Correlations between plasma noradrenaline concentrations, antioxidants, and neutrophil counts after submaximal resistance exercise in men

A Ramel, K-H Wagner, I Elmadfa

Methods
Subjects
A mixed sample of 17 male participants was included into the study (table 1). Ten participants were not resistance trained, while five reported performing endurance exercise—that is, running or bicycling two to three times a week. Seven participants were resistance trained, and five reported to practising endurance exercise two to three times a week as well. 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 ethics committee of the Medical Faculty, University of Vienna.

Experimental protocol
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 exercise intensity for the main test was defined individually at 75% of the 1-RM; the mean number of repetitions was 9. For the exercise crunch, 75% of the maximum repetitions were done in the main test.

For the main test, subjects came to the resistance exercise circuit at 07.30 after an overnight fast and having abstained from alcohol since the day before. The participants had not done any exercise for two days before the experiment. After a warm up on a cycle ergometer (15 minutes, 75 W), each subject carried out the submaximal resistance circuit exercise once at the defined intensity. The recovery time between the different exercise stations was set at one minute. The mean (SD) exercise time (without warm up) was 18.6 (1.1) minutes. A catheter was introduced into an antecubital vein shortly before the test. Blood samples were drawn 30 minutes before the resistance training circuit and immediately after.

Biochemical analyses
All measurements were made in duplicate. Fat soluble antioxidants (α and γ tocopherol, β carotene, and lycopene) were measured by the method of Jacob and Elmadfa. Ascorbic acid was measured by the method of Denson and Bowers. Malondialdehyde (MDA) was measured according to Wong et al. Conjugated dienes of linoleic acid were measured by the method of Banni et al. Noradrenaline concentrations were measured using the method of Hollenbach et al. Neutrophils were counted with a blood cell counter shortly before the test (8). Neutrophil counts were performed using a blood cell counter (21).

Abbreviations: MDA, malondialdehyde; ROS, reactive oxygen species; 1-RM, one repetition maximum

Methods
Subjects
A mixed sample of 17 male participants was included into the study (table 1). Ten participants were not resistance trained, while five reported performing endurance exercise—that is, running or bicycling two to three times a week. Seven participants were resistance trained, and five reported to practising endurance exercise two to three times a week as well. 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 ethics committee of the Medical Faculty, University of Vienna.

Experimental protocol
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 exercise intensity for the main test was defined individually at 75% of the 1-RM; the mean number of repetitions was 9. For the exercise crunch, 75% of the maximum repetitions were done in the main test.

For the main test, subjects came to the resistance exercise circuit at 07.30 after an overnight fast and having abstained from alcohol since the day before. The participants had not done any exercise for two days before the experiment. After a warm up on a cycle ergometer (15 minutes, 75 W), each subject carried out the submaximal resistance circuit exercise once at the defined intensity. The recovery time between the different exercise stations was set at one minute. The mean (SD) exercise time (without warm up) was 18.6 (1.1) minutes. A catheter was introduced into an antecubital vein shortly before the test. Blood samples were drawn 30 minutes before the resistance training circuit and immediately after.

Biochemical analyses
All measurements were made in duplicate. Fat soluble antioxidants (α and γ tocopherol, β carotene, and lycopene) were measured by the method of Jacob and Elmadfa. Ascorbic acid was measured by the method of Denson and Bowers. Malondialdehyde (MDA) was measured according to Wong et al. Conjugated dienes of linoleic acid were measured by the method of Banni et al. Noradrenaline concentrations were measured using the method of Hollenbach et al. Neutrophils were counted with a blood cell counter shortly before the test (8). Neutrophil counts were performed using a blood cell counter (21).

Abbreviations: MDA, malondialdehyde; ROS, reactive oxygen species; 1-RM, one repetition maximum
analysed in the department of haematology at the General Hospital Vienna. Plasma volume changes were calculated using the formula of Van Beaumont et al.\textsuperscript{16}

Statistical analyses
Statistical analyses were done using SPSS/PC 10.0. The effect of exercise was tested using Wilcoxon's signed rank test. Correlations were calculated using Pearson's correlation coefficient $r$. Statistical significance was set at $p<0.05$.

RESULTS
Neutrophils, noradrenaline concentrations, fat soluble antioxidants, MDA, and conjugated dienes all increased after exercise. Ascorbic acid decreased after exercise, although not significantly. Plasma volume decreased slightly after exercise ($-2.5\%$); correction for plasma volume therefore attenuated the changes in the investigated variables (table 2). There were no differences between resistance trained and untrained participants in the investigated variables before and after exercise.

The increase in noradrenaline concentrations after exercise correlated positively with the increase in $\gamma$ tocopherol ($r = 0.584$, $p = 0.046$), $\beta$ carotene ($r = 0.710$, $p = 0.007$), and lycopene ($r = 0.553$, $p = 0.050$) after exercise. Ascorbic acid concentrations correlated positively with the increase in noradrenaline concentrations after exercise ($r = 0.647$, $p = 0.031$). The increase in neutrophils after exercise was related to conjugated dienes ($r = 0.601$, $p = 0.018$).

