Chronic Eccentric Exercise and Antioxidant Supplementation: Effects on Lipid Profile and Insulin Sensitivity

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Abstract

Eccentric exercise has been shown to exert beneficial effects in both lipid profile and insulin sensitivity. Antioxidant supplementation during chronic exercise is controversial as it may prevent the physiological training-induced adaptations. The aim of this study was to investigate: 1) the minimum duration of the eccentric exercise training required before changes on metabolic parameters are observed and 2) whether antioxidant supplementation during training would interfere with these adaptations. Sixteen young healthy men were randomized into the Vit group (1 g of vitamin C and 400 IU vitamin E daily) and the placebo (PL) group. Subjects received the supplementation for 9 weeks. During weeks 5-9 all participants went through an eccentric exercise training protocol consisting of two exercise sessions (5 sets of 15 eccentric maximal voluntary contractions) per week. Plasma triglycerides (TG), total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), apolipoproteins (Apo A1, Apo B and Lpa) and insulin sensitivity (HOMA) were assessed before the supplementation (week 0), at weeks 5, 6, 7, 8 and 9. TG, TC and LDL were significantly lower compared to pre supplementation at both weeks 8 and 9 (P<0.05) in both groups. HDL was significantly elevated after 4 weeks of training (p < 0.005) in both groups. There was no effect of the antioxidant supplementation in any of the variables. There was no effect of either the training or the supplementation protocol in apolipoproteins levels and insulin sensitivity. A minimum duration of 3 weeks of eccentric exercise training is required before beneficial effects in lipid profile can be observed in healthy young men. Concomitant antioxidant supplementation does not interfere with the training-induced adaptations.

Key words: Eccentric exercise, insulin sensitivity, metabolic profile, vitamin supplementation.

Introduction

Exercise has been established as a very important tool in the prevention and treatment of a vast variety of chronic diseases. Although for many years concentric exercise, both aerobic and resistance, has been given all the attention, eccentric exercise has recently raised the interest of researchers (Jamurtas et al., 2013; Paschalidis et al., 2011; Silva et al., 2013). This is mainly due to the fact that eccentric exercise is characterized by lower metabolic cost and can therefore be more suitable for specific populations who are not able to participate in all types of exercise such as elderly individuals or patient populations who are characterized by muscle atrophy e.g. cancer patients or individuals in rehabilitation after sports injuries (LaStayo et al., 2014; Lim, 2016).

Although eccentric exercise has been used as a means to study mainly muscle damage and muscle function, the focus of the researchers has shifted lately towards the relation of eccentric exercise and metabolic regulation. Smith et al reported already in 1994 that an acute bout of eccentric exercise leads to a significant reduction in total cholesterol which can last up to 72h post exercise (Smith et al., 1994). More than a decade later, our group as well as others, confirmed the same positive results in systemic lipid profile after acute eccentric stimulus, regardless of the form of eccentric exercise and protocol used (Nikolaidis et al., 2008b; Papili et al., 2009; Paschalidis et al., 2010a). In addition, insulin sensitivity is another main end point that has been investigated in relation to eccentric exercise (Drexel et al., 2008). The results are homogenous across studies showing that an acute bout of eccentric exercise leads to temporary insulin resistance that can last up to 4 days (Kirwan et al., 1992; Jamurtas et al., 2013). This unfavourable effect is a result of impaired stimulation of the insulin signalling cascade in the skeletal muscle due to the increased inflammation that occurs after muscle damage during eccentric contractions (Del Aguila et al., 2000). However, these negative effects on insulin sensitivity are abolished when eccentric exercise is repeated in a chronic fashion. A number of studies has shown that chronic eccentric exercise has beneficial effects in glucose tolerance and clearance of LDL cholesterol (Drexel et al., 2008; Zeppetzauer et al., 2013), as well as in mitochondrial function (Silva et al., 2013). Interestingly, our group has previously shown that only a single bout of eccentric exercise every week for eight weeks is sufficient to improve blood lipid profile and insulin sensitivity without, however, altering the apolipoproteins levels (Paschalidis et al., 2011).

