Acute Antioxidant Supplementation Improves Endurance Performance in Trained Athletes

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ORIGINAL RESEARCH

Acute Antioxidant Supplementation Improves Endurance Performance in Trained Athletes

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This study examined the acute effects of a single dose of an antioxidant (AO; Lactaway containing pycnogenol) on time to fatigue (TTF). Nine trained cyclists [mean ± SD age 35 ± 10 yrs; body mass 71.6 ± 10.2 kg; VO₂peak 63 ± 11 ml/kg/min] performed on two separate occasions a continuous protocol of 5 min at 50% of peak power output (PPO), 8 min at 70% of PPO, and

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then cycled to fatigue at 95% PPO. Four hours prior to the exercise protocol, the subjects consumed the supplement or a placebo (counterbalanced, double blind protocol). Cyclists, on average, rode for 80 s more in the Lactaway trial than they did in the placebo trial. There was considerable evidence (chances ≥ 94.5%) for substantial positive treatment effects for TTF and the other performance-related variables (excluding [BLa] at 95% PPO). Other studies are necessary to confirm these results and identify the mechanisms underlying the observed effects.

KEYWORDS ergogenic, fatigue, metabolism, stress

INTRODUCTION

Peripheral muscle fatigue has been defined as “the progressive decline of performance which largely recovers after a period of rest” (Allen, Lamb, & Westerblad, 2008). Fatigue has been implicated with a variety of metabolic or neural factors of central (brain) or peripheral (skeletal muscle) origin (Allen et al., 2008; Robergs, Ghiasvand, & Parker, 2004; Taylor, Todd, & Gandevia, 2006). During sustained intensive whole-body exercise, there is the coordination of a number of physiological systems to facilitate gas exchange, energy supply, and, in turn, repetitive skeletal muscle contraction, improved task performance, and elimination of a premature reduction in power output. In this regard, muscle fatigue traditionally has been implicated with skeletal muscle acidosis due to an imbalance between lactic acid production and removal (metabolism) or substrate (glycogen) depletion (Juel et al., 2004; Pilegaard et al., 1999; Robergs et al., 2004; Sahlin, Tonkonogi, & Soderlund, 1998).

Reactive oxygen species (ROS) are continuously produced in skeletal muscle in a basal state but increase to a larger extent during exercise (Reid, 2008). The accumulation of ROS in contracting skeletal muscle is associated with the magnitude of the exercise task but also the AO capacity (Reid, 2008). At lower levels, ROS appears to improve muscle contractility in vitro, whereas at high levels muscle function may be impeded (Reardon & Allen, 2009; Reid et al., 1992).

Recent commentary on the effects of AO supplementation has suggested this as a means of improving skeletal muscle performance and reducing oxidative stress during exercise (Peake, Suzuki, & Coombes, 2007; Reid, 2008). Most studies to date however, have not shown any beneficial effect of AO supplementation directly or by way of consumption of diets containing a large amount of AOs (for review see Peake et al., 2007). The studies that have shown positive effects of AOs on skeletal muscle (endurance) performance have been with continuous administration
of a pharmacological (venous) infusion during exercise (Medved, Brown, Bjorksten, & McKenna, 2004a; Medved et al., 2004b). No studies to date have examined the acute effects on endurance performance of a single ingestion (dose) of a supplement containing AOs in athletes.

Lactaway is a new orally administered sports supplement containing Pycnogenol, which has not yet been assessed in terms of its ability to promote high-intensity endurance performance. Pycnogenol is the registered trade name for a natural extract from the bark of a French maritime pine (Pinus Pinaster). The extract contains a combination of phenolic acids, catechin, taxifolin, and procyanidins with each component exerting unique biological effects. Pycnogenol has a high bioavailability and exerts pronounced AO effects in vitro (Packer, Rimbach, & Virgili, 1999; Devaraj et al., 2002). Hence Lactaway, if consumed prior to exercise, may have the potential to improve AO capacity and promote performance during demanding exercise. No data are available, however, examining the acute effects of Lactaway on endurance performance during high-intensity endurance exercise. Only one previous study has been conducted examining the effect of pycnogenol on endurance performance (Pavlovic, 1999). In this study, a significant increase in endurance performance was observed in recreationally trained athletes after pycnogenol was consumed orally compared with a placebo preparation. This study was, however, conducted with long-term supplementation (30 days). There are no other studies that have examined a single dose of a supplement containing pycnogenol on endurance performance in athletes.

