Eur J Nutr (2004) 43 :2–6
DOI 10.1007/s00394-004-0432-z

ORIGINAL CONTRIBUTION

Alfons Ramel
Karl-Heinz Wagner
Ibrahim Elmadfa

Plasma antioxidants and lipid oxidation after submaximal resistance exercise in men

Summary *Background* An increased generation of reactive oxygen species occurs during exercise. *The aim of the study* We investigated whether changes in plasma antioxidants and lipid oxidation products after submaximal resistance exercise are detectable, and whether training status has any effect on changes. *Methods* Seven resistance trained (RT, 31.3 ± 10.2 yrs) and ten non-resistance trained male subjects (NRT, 28.2 ± 3.9 yrs) performed a submaximal resistance exercise circuit (10 different exercises, 75 % of 1-repetition maximum, 18.6 ± 1.1 minutes). Blood samples were taken before and immediately after exercise. Plasma antioxidants (AO), lipid oxidation products malondialdehyde (MDA) and conjugated dienes (CD) were measured using HPLC and/or photometric detection. Groups were compared using the Mann-Whitney U test, the exercise effect was tested using the Wilcoxon signed ranks test. $P < 0.05$ was regarded as significant. *Results* α-Tocopherol, γ-tocopherol, β-carotene, lycopene, ascorbic acid, MDA and CD concentrations did not differ between groups at rest. There was a similar increase of fat soluble plasma AO in both groups after exercise, but not ascorbic acid. MDA increased also in both groups after exercise, but CD increased only in NRT. *Conclusion* There is no difference in plasma AO and lipid oxidation products in RT and NRT at rest. After short time resistance exercise there is a mobilization of fat soluble AO. Despite mobilization of AO, oxidative stress occurs during submaximal resistance exercise, which is indicated by increased MDA and CD concentrations. As exercise induced an increase of CD only in NRT, it seems that regular resistance training partly prevents lipid peroxidation during exercise.

Key words resistance exercise – reactive oxygen species – antioxidants – lipid oxidation

Received: 7 February 2003
Accepted: 16 May 2003
Published online: 6 January 2004

A. Ramel (✉)
Unit for Nutrition Research
University of Iceland
Eiriksgata 29
101 Reykjavik, Iceland
E-Mail: ramel@hi.is
Tel.: 3 54/8 64-83 30
Fax: 3 54/5 43-48 24

K.-H. Wagner · I. Elmadfa
Institute of Nutritional Sciences
University of Vienna
Vienna, Austria

Introduction

Although physical activity is known to have beneficial health effects [1], many studies have reported that physical exercise induces oxidative stress by increasing the generation of reactive oxygen species (ROS) [2, 3]. Aerobic exercise causes an increase in oxygen consumption and can result in elevated levels of free radicals. It has been demonstrated that whole body oxygen consumption increases 10- to 20-fold over the resting state during aerobic exercise [4]. To minimize oxidative stress and remove free radicals the body uses a very effective antioxidative defense system containing nutritional antioxidants (AO) like tocopherols, ascorbic acid or polyphenols and endogenous antioxidative enzymes like catalase or glutathione peroxidase. If free radical formation exceeds antioxidant capacity, lipids, proteins and other cell components may be oxidized [5–7].

Most of the experimental studies which have investigated the association between exercise-induced oxidative stress and its acute effects on plasma antioxidants (AO) or lipid peroxidation have used endurance exercise [3, 8–10]. Only a few studies which used resistance exercise to investigate this matter have been published [11, 12]. Allessio et al. [11] investigated the effects of isomet-

EJN 432

ric exercise, and Mc Bride et al. [12] investigated high intensity resistance exercise with respect to lipid oxidation. Both studies did not measure plasma AO. Submaximal resistance exercise has been recommended for maintaining health [13], and is performed regularly by many recreational athletes. Despite the lower oxygen demands during resistance exercise compared to aerobic exercise, generation of free radicals through other mechanisms is possible: 1) xanthine/xanthine oxidase pathway, 2) respiratory burst of neutrophils, 3) catecholamine auto-oxidation, 4) local muscle ischemia/hypoxia, and 5) conversion of the weak superoxide to the strong hydroxyl radical by lactic acid [5–7]. To increase the knowledge in this field, this study investigated 1) the effects of acute submaximal resistance exercise on plasma antioxidants and lipid oxidation products, and 2) the differences between resistance trained (RT) and non-resistance trained subjects (NRT) at rest and after exercise.