However, the minimum duration of eccentric exercise training required before the beneficial effects on the metabolic parameters are detected is still not known. It is widely known that eccentric exercise is associated with oxidative stress and muscle damage (Nikolaidis et al., 2008a) which results in impaired muscle function and performance. This notion has led both athletes as well as recreationally active individuals to extensive use of antioxidant supplements during exercise in an attempt to prevent these unfavourable effects. Con-
sumption of antioxidant vitamins has been proven to be beneficial in relation to muscle damage (Pereira Panza et al., 2015), muscle function (Jakeman and Maxwell, 1993; Shafat et al., 2004), inflammation (Goldfarb et al., 2005; Michailidis et al., 2013) and antioxidant defence. Nevertheless, during the last decade, a vast array of references has shown that use of antioxidant supplements during exercise might have detrimental effects on the exercise-induced adaptations, namely insulin sensitivity (Ristow et al., 2009), performance (Gomez-Cabrera et al., 2008; Ristow et al., 2009), mitochondrial biogenesis (Gomez-Cabrera et al., 2008; Gomez-Strobel et al., 2011; Paulsen et al., 2014a), and antioxidant defence mechanisms (Cumming et al., 2014; Gomez-Cabrera et al., 2008; Ristow et al., 2009; Yfanti et al., 2010; 2012). The hypothesis behind this notion is that use of antioxidants in relation to exercise blocks the free radicals that are produced during exercise exerting signalling effects for the exercise-induced adaptations.

Based on the above, the aim of the present study was two-fold; to investigate the minimum duration of the eccentric exercise training required before changes on metabolic parameters are observed and to further test whether antioxidant supplementation during training would interfere with these adaptations. For the purposes of this study, the antioxidant supplements that were used are vitamin C and E as these are the most commonly used among individuals. Furthermore, in order to have as more direct comparison as possible, discussion of the results is limited to studies where the same vitamin supplements have been used.

Methods

Subjects

Sixteen healthy men were randomised into two groups, the Vitamins group (Vit) (n = 8) and the Control group (Con) (n = 8). The two groups were randomized according to their age, body mass index and maximum isometric torque. Participants were moderately trained (physical activity 2-3 times/week). The characteristics of the two groups are shown in Table 1. There were no significant differences between the Vit and the Con group (Unpaired t-test).

Table 1. Personal characteristics of subjects. Data are means (±SEM).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Con</th>
<th>Vit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>25.9 (2.0)</td>
<td>24.6 (1.0)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.75 (.01)</td>
<td>1.75 (.01)</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>75.1 (1.9)</td>
<td>70.6 (8)</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>13.1 (2.0)</td>
<td>9.8 (1.4)</td>
</tr>
</tbody>
</table>

Written informed consent was obtained by all individual participants included in the study. The study was approved by the Institutional Review Board of the University of Thessaly and all procedures were in accordance with the 1975 Declaration of Helsinki, as revised in 2000.

During the first visit, body mass was measured to the nearest 0.5 kg (Beam Balance 710; Seca, Birmingham, United Kingdom) while height was measured to the nearest 0.5 cm (Stadiometer 208, Seca). Percent body fat was measured using a Harpenden skin fold caliper (John Bull, St Albans, United Kingdom). Measures from seven skinfolds were obtained (average of two measures on each site) and the Siri skinfold-thickness equation was used to calculate body fat.

Subjects were instructed to abstain from any other type of exercise during the investigational period.

Supplementation

Participants in the Vit group consumed 1 tablet of 1 g of vitamin C (ascorbic acid; Lamberts Health Care Ltd, Kent, United Kingdom) and 1 tablet of 400 IU vitamin E (d-α tocopherol; Lamberts Health Care Ltd) daily for 9 weeks while the PL group received placebo tablets (lactose).

All participants were instructed to take the supplementation once a day before breakfast. Each participant received the tablets pre-packed in daily doses labelled with the day of consumption. The supplementation started five weeks before onset of the training and continued throughout the training period. The participants were instructed to maintain their habitual diet.

Supplementation with vitamin C and E for >4 weeks has previously been used and proven as an adequate duration in order to increase significantly the plasma levels of both vitamin C and E (Theodorou et al. 2011; Yfanti et al., 2010; 2011; 2012).

