathione (49), has recently been reported. The same authors could not find any post-exercise increase of MDA. The obvious discrepancy between this finding and several other reports on the post-exercise MDA increase in animals and humans has been explained as dependent on differences in method (49). However, our findings are very consistent and, in accordance with other reports on the MDA increase, they also show that the increase of MDA is very transitory and depends very much on the time of sampling and intensity of exercise.

Our study has shown that the decrease in muscle soreness parallels the decrease in the concentration of lipid peroxides and the decrease in lactate production. The improvement of the symptoms (muscle pain, tenderness and oedema) occurred after a time period of sufficient to eliminate any influence of familiarization with the test situation and learning, both possibly improving the efficiency of the performed exercise. This was also suggested by the observations that the level of physical fitness and increases of heart rate and blood pressure were not different before and after treatment. The degree of oedema was verified by the objective blind measurements by the non-involved observer and confirmed to be significantly different before and after the treatment with the antioxidant but not after the treatment with placebo. Thus, at least this variable was in harmony with the results obtained by means of visual analogue scales. These scales are otherwise commonly used (also by us for measurements of pain, hunger feelings, etc.) and generally accepted as a good measure of subjective feelings.

The addition of pollen extract to the red blood cell incubation medium, with and without a free radical-generating system, caused a significant reduction of the concentration of MDA. The decrease of MDA was parallel and significantly correlated to the improvement of the erythrocyte deformability (50). A similar improvement in blood rheology has also been observed in volunteers after 3 weeks of oral administration of pollen extract (50).

Whatever the underlying mechanism, the improved blood rheological properties could influence the availability of the oxygen derived from the red blood cells and, in that way, ameliorate ischaemia and lactate production in the working muscle. It seems probable that the administration of pollen extract does not influence the production of lipid peroxides or the concentration of MDA during the late reperfusion period during recovery but acts only during strenuous exercise and possibly directly after its termination.

The concentration of MDA was already normal within 5 min after terminating exercise. This is in accordance with the findings of Lovlin et al. (44), who also stressed the dependence of MDA production on the intensity of exercise. The rapid decrease in MDA concentration most probably indicates that the cytosolic NADH increases and the formation of free radicals decreases very soon after the termination of exercise, due to an increased uptake of lactate and increased activity of enzymes that inhibit peroxidative processes. The observation that lactate production was diminished and the glycogen content spared (Fig. 2, 4) suggests that the mechanism of action of pollen extract is the facilitation of the aerobic metabolism in muscles with a glycogen-sparing and lactate production-diminishing effect.

In summary, the administration of pollen extract, the antioxidant-containing preparation, effectively ameliorated the subjective symptoms of muscle soreness parallel to both the decrease in enzyme leakage and decrease in MDA and lactate concentrations in the muscle tissue. The results of the study strongly suggest the involvement of lipid peroxides in the pathogenesis of muscle soreness and indicate the potential of free radical scavengers in its prophylaxis. Further studies are needed to confirm the usefulness of the present study design as a model for reperfusion injury.

Acknowledgements

This study was supported by the Swedish Sports Council and the Askers Foundation.

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