Methods

Subjects

Seventeen male participants were included in the study. They were students of the University of Vienna and/or customers of a local gym, who had been asked to participate. Ten of them were non-resistance trained (NRT), i. e., they had not performed resistance exercise or weight lifting at regular intervals for 6 months or longer before this study. Five men of the NRT group reported themselves to be sedentary and 5 reported to perform endurance exercise, i. e., running or bicycling, 2–3 times a week. Seven male participants were resistance trained (RT), i. e., they had performed resistance exercise or weight lifting at least three times per week for 6 months or longer before this trial. Five subjects of the RT group reported engaging in endurance exercise 2–3 times a week. Six subjects of the NRT group and 4 of the RT group were smokers. The participants were informed about the purpose, nature and potential risks and gave their written informed consent before participating. The study protocol was approved by the Ethic Committee of the Medical Faculty, University of Vienna. Characteristics of the participants are shown in Table 1.

Procedures

One week before the start of the study, subjects were shown the 10 exercises of the resistance exercise circuit (bench press, leg press, latissimus dorsi pull, leg extension, shoulder press, triceps exercise, crunch, vertical row, biceps curl and pull up) and their one repetition maximum (1-RM) for each exercise was evaluated. For the exercise *crunch* the maximum number of repetitions was evaluated. The participants of the NRT group in particular were instructed how to perform the various exercises. The intensity for the main test was defined individually at 75 % of the 1-RM, the median number of repetitions was 8 for NRT, and 11 for RT. Seventy five percent of the maximum repetitions were performed in the main test for the exercise *crunch*.

Table 1 Group characteristics (mean ± SD)

	NRT	RT	*P*-value*
Age (years)	28.2 ± 3.9	31.3 ± 10.2	0.669
Height (cm)	182 ± 5.0	182 ± 8.0	0.962
Body mass (kg)	81.5 ± 6.0	82.9 ± 10.0	0.887
Body fat (%)	18.6 ± 5.0	14.5 ± 4.5	0.091

RT ($n = 7$) resistance trained subjects; *NRT* ($n = 10$) nonresistance trained subjects;
* Mann-Whitney U test

For the main test subjects came to the resistance exercise circuit at 07.30 in the morning after an overnight fast and abstinence from alcohol since the previous day. The participants had not exercised for two days before the experiment. After a warm up on a cycle ergometer (15 min, 75 W), each subject performed the submaximal resistance circuit exercise once at the defined intensity. The recovery time between the different exercise stations was set to one minute. The exercise time (without warm up) was 18.6 ± 1.1 minutes. There was no fluid intake during exercise. A catheter was introduced into an antecubital vein in the morning preliminary to the test. Blood samples were drawn 30 minutes before and immediately after the resistance training circuit.

Measurements

All measurements were made in duplicate. Venous blood was collected into heparin-containing vacuum tubes (Vacuette; Greiner, Vienna, Austria). Blood was centrifuged to receive platelet poor plasma for analyses and stored frozen at –70 °C until analysis. Plasma ascorbic acid concentrations were analyzed within the same day.

Fat soluble AO (α- and γ-tocopherol, β-carotene and lycopene) were measured by the method of Jacob and Elmadfa [14]. Values were expressed in μmol · L plasma$_{-1}$. The intra-assay coefficients of variation (CV) for α- and γ-tocopherol, β-carotene and lycopene were 4.5, 5.1, 5.7 and 6.4 %, respectively.

Ascorbic acid was measured by the method from Denson and Bowers [15]. Plasma ascorbic acid values are expressed in μmol · L$_{-1}$. The intra-assay CV for this method was 2.2 %.

Lipid peroxidation was measured as MDA and CD of linoleic acid.

MDA was measured according to the method of

European Journal of Nutrition (2004) Vol. 43, Number 1

Wong et al. [16]. MDA values are expressed in μmol · L_{-1}. The intra-assay CV for this method was 5.7 %. CD formation of linoleic acid was measured using the method of Banni et al. [17]. Plasma values are expressed in μg · ml_{-1}. The intra-assay CV for this method was 3.9 %.

Statistical methods

Nonparametric tests were used for analyses. The groups were compared using the Mann-Whitney U test. Before-after differences were tested using the Wilcoxon signed ranks test. Correlations were calculated using Spearman's rho. All tests were performed using statistical software from SPSS/PC 10.0 (Statistical Package for the Social Science, SPSS Incorp., Chicago Ill., USA). The results are expressed as mean ± standard deviation (SD). Statistical significance was set at $P < 0.05$.

Results

Effect of exercise, all subjects together

α-Tocopherol, γ-tocopherol, β-carotene, and lycopene concentrations increased after exercise. MDA and CD concentration were also elevated after exercise. Ascorbic acid concentrations did not change significantly after exercise. The exact values are shown in Table 1.