Study design

Before beginning of supplementation, muscle function measurements and blood samples were drawn from all participants. After 5 weeks, the participants went through an eccentric exercise training protocol consisting of two exercise sessions per week for four weeks. The exercise was performed on both legs and was supervised. Blood samples and physiologic measurements were performed before the supplementation (week 0), at weeks 5, 6, 7, 8 and 9. A schematic presentation of the study design is shown in Figure 1.

Training protocol

The eccentric exercise was performed twice per week with two days rest between sessions, therefore, either every Monday and Thursday or every Tuesday and Friday. In case the participant was not able to perform the exercise the first scheduled day of the week, both sessions were performed one day later respectively. All participants completed all scheduled visits.

The exercise was performed on the isokinetic dynamometer which was calibrated weekly according to the manufacturer’s instructions. Before the eccentric exercise sessions, subjects performed a warm-up of 8 min on a Monark cycle ergometer (Monark, Vansbro, Sweden) at 70rpm and 50W followed by 5 min of stretching exercises of the major muscle groups of the lower limbs.
The exercise protocol that was used is previously described in (Theodorou et al., 2011) and has been shown to induce severe muscle damage and oxidative stress (Nikolaidis et al., 2007; Paschalis et al., 2010b; 2011; Theodorou et al., 2010; 2011). More specifically, during the eccentric exercise sessions, subjects were seated (120° hip angle) with the lateral femoral condyle aligned with the axis of rotation of the isokinetic dynamometer (Cybex, Ronkonkoma, NY) and were coupled to the dynamometer by an ankle cuff attached at the proximal to the lateral malleolus. The position of each subject was recorded and used in the next measurements. Each subject’s functional range of motion (ROM) was set electronically between full extension (0°) and 120° of knee flexion to prevent hyperextension and hyperflexion. Gravitational corrections were made to account for the effect of limb weight on torque measurements. Feedback of the intensity and duration of the eccentric exercise was provided automatically by the dynamometer. Subjects had to perform 5 sets of 15 eccentric maximal voluntary contractions with each leg at an angular velocity of 60/s in the seated position. There was a 2-min rest interval between sets.

Muscle function
Muscle function was evaluated by measuring the isometric knee extensor peak torque at 90° knee flexion. The average of three maximal voluntary contractions was used to calculate the peak torque. In case there was >10% difference between the lower and the higher torque values, the measurement was repeated. There was a 2 min rest break between repetitions. The test-retest reliability of the isometric peak torque measurement was 0.98.

Dietary analysis
Subjects were instructed to record their diet for 3 days before start of supplementation, at week 5 and after the training period. Each subject was provided with written guidelines for monitoring diet consumption and a record sheet to record food intake during these days. Subjects were asked to record their food intake two week days and one weekend day. Data were analysed using the nutritional analysis system Science Fit Diet 200A (Sciencefit, Athens, Greece).

Blood samples
Blood samples were drawn in the morning, after an overnight fast and after the subjects had abstained from alcohol and caffeine for 3 days. Blood was collected in EDTA-containing tubes and immediately centrifuged at 1370 X g for 10 min at 4°C and plasma was collected. Samples were stored in aliquots at -80°C and thawed only once before analysis. On the days of blood sampling, supplements were consumed after the blood was drawn.

Laboratory analyses
Vitamin concentration: Vitamin concentrations were measured as previously described (Theodorou et al. 2011). Specifically, vitamin C concentration in plasma was measured with the use of spectrophotometry using a ferric acid reducing ascorbate assay kit (K 671-1000 from Bio Vision (Mountain View), CA). Vitamin E concentration was measured according to the method of Talwar et al., 1998 (Talwar et al. 1998) using HPLC.

Insulin and Glucose: Both plasma glucose and insulin were measured photometrically using a biochemistry analyser Cobas Integra Plus 400 (Roche Diagnostics, Germany). HOMA was calculated according to the equation: glucose (mM) x insulin (μIU/ml)/22.5 (Matthews et al. 1985).

Lipids, lipoproteins and Apolipoproteins: Triglycerol (TG), total cholesterol (TC) and high-density lipoprotein cholesterol (HDL) were measured photometrically using a biochemistry analyser Cobas Integra Plus 400 (Roche Diagnostics, Germany). Finally, low density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation (Friedewald et al. 1972).