The purpose of this study was to examine the effect of an oral dose of Lactaway on TTF and related physiological parameters during high-intensity exercise in well-trained cyclists. It was hypothesized that a single dose of the supplement would improve TTF during high-intensity exercise.

**METHODS**

**Subjects**

Nine male cyclists [mean ± SD age 35 ± 10 yrs; body mass 71.6 ± 10.2 kg; peak oxygen uptake (VO₂peak; ml·kg⁻¹·min⁻¹) 63 ± 11] accustomed to high-intensity exercise voluntarily participated in the study. Subjects were informed verbally and in writing about the supplement and the study procedures. All the subjects then signed an informed consent document prior to commencing the experiment. Approval for the study was granted by an investigational review board. Prior to the commencement of any exercise testing, a medical questionnaire was administered to each subject to establish whether they were free from illness and injury. All subjects were well trained cyclists or triathletes, however, accustomed to demanding physical activity with no recognized pathology.
Pretesting and Familiarization

Prior to the experimental testing, each subject performed a progressive incremental exercise test to exhaustion on a cycle ergometer (Danpri, Melbourne, Australia) equipped with a portable power measurement system (Schoberer Rad Messtechnik, Science, Julich, Germany) for determination of PPO [Watts (W)] and VO₂max (ml·kg⁻¹·min⁻¹). Standard calibration including warm-up (performed by the researcher) and zero offset was set as per the manufacturer and as referenced by Gardner et al., (2004). Other studies have also shown that the reliability of the SRM system is high in the same testing time frame as that which was used in our study (Bertucci, Duc, Villerius, Pernin, & Grappe, 2005). All subjects previously had ridden the ergometer for the purposes of both incremental testing and interval training. The ergometer also was adjusted to the subject’s specific requirements prior to testing. The incremental test commenced at a workload of 1 W·kg⁻¹ with 30 W increments every 3 min (Bentley, Newell, & Bishop, 2007). During the test heart rate (HR), b·min⁻¹ was continuously measured with an HR monitor (Polar s810, Polar, Kempele, Finland). The subjects breathed into a one-way valve that was connected to a metabolic measurement system (Parvo Medics, TrueMax 2400, USA). The gas analyzers of the system were calibrated immediately prior to each test using ambient air and gases containing known concentrations of oxygen (16.0%) and carbon dioxide (3.98%). The turbine flow meter used for the determination of minute ventilation was calibrated with a 3-L syringe (Hans Rudolph, Shawnee, KS, USA). During the test, VO₂ was measured every breath then averaged every 60 s. The VO₂ peak was measured as the highest 60 s average value during the test, which was always located in the last workload before fatigue. Peak power output (PPO) was measured as the highest 3 min average power output (W) value during the test, which was the last 3 min of the test (Bentley et al., 2007).

After the incremental test, each subject completed a familiarization of the experimental exercise protocol to eliminate any learning effect. Specifically, each subject performed exercise to exhaustion at 95% of PPO. Each subject was tested in a climate controlled laboratory (temperature ~20°C; relative humidity ~50%). Following the pretest session, the subjects were asked to consume one sample of Lactaway (150 ml) 4 hrs prior to completion of their first experimental exercise session of either the supplement (containing 2.4 g of pycnogenol per 1 L) or a placebo that was of the same composition as the supplement without the presence of the pycnogenol. The time frame for consumption of the supplement as well as the amount was deemed optimal in terms of effects and gastrointestinal comfort in pilot examinations. The supplement was consumed with the subject’s normal precompetition meal. The subjects then fasted for the 4 hr duration prior to testing, with the exception of water intake. The subjects were also asked to ensure that the pretest meal consumed was the same on both occasions; this was verified via questionnaire and record. The administration of
the supplement was done in counterbalanced order and double blind so that the researchers and cyclists did not know what was consumed on each test occasion.