Effect of exercise, NRT and RT separately

Fat soluble plasma antioxidants increased similarly in both groups, although a significant effect of exercise could only be observed in NRT. Ascorbic acid concentrations decreased in NRT, but this was not significant. MDA concentrations increased in both groups and CD concentrations increased only in NRT (Table 2).

Comparison between groups

CD concentrations after exercise were higher in NRT, although this was not statistically significant ($P = 0.09$). There were no other differences between groups (Table 2).

Correlation analyses

MDA concentrations after exercise correlated with ascorbic acid concentrations (rho = –0.719, 0.004). Other plasma AO were not related to CD or MDA concentrations.

Discussion

There was no difference in plasma AO and lipid oxidation products between the two groups at rest. Although there is an increasing body of evidence which shows that exercise can elevate oxidative stress [3, 11], the plasma AO status of athletes and sedentary individuals is controversial in the literature [8, 18–21]. It is not clear why studies examining antioxidant status in athletes show various results. It has been suggested that this variability may be due to the differences in the mode of exercise used, the time points examined, the level of training of the subjects, environmental factors and the chosen methodology [5, 6, 22]. Nutrition is known to influence AO status, which might explain some of the inconsistencies observed in these studies [23, 24].

Table 2 Exercise effect** on plasma antioxidants and lipid oxidation products, NRT ($n = 10$) and RT ($n = 7$; mean ± SD)

	Goup	Before exercise		After exercise	*P*-value*
Ascorbic acid	(μmol · L plasma^{-1})	NRT	51.0 ± 27.1	39.4 ± 23.8	0.139
		RT	49.8 ± 29.4	51.1 ± 28.6	0.893
α-Tocopherol	(μmol · L plasma^{-1})	NRT	16.2 ± 5.6	18.7 ± 6.2	0.021
		RT	18.5 ± 3.5	20.7 ± 6.3	0.063
γ-Tocopherol	(μmol · L plasma^{-1})	NRT	1.23 ± 0.48	1.39 ± 0.54	0.051
		RT	1.10 ± 0.41	1.23 ± 0.46	0.237
β-Carotene	(μmol · L plasma^{-1})	NRT	0.227 ± 0.148	0.266 ± 0.170	0.021
		RT	0.245 ± 0.135	0.298 ± 0.141	0.091
Lycopene	(μmol · L plasma^{-1})	NRT	0.177 ± 0.151	0.202 ± 0.164	0.028
		RT	0.252 ± 0.172	0.290 ± 0.131	0.235
MDA	(μmol · L plasma^{-1})	NRT	2.09 ± 1.18	2.49 ± 0.72	0.386
		RT	1.60 ± 0.38	2.00 ± 0.59	0.735
Conjugated dienes	(mg · L plasma^{-1})	NRT	12.1 ± 5.2	15.4 ± 8.1	0.028
		RT	8.6 ± 3.4	8.48 ± 1.6	0.600

RT resistance trained subjects; *NRT* resistance untrained subjects
* Wilcoxon signed ranks test
** Taking all subjects together (N = 17), the exercise effect was significant for all variables but ascorbic acid

Our study shows that fat soluble plasma antioxidants increase after acute submaximal resistance exercise. Similar results have been reported after aerobic exercise and isometric exercise in humans [9, 11, 20]. However, elevated plasma antioxidants after short-term resistance exercise have not been previously reported. It is known that there is a redistribution of AO towards the site of oxidative stress [25, 26]. The increase of fat soluble plasma antioxidants did not correlate with the increase of total plasma lipids (results not shown). Normally, the largest proportion of fat soluble AO is transported in various lipoproteins, e. g., HDL and LDL, which have been reported to increase directly after exercise [27], which could also increase fat soluble AO in plasma. In the present study, lipoprotein fractions were not measured; thus it is not possible to know whether the increase in fat soluble AO was due to higher LDL or HDL levels, or due to higher AO concentrations in the lipoproteins. When changes of fat soluble AO of NRT and RT were analyzed separately, the increase was significant only in NRT. However, because the mean changes were similar, we conclude that a lack of statistical power due to the smaller size of the RT group (7 vs. 10) was responsible for the fact that the effect of exercise was not significant in RT.

No significant changes in ascorbic acid concentrations could be observed during exercise. Other studies have reported higher ascorbic acid levels after endurance exercise. Gleeson et al. have suggested that the increase in ascorbic acid is a result of the release of stress hormones [28]. A weaker hormonal response during resistance exercise than during endurance exercise [29] could explain the unchanged ascorbic acid concentrations. Although there was no increase in ascorbic acid concentrations, we observed a negative correlation between MDA and ascorbic acid after exercise. It is widely accepted that ascorbic acid is an important antioxidant [30].