Lipoproteins Lipoprotein(a) ((Lp(a)), apolipoprotein B-100 (apo B-100) and apolipoprotein A-1 (apo A-1) were measured using a immunoturbidimetric method in a biochemistry analyser Olympus 640 (Medicon).

Statistical analysis
Data were tested for normality of distribution before further analysis using the Shapiro-Wilk test and it was confirmed that all data were normally distributed. A 2X2 repeated measures ANOVA was used to examine whether the vitamin supplementation (Vit vs Con) only during the first five weeks (week 0 vs week 5) had an effect on the assessed variables. Furthermore, a 2X6 repeated measures
ANOVA was used to identify differences between the two groups (Vit vs Con) across time (weeks 0-9). Data are presented as means ± SEM. The level of significance was set at α = 0.05.

Results

Vitamin C and E concentration in diet and plasma
There were no significant differences (p > 0.05) in the dietary intake of vit C and vit E neither between the two groups nor across time. That is depicted also by the plasma values of the two vitamins.

Vitamin concentration was measured at baseline (pre supplementation, after 4 weeks of supplementation without training and after 4 weeks of training (end of the training period, week 9). The Con and the Vit groups did not differ at baseline. Four weeks of supplementation with vitamin C and E resulted in significantly increased levels in the Vit group which remained elevated until the end of the training period (Table 2).

More specifically, plasma ascorbic acid increased in the Vit group by 22.7% after 4 weeks of supplementation and remained elevated until the end of the training period 818.6% increase9. Similarly, d-α tocopherol increased in the Vit group by 46% after 4 weeks of supplementation and remained elevated until the last week of training (32.9% increase). Plasma ascorbic acid and d-α tocopherol in the Con group did not change significantly throughout the study period.

Muscle function
Peak torque was increased significantly at week 8 and week 9 compared to pre-supplementation in both groups (Table 3). More specifically, peak torque increased by 4.9% at week 8 and by 6.6% at week 9 (p < 0.05) in the Con group. Respectively, in the Vit group, peak torque increased by 3.2% at week 8 and by 7.9% at week 9 (p < 0.05). There was no significant difference between the two groups.

Table 2. Plasma concentration of vitamin C and E levels in the Vit and Con groups. Data are means (±SEM).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Presup</th>
<th>Postsup</th>
<th>Week 6</th>
<th>Week 7</th>
<th>Week 8</th>
<th>Week 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vit C (mmol L⁻¹)</td>
<td>Con</td>
<td>56.7 (8.2)</td>
<td>61.9 (7.2)</td>
<td>63.5 (9.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vit</td>
<td>71.9 (6.0)</td>
<td>88.2 (6.0)</td>
<td>85.3 (5.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vit E (mmol L⁻¹)</td>
<td>Con</td>
<td>24.1 (2.0)</td>
<td>22.6 (2.1)</td>
<td>24.2 (1.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vit</td>
<td>21.6 (2.1)</td>
<td>31.5 (2.1)</td>
<td>28.7 (2.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Vit: Vitamin group; Con: Control group; Presup: pre supplementation; Postsup: post supplementation; mmol L⁻¹: millimole/liter. *Sig. different vs. Con.

Glucose, Insulin and HOMA
The results for plasma glucose and insulin concentration as well as for HOMA are presented in Table 3. The results show that there was no effect of either the training or the supplementation to any of these variables (p > 0.05 compared to pre-supplementation).

Lipids, lipoproteins and apolipoproteins
There was a significant time effect for the plasma triglycerides (TG), total cholesterol (TC), high density lipoprotein (HDL) and low density lipoprotein (LDL) for both the Con and Vit groups (Table 4). More specifically, TG were significantly lower compared to pre-supplementation at both weeks 8 and 9 (p < 0.05) for the Con group and for the Vit group. The same effect was detected for TC.