DISCUSSION
Acute resistance exercise increases lipid oxidation products, antioxidant concentrations, neutrophils, and noradrenaline concentrations in plasma, which cannot be explained by plasma volume changes. Despite mobilisation of fat soluble antioxidants during exercise, the plasma concentrations of MDA increased after exercise. This is in agreement with previous investigations involving resistance exercise,\textsuperscript{12} indicating inadequacies in the antioxidant defence system. The increase in $\gamma$ tocopherol, $\beta$ carotene, and lycopene concentrations during exercise correlated with the increase in noradrenaline. $\alpha$ Tocopherol, quantitatively the most important vitamin E vitamer, did not correlate with noradrenaline concentrations. It has been suggested that there is a rapid flux of $\alpha$ tocopherol between plasma and tissue,\textsuperscript{12} and this could explain why we could not detect any association between plasma noradrenaline and $\alpha$ tocopherol. There is a large variability in the response of plasma ascorbic acid to resistance circuit training and thus no significant differences between before and after the exercise, but higher ascorbic acid concentrations were also associated with the increase in noradrenaline concentrations.

The association between noradrenaline concentrations and antioxidants could represent a physiological mechanism ensuring adequate antioxidant defence during physical activity. This type of study and statistical analysis do not prove causality between noradrenaline and antioxidant concentrations; the observed correlations could also reflect the possibility that both factors—noradrenaline and antioxidants—respond independently of each other to exercise. However, it has been speculated that the release of catecholamines leads to upregulation of antioxidant defence.\textsuperscript{11} Catecholamine secretion parallels secretion of ascorbic acid from the adrenal gland,\textsuperscript{14} and there is research suggesting that noradrenaline secretion induces antioxidant defence—for example, superoxide dismutase expression.\textsuperscript{13}

In our study no significant correlation between the stress hormone and the lipid oxidation products conjugated dienes and MDA could be observed after exercise, although it has been suggested that auto-oxidation of catecholamines, which generates a superoxide anion radical, could result in lipid oxidation.\textsuperscript{15} During short term resistance exercise, noradrenaline concentrations increase only moderately. We cannot rule out the possibility that during intensive endurance exercise, high noradrenaline concentration may contribute to oxidative stress. After short time exercise there are other possible sources of free radicals causing lipid oxidation.\textsuperscript{16} For example, in our study there was a positive correlation between the neutrophil count and the concentrations of conjugated dienes; it is well known that neutrophils can

Table 1
Characteristics of the participants ($n = 17$)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>29.5 (7.1)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.82 (0.06)</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>82.1 (7.6)</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>16.8 (5.0)</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>24.8 (1.8)</td>
</tr>
</tbody>
</table>

BMI, body mass index.

Table 2
Plasma antioxidants, noradrenaline, neutrophils, and lipid oxidation products before and after exercise ($n = 17$)

<table>
<thead>
<tr>
<th></th>
<th>Before exercise</th>
<th>After exercise</th>
<th>$p$ Value</th>
<th>$p$ Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noradrenaline (nmol/l)</td>
<td>1.46 (0.53)</td>
<td>2.32 (0.90)</td>
<td>0.006</td>
<td>0.006</td>
</tr>
<tr>
<td>Neutrophils (cells × 10$^9$ ml)</td>
<td>2.66 (1.01)</td>
<td>3.83 (1.48)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid (µmol/l plasma)</td>
<td>50.6 (32.1)</td>
<td>43.6 (25.2)</td>
<td>0.363</td>
<td>0.099</td>
</tr>
<tr>
<td>$\alpha$ Tocopherol (µmol/l plasma)</td>
<td>17.2 (4.8)</td>
<td>19.6 (6.1)</td>
<td>0.026</td>
<td>0.156</td>
</tr>
<tr>
<td>$\gamma$ Tocopherol (µmol/l plasma)</td>
<td>1.17 (0.44)</td>
<td>1.32 (0.50)</td>
<td>0.017</td>
<td>0.020</td>
</tr>
<tr>
<td>$\beta$ Carotene (µmol/l plasma)</td>
<td>0.235 (0.138)</td>
<td>0.280 (0.154)</td>
<td>0.004</td>
<td>0.011</td>
</tr>
<tr>
<td>Lycopene (µmol/l plasma)</td>
<td>0.210 (0.159)</td>
<td>0.241 (0.153)</td>
<td>0.023</td>
<td>0.061</td>
</tr>
<tr>
<td>MDA (µmol/l plasma)</td>
<td>1.90 (0.98)</td>
<td>2.28 (0.69)</td>
<td>0.034</td>
<td>0.049</td>
</tr>
<tr>
<td>Conjugated dienes (mg/l plasma)</td>
<td>10.7 (5.2)</td>
<td>12.6 (7.1)</td>
<td>0.036</td>
<td>0.069</td>
</tr>
</tbody>
</table>

Values are mean (SD).

*After correction for plasma volume changes.
generate ROS through an oxidative burst, which might worsen exercise induced damage and contribute to lipid peroxidation.

Authors’ affiliations
A Ramel, Unit for Nutrition Research, University of Iceland, Reykjavik, Iceland
K-H Wagner, I Elmadfa, Institute of Nutritional Sciences, University of Vienna, Vienna, Austria

Correspondence to: Dr Alfons Ramel, Haedargardur 18, Reykjavik, Iceland; ramel@hi.is

Accepted 29 August 2003

REFERENCES