Despite mobilization of fat soluble AO during exercise, the plasma concentrations of the lipid oxidation product MDA increased after exercise. This is in agreement with some of the previous investigations involving aerobic exercise or resistance exercise [12, 31] indicating inadequacies in the antioxidant defense system [32]. Some studies have not found an elevated MDA concentration after exercise, which might be caused by delayed blood sampling several hours after exercise [32]. CD increased in NRT subjects after exercise, but not in RT subjects. Due to the large variability of CD between subjects, the difference was not significant. Other studies have not found higher CD in trained subjects after endurance exercise [33, 34]. There are several studies in humans [35, 36] which have documented that regular exercise reduces lipid oxidation after acute exercise. A protection of athletes could represent an adaptation which ensures adequate antioxidant defense during acute exercise.

Conclusions

There is no difference in plasma AO and lipid oxidation in RT and NRT at rest. After short time resistance exercise there is a mobilization of fat soluble AO. Despite this mobilization, plasma MDA and CD concentrations increase after exercise.

Acknowledgements The authors thank Ing. Markus Spannbruckner for technical assistance and help.

References

1. Sato Y (2000) Diabetes and life-styles: role of physical exercise for primary prevention. Br J Nutr 84 (suppl): 187S–190S. Review
2. Witt EH, Reznick AZ, Viguie CA, Starke Reed P, Packer L (1992) Exercise, oxidative damage and effects of antioxidant manipulation. J Nutr 122:766–773
3. Sanchez-Quesada JL, Holms-Serradesanferm R, Serrat-Serrat J, Serra-Grima JR, Gonzalez-Sastre J, Ordonez-Llanos J (1995) Increase of LDL susceptibility to oxidation occurring after intense, long duration aerobic exercise. Atherosclerosis 118:297–305
4. Konig D, Wagner KH, Elmadfa I, Berg A (2001) Exercise and oxidative stress: significance of antioxidants with reference to inflammatory, muscular, and systemic stress. Exerc Immunol Rev 7:108–133
5. Clarkson PM, Thompson HS (2000) Antioxidants: what role do they play in physical exercise and health? Am J Clin Nutr 72 (suppl):637S–646S
6. Ji LL (2000) Free radicals and antioxidants in exercise and sports. In: Garrett WE, Kirkendall DT (eds) Exercise and Sport Science. Lippincott Williams and Wilkin, Philadelphia, pp 299–317
7. Smith LL, Miles MP (2000) Exercise-induced muscle injury and inflammation. In: Garrett WE, Kirkendall DT (eds) Exercise and Sport Science. Lippincott Williams and Wilkin, Philadelphia, pp 401–411
8. Bergholm R, Mäkimattila S, Valkonen M, Liu M, Lahdenperä S, Taskinen MR, Sovijärvi A, Malmberg P, Yki-Järvinen H (1999) Intense physical training decreases circulating antioxidants and endothelium dependent vasodilatation in vivo. Atherosclerosis 145:341–349
9. Kaikkonen J, Kosonen L, Nyyssönen K, Porkkala-Sarataho E, Salonen R, Korpela H, Salonen JT (1998) Effect of combined coenzyme Q 10 and d-α-tocopheryl acetate supplementation on exercise-induced lipid peroxidation and muscular damage: a placebo controlled double blind study in marathon runners. Free Rad Res 29:85–92
10. Case D, Baer JT, Subbiah MT (1999) The effect of prolonged exercise on lipid peroxidation in eumenorrheic female runners. Med Sci Sports Exerc 31: 1390–1393
11. Alessio HM, Hagerman AE, Fulkerson BK, Ambrose J, Rice RE, Wiley RL (2000) Generation of reactive oxygen species after exhaustive aerobic and isometric exercise. Med Sci Sports Exerc 32: 1576–1581