Table 3. Peak torque and HOMA values and plasma concentration of glucose and insulin. Data are means (±SEM).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Presup</th>
<th>Postsup</th>
<th>Week 6</th>
<th>Week 7</th>
<th>Week 8</th>
<th>Week 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak Torque (Nm)</td>
<td>Con</td>
<td>200.9 (12.4)</td>
<td>199.1 (11.8)</td>
<td>192.6 (12.0)</td>
<td>203.3 (13.2)</td>
<td>210.8 (12.8)</td>
<td>214.3 (11.6)</td>
</tr>
<tr>
<td></td>
<td>Vit</td>
<td>202.8 (11.3)</td>
<td>200.0 (10.3)</td>
<td>203.1 (12.4)</td>
<td>205.3 (10.7)</td>
<td>209.1 (12.1)</td>
<td>218.9 (11.7)</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>Con</td>
<td>4.74 (1.18)</td>
<td>4.79 (1.18)</td>
<td>4.73 (1.17)</td>
<td>4.74 (1.19)</td>
<td>4.68 (1.18)</td>
<td>4.75 (2.0)</td>
</tr>
<tr>
<td></td>
<td>Vit</td>
<td>4.41 (1.17)</td>
<td>4.42 (1.18)</td>
<td>4.41 (1.17)</td>
<td>4.35 (1.16)</td>
<td>4.32 (1.16)</td>
<td>4.33 (1.16)</td>
</tr>
<tr>
<td>Insulin (μIU/mL)</td>
<td>Con</td>
<td>15.31 (9.66)</td>
<td>14.98 (7.86)</td>
<td>14.88 (7.00)</td>
<td>14.47 (5.99)</td>
<td>14.29 (4.87)</td>
<td>14.59 (6.11)</td>
</tr>
<tr>
<td></td>
<td>Vit</td>
<td>14.69 (1.32)</td>
<td>14.33 (0.66)</td>
<td>15.08 (7.00)</td>
<td>14.04 (0.63)</td>
<td>14.78 (6.21)</td>
<td>14.29 (5.71)</td>
</tr>
<tr>
<td>HOMA</td>
<td>Con</td>
<td>3.24 (0.28)</td>
<td>3.20 (0.23)</td>
<td>3.14 (0.22)</td>
<td>3.05 (0.19)</td>
<td>2.97 (0.16)</td>
<td>3.08 (0.18)</td>
</tr>
<tr>
<td></td>
<td>Vit</td>
<td>2.85 (0.23)</td>
<td>2.79 (0.10)</td>
<td>2.92 (0.03)</td>
<td>2.60 (0.07)</td>
<td>2.82 (0.09)</td>
<td>2.72 (0.06)</td>
</tr>
</tbody>
</table>

Presup: pre supplementation; Postsup: post supplementation; Nm: Newton meter; mM: millimol; μIU/mL: micro international units/milliliter. *Significant different vs. Presup, p < 0.05.

Table 4. Plasma concentration of lipids and lipoproteins. Data are means (±SEM).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Presup</th>
<th>Postsup</th>
<th>Week 6</th>
<th>Week 7</th>
<th>Week 8</th>
<th>Week 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG (mm/L)</td>
<td>Con</td>
<td>71.0 (0.6)</td>
<td>72.0 (0.6)</td>
<td>65.0 (0.4)</td>
<td>64.0 (0.5)</td>
<td>64.0 (0.4)</td>
<td>64.0 (0.5)</td>
</tr>
<tr>
<td></td>
<td>Vit</td>
<td>80.0 (0.5)</td>
<td>79.0 (0.4)</td>
<td>76.0 (0.3)</td>
<td>78.0 (0.3)</td>
<td>74.0 (0.4)</td>
<td>72.0 (0.4)</td>
</tr>
<tr>
<td>TC (mm/L)</td>
<td>Con</td>
<td>4.73 (0.27)</td>
<td>4.74 (0.26)</td>
<td>4.70 (0.30)</td>
<td>4.63 (0.26)</td>
<td>4.50 (0.26)</td>
<td>4.48 (0.28)</td>
</tr>
<tr>
<td></td>
<td>Vit</td>
<td>4.55 (0.28)</td>
<td>4.58 (0.26)</td>
<td>4.56 (0.27)</td>
<td>4.40 (0.28)</td>
<td>4.36 (0.24)</td>
<td>4.26 (0.27)</td>
</tr>
<tr>
<td>HDL (mm/L)</td>
<td>Con</td>
<td>1.23 (0.11)</td>
<td>1.22 (0.11)</td>
<td>1.36 (0.13)</td>
<td>1.35 (0.13)</td>
<td>1.35 (0.11)</td>
<td>1.40 (0.11)</td>
</tr>
<tr>
<td></td>
<td>Vit</td>
<td>1.31 (0.12)</td>
<td>1.33 (0.11)</td>
<td>1.39 (0.13)</td>
<td>1.37 (0.12)</td>
<td>1.36 (0.12)</td>
<td>1.42 (0.12)</td>
</tr>
<tr>
<td>LDL (mm/L)</td>
<td>Con</td>
<td>3.36 (0.29)</td>
<td>3.37 (0.27)</td>
<td>3.21 (0.30)</td>
<td>3.16 (0.27)</td>
<td>3.02 (0.27)</td>
<td>2.95 (0.29)</td>
</tr>
<tr>
<td></td>
<td>Vit</td>
<td>3.09 (0.23)</td>
<td>3.09 (0.23)</td>
<td>3.02 (0.21)</td>
<td>2.88 (0.22)</td>
<td>2.85 (0.21)</td>
<td>2.70 (0.23)</td>
</tr>
</tbody>
</table>

TG: triglycerides; TC: total cholesterol; HDL: high density lipoprotein; LDL: low density lipoprotein; mm/L: millimol/liter. *Significant different vs. Presup, p < 0.05. ** Significant different vs. Presup, p < 0.001. ¥ Significant different vs. Presup, p < 0.005.
TC levels were significantly lower compared to pre-supplementation at both weeks 8 and 9 (p < 0.05) for the Con group and for the Vit group, respectively. In addition, there was a 10% reduction at week 8 of LDL in the Con and Vit groups, respectively. This reduction was even higher at week 9 for both groups as it reached 12% for the Con group and 13% for the Vit group (p < 0.001). The same training effect was detected in plasma HDL. However, the increase reached significance only after 4 weeks of training. In the Con group, the increase was 14% whereas in the Vit group the values increased by 8% (p < 0.005). There was no effect of the antioxidant supplementation for any of the above variables. In addition, no significant differences were detected in any of the apolipoproteins measured, either in response to the antioxidant supplementation or the eccentric training. The results are presented in Table 5.

In addition, Pearson correlation between the torque and glucose, insulin, lipids, lipoproteins and apolipoproteins was also performed. There were no significant correlations between the assessed variables (data not reported).

**Discussion**

**Changes over time**

The results of the present study demonstrate that four weeks of eccentric exercise training result in beneficial effects in lipid profile, regardless of the concomitant antioxidant supplementation. There is a number of studies which have shown the same beneficial effect in lipid markers after a training period with eccentric exercise (Drexel et al., 2008; Paschalis et al., 2011; Zeppetzauer et al., 2013). However, the novelty of our study is the fact that is the first one to examine changes on a weekly basis during the entire training period in contrast to the previous studies where differences were reported only before and after the training period. More specifically, it is shown that 3 weeks of eccentric exercise training are required before significant reductions in TG, TC and LDL are noted. In addition, an additional week is required before increases in HDL reach significance.

Therefore, our results provide an essential component for the design of future studies where eccentric exercise training will be used as stimulus for training adaptations. Furthermore, these results will comprise a tool for the design of physical activity programs which aim to improve metabolic fitness, providing the minimum duration of eccentric exercise training before the effects on lipid profile can be detected.

Although the beneficial effects of the training protocol used in this study are very clear on lipid profile, the same effect was not present with regards to insulin sensitivity and apolipoproteins. Our group has shown previously a very clear beneficial effect on insulin sensitivity and other health parameters after eight weeks of eccentric exercise training in young women (Paschalis et al., 2011). The same beneficial effect was noted in the studies of Drexel et al. (2008) and Zeppetzauer et al. (2013). One of the reasons for this discrepancy could be the difference in the duration of the training period. The duration in our study was only four weeks while in the studies mentioned above it was double. Therefore, it is possible that a longer training period is required before changes in insulin sensitivity can be detected.