12. McBride JM, Kraemer WJ, Triplett-McBride T, Sebastianelli W (1998) Effect of resistance exercise on free radical production. Med Sci Sports Exerc 30:67–72
13. American College of Sports Medicine: Position stand (1993) Physical activity, physical fitness, and hypertension. Med Sci Sports Exec 25:i–x
14. Jakob E, Elmadfa I (1995) Rapid HPLC-assay of vitamin K1, A, E and beta-carotene status in children (7–19 years). Internat J Vit Nutr Res 65:31–35
15. Denson KW, Bowers EF (1961) The determination of ascorbic acid in white blood cells. Clin Sci 21:157–162
16. Wong SHY, Knight JA, Hopfer SM, Zaharia O, Leach CN Jr, Sunderman FW Jr (1987) Lipoperoxides in plasma measured by liquid chromatographic separation of malondialdehyde-thiobarbituric acid adduct. Clin Chem 33: 214–220
17. Banni S, Lucchi L, Baraldi A, Botti B, Cappelli G, Corongin FP, Dessi MA, Tomasi A, Lusvarghi E (1996) No direct evidence of increased lipid peroxidation in hemodialysis patients. Nephron 72:177–183
18. Thomas TR, Ziogas G, Yan P, Schmitz D, LaFontaine T (1998) Influence of activity level on vitamin E status in healthy men and women and cardiac patients. J Cardiopulm Rehabil 18:52–59
19. Dernbach AR, Sherman WM, Simonsen JC, Flowers KM, Lamb DR (1993) No evidence of oxidative stress during high intensity rowing training. J Appl Physiol 74:2140–2145
20. Hernández R, Mahedero G, Caballero MJ, Rodríguez J, Manjón I, Rodríguez I, Maynar M (1999) Effects of physical exercise in pre- and postmenopausal women on lipid peroxidation and antioxidant systems. End Res 25:153–161
21. Sen CK (1995) Oxidants and antioxidants in exercise. J Appl Physiol 79:675–686
22. Jenkins RR (2000) Exercise and oxidative stress methodology: a critique. Am J Clin Nutr 72 (suppl):670S–674S
23. Peters EM, Anderson R, Nieman DC, Fickl H, Jogessar V (2001) Vitamin C supplementation attenuates the increases in circulating cortisol, adrenaline and anti-inflammatory polypeptides following ultramarathon running. Int J Sports Med 22:537–543
24. Huang HY, Appel LJ, Croft KD, Miller ER 3rd, Mori TA, Puddey IB (2002) Effects of vitamin C and vitamin E on in vivo lipid peroxidation: results of a randomized controlled trial. Am J Clin Nutr 76:549–555
25. Elsayed NM (2001) Antioxidant mobilization in response to oxidative stress: a dynamic environmental-nutritional interaction. Nutrition 17:828–834
26. Swift JN Jr, Kehrer JP, Seiler KS, Starnes JW (1998) Vitamin E concentration in rat skeletal muscle and liver after exercise. Int J Sport Nutr 8:105–112
27. Krum H, Conway EL, Howes LG (1991) Acute effects of exercise on plasma lipids, noradrenaline levels and plasma volume. Clin Exp Pharmacol Physiol 18:697–701
28. Gleeson M, Robertson JD, Maugham RJ (1987) Influence of exercise on ascorbic acid status in man. Clin Sci 73:501–505
29. Stock C, Schaller K, Baum M, Liesen H, Weiss M (1995) Catecholamines, lymphocyte subsets, and cyclic adenosine monophosphate production in mononuclear cells and CD4+ cells in response to submaximal resistance exercise. Eur J Appl Physiol Occup Physiol 71:166–172
30. Wilson JX (2002) The physiological role of dehydroascorbic acid. FEBS Lett. 527:5–9 (Review)
31. Child R, Wilkinson DM, Brown S, Fallowfield JL, Donnelly A (1998) Elevated serum antioxidant capacity and plasma malondialdehyde concentration in response to a simulated half-marathon run. Med Sci Sports Exerc 30:1603–1607
32. Lenn J, Uhl T, Mattacola C, Boissonneault G, Yates J, Ibrahim W, Bruckner G (2002) The effects of fish oil and isoflavones on delayed onset muscle soreness. Med Sci Sports Exerc 34: 1605–1613
33. Case D, Baer JT, Subbiah MT (1999) The effect of prolonged exercise on lipid peroxidation in eumenorrheic female runners. Med Sci Sports Exerc 31: 1390–1393
34. Sacheck JM, Decker EA, Clarkson PM (2000) The effect of diet on vitamin E intake and oxidative stress in response to acute exercise in female athletes. Eur J Appl Physiol 83:40–46
35. Miyazaki H, Oh-ishi S, Ookawara T, Kizaki T, Toshinai K, Ha S, Haga S, Ji LL, Ohno H (2001) Strenuous endurance training in humans reduces oxidative stress following exhausting exercise. Eur J Appl Physiol 84:1–6
36. Chang CK, Tseng HF, Hsuuw YD, Chan WH, Shieh LC (2002) Higher LDL oxidation at rest and after a rugby game in weekend warriors. Ann Nutr Metab 46:103–107