Furthermore, the study population among the above-mentioned studies is different. In the study of Paschalis et al. (2011), female subjects were used in opposition to males that took part in this study. It is well known that there is a distinct sex difference with regards to the molecular mechanisms that are involved in insulin sensitivity with female subjects having a higher capacity for glucose uptake by the skeletal muscle as well as a higher metabolic flexibility (Lundsgaard and Kiens, 2014). In addition, the study population in the studies of Drexel et al. (2008) and Zeppetzauer et al. (2013) were sedentary and the average age was significantly older compared to the participants in the present study. The participants in this study were healthy recreationally moderately trained men performing physical activity 2-3 times per week making this sample group different than the previously mentioned study participants. Taken into account that age and sedentary lifestyle are both negatively correlated with overall health status (Wirth et al., 2016), the likelihood of achieving training adaptations of higher magnitude or in less time in such population compared to young healthy individuals is much higher.

**Effect of antioxidant supplementation on training adaptations**

Another purpose of the study was to examine whether concomitant antioxidant supplementation would interfere with the training-induced adaptations. There were no significant differences (p > 0.05) in the dietary intake of vit C and vit E neither between the two groups nor across time. That is depicted in the plasma values of the two vitamins which suggest that there was no hypovitaminosis at the beginning of the study. The supplementation protocol used in this study has been shown previously to pro-

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**Table 5. Plasma concentration of apolipoproteins. Data are means (±SEM).**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Week 6</th>
<th>Week 7</th>
<th>Week 8</th>
<th>Week 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lpa (mg/dL)</td>
<td>Con</td>
<td>10.08 (2.76)</td>
<td>11.13 (2.87)</td>
<td>10.13 (2.37)</td>
<td>9.25 (2.53)</td>
</tr>
<tr>
<td></td>
<td>Vit</td>
<td>8.75 (1.88)</td>
<td>8.75 (1.71)</td>
<td>9.50 (1.90)</td>
<td>7.38 (0.97)</td>
</tr>
<tr>
<td>ApoB-100 (mg/dL)</td>
<td>Con</td>
<td>90.63 (2.59)</td>
<td>95.63 (4.10)</td>
<td>93.25 (4.20)</td>
<td>90.63 (3.43)</td>
</tr>
<tr>
<td></td>
<td>Vit</td>
<td>84.13 (4.79)</td>
<td>83.13 (4.30)</td>
<td>89.00 (3.67)</td>
<td>89.25 (5.61)</td>
</tr>
<tr>
<td>ApoA-1 (mg/dL)</td>
<td>Con</td>
<td>117.50 (3.16)</td>
<td>110.88 (3.18)</td>
<td>110.25 (2.99)</td>
<td>110.25 (2.16)</td>
</tr>
<tr>
<td></td>
<td>Vit</td>
<td>120.63 (4.12)</td>
<td>122.50 (4.47)</td>
<td>124.00 (5.20)</td>
<td>122.00 (5.03)</td>
</tr>
<tr>
<td>ApoB/ApoA1 (mg/dL)</td>
<td>Con</td>
<td>0.82 (0.02)</td>
<td>0.87 (0.03)</td>
<td>0.85 (0.04)</td>
<td>0.83 (0.05)</td>
</tr>
<tr>
<td></td>
<td>Vit</td>
<td>0.71 (0.06)</td>
<td>0.70 (0.05)</td>
<td>0.74 (0.05)</td>
<td>0.75 (0.06)</td>
</tr>
</tbody>
</table>

Lpa: Lipoprotein a; Apo B-100: apolipoprotein B-100; apo A-1: apolipoprotein A-1; mg/dL: milligram/decilitre; Presup: pre supplementation; Postsup: post supplementation.
vide adequate antioxidant protection and this is confirmed by the increased levels of vitamin C and E in the blood after 4 weeks of supplementation (results Table 2). In addition, the training protocol used was effective in achieving a clear training effect, as it is shown by the significant increases in peak torque values (Table 3) after already 3 weeks of training. Nevertheless, the results of the present study clearly demonstrate that consumption of vitamin C and E during eccentric exercise training has no effect on any of the metabolic variables measured.

This is the first study to test the interrelation of antioxidant supplementation and chronic eccentric exercise on metabolic adaptations. The effect of the same combination of vitamins during training on metabolic parameters has been investigated in earlier studies (Higashida et al. 2011; Picklo and Thyfault 2015; Ristow et al. 2009; Yfanti et al. 2011), however, this was in relation to endurance concentric exercise training. Ristow et al. (2009) reported a detrimental effect of the antioxidant supplementation during a combined endurance and circuit training on insulin sensitivity in young healthy individuals. However this attenuating effect was not present after endurance training in the human study of Yfanti et al. (2011) or in the animal studies where the same combination of vitamins was used (Higashida et al., 2011; Picklo and Thyfault, 2015). The discrepancy among the results can be attributed to the differences in the training protocol, the study population and the vitamin dose that was used between the studies. Although a direct comparison of our study with the above-mentioned studies would not be optimal as the exercise type is very different, our study is another evidence of the complexity that characterizes this field. In the present study we were not able to detect the beneficial effect of the training on insulin sensitivity in either of the groups. This alone, leaves a very small window for detecting an additive effect of the antioxidant supplementation. The interrelation of insulin sensitivity mechanisms, eccentric exercise and antioxidant consumption has not been studied before. It is well-known though that an acute bout of eccentric exercise leads to temporary insulin resistance (Jamurtas et al., 2013). This effect is diminished after chronic exercise, however, this could be an underlying reason why changes in insulin sensitivity, either as a result of training or antioxidant effect, would take longer time to be detected. Therefore, it could be a possibility that significant changes in insulin sensitivity might require longer than four weeks of eccentric exercise training to appear.

Furthermore, data regarding the effect of antioxidant supplements during chronic exercise on lipid profile are scarce, and therefore interpretation of the results becomes difficult. As for the other variables measured in this study, there was no effect of the vitamins on the beneficial training effect observed in the lipid profile. This is in accordance with the results in the study of Yfanti et al. (2011). Of note, in the latter study, the training effect was only visible in the HDL.

As mentioned earlier in the introduction of this article, there is a lot of focus on the antioxidant effect during training on the adaptive responses with regards to performance (Asha Devi et al., 2003; Aguiló et al., 2007; Jourkesh et al., 2007; Nalbant et al., 2009; Yfanti et al., 2010; Zoppi et al., 2006), insulin sensitivity (Higashida et al., 2011; Picklo and Thyfault 2015; Ristow et al., 2009; Yfanti et al., 2011), antioxidant defence (Cumming et al., 2014; Gomez-Cabrera et al., 2008; Ristow et al., 2009; Yfanti et al., 2010; 2012), mitochondrial biogenesis (Gomez-Cabrera et al., 2008; Paulsen et al., 2014b; Strobel et al., 2011), and muscle hypertrophy (Paulsen et al., 2014b). In most of these studies the exercise mode used is endurance exercise, thus primarily concentric type of exercise. The only study that has used the same type of exercise is a previous study from our laboratory where also the same antioxidant protocol was used (Theodorou et al., 2011). Although the focus of the latter study was primarily on muscle performance and redox status, the results between the two studies support the lack of effect of the antioxidant supplements.

Conclusion

We investigated the minimum duration of eccentric exercise training that is required for changes in metabolic parameters to be detected. In addition, we investigated the possible effect of antioxidant supplementation on the adaptations that is attained after chronic eccentric training. Our results demonstrate that a minimum duration of three weeks of eccentric exercise are required for significant improvements in blood lipid profile to occur. Furthermore, we found that supplementation of vitamin C and E during the training period has no effect either on lipid profile or insulin sensitivity. There is certainly a great need for investigating further the eccentric exercise-induced adaptations in metabolic profile in various population groups. However, based on the results of this study, as well as on previous work on the synergetic effect of antioxidant supplementation with exercise training, we would like to emphasize a critical view towards consumption of vitamin supplementation during physical activity.

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**Key points**

- Eccentric training results in improvements in blood lipid profile.
- A minimum of three weeks is needed to see improvements in blood lipid profile following eccentric training.
- Vit C and E supplementation concomitant with eccentric training has no effect on blood lipid profile or insulin sensitivity.