Experimental Procedures

The subjects attended the laboratory 30 min prior to the scheduled testing time, and the cycle ergometer was adjusted to their requirements. On both testing occasions the subjects performed a continuous, stepwise exercise protocol consisting of 4 min at 50% of PPO (warm-up), 8 min at 70% of PPO, and then cycled to fatigue at 95% PPO (TTF; sec). The reliability of TTF at this intensity and duration (~10) has been previously reported (Hopkins, Schabort, and Hawley, 2001). During the cycle ride to fatigue subjects were instructed to maintain the set work (W) and cycle for as long as possible. The subject was not aware of elapsed time or any physiological measure, only the power output required. During exercise, each subject maintained their “preferred pedaling cadence,” which was similar (~ 90 rpm) between trials. A fingerprick sample of blood was obtained in the last 60 s of each workload and immediately upon fatigue. The blood samples were collected into microcurvettes (Sarstedt, Numrecht, Germany) lined with Li-Heparin and analyzed immediately in duplicate, for lactate (mmol·l⁻¹) using a YSI 2700 Stat (Yellow Springs Instruments, Yellow Springs, OH, USA). In addition, a reliable subjective rating (6–20) of perceived exertion (RPE; Borg scale) was assessed at the end of exercise at 70% PPO and at fatigue (95% PPO; Eston & Williams, 1988). Heart rate (HR) (b·min⁻¹), VO₂ (l·min⁻¹), and pulmonary ventilation (VE) (l·min⁻¹) were measured continuously (as previously described) then averaged in the last 2 min of exercise at 70% and 95% PPO. Economy was measured as the ratio of work performed (Watts) to VO₂ (W·l⁻¹·min⁻¹). Both experimental tests were always completed with at least 72 hr but no more than a 5-day interval. Each subject rested and performed no activity in the 24 hrs prior to each test session.

Statistical Analysis

Unless otherwise stated, statistical analyses were performed using SPSS® for Windows software (release 16.0; SPSS Inc., Chicago, IL, USA). Normality of the raw scores and the difference scores (between conditions) was checked using Q-Q plots and deemed plausible in each instance. Central tendency and dispersion were reported as the mean and between-subject standard deviation. Inferential statistics were based upon magnitudes of minimum worthwhile effects and 90% confidence intervals, calculated using a spreadsheet (Hopkins, 2007), which incorporated effect sizes (mean differences) and significance probabilities obtained from two-sided, one-sample t tests. Minimal worthwhile effects were regarded as the minimum improvements in performance and physiological variables between the placebo
and supplement trials that would be deemed practically important from a physical performance or physiological perspective. This value was set at 1% for TTF, VO₂, economy, and blood lactate concentration (Paton & Hopkins, 2005). Ninety percent confidence intervals were used to help focus attention on the plausible ranges for the magnitude of the population differences, instead of on statistical significance (Sterne & Smith, 2001). The spreadsheet also calculated the chances that the population values of the effects for these variables were substantially positive. Qualitative interpretations of the chances of substantial effects were reported using the following percentage thresholds, where \( d_w \) is the absolute difference between experimental conditions for a particular variable: 
- \( d_w < 0.5 \), most unlikely;
- \( 0.5 \leq d_w < 5 \), very unlikely;
- \( 5 \leq d_w < 25 \), unlikely;
- \( 25 \leq d_w < 75 \), possibly;
- \( 75 \leq d_w < 95 \), likely;
- \( 95 \leq d_w < 99.5 \), very likely;
- \( d_w > 99.5 \), most likely (Hopkins, 2007).

Inferences on the minimum worthwhile effect were not made for minute ventilation, heart rate, and ratings of perceived exertion, as the minimum worthwhile effect could not be discerned.

**RESULTS**

The mean power output at 95% PPO was 301 ± 51 W in the placebo trial and 302 ± 50 W in the Lactaway trial (mean difference = 1 W; 90% CI -2, 5), which suggests that there was a negligible difference between experimental conditions. Table 1 shows the mean ± SD and 90% confidence intervals for the mean difference for TTF at 95% PPO and the physiological variables and RPE at 70% and 95% PPO. The chance that the population mean difference is substantial is also shown for TTF, VO₂, economy, and [BLa] at 70% and 95% PPO.

There was considerable evidence (chances ≥94.5%) for substantial positive treatment effects for performance-related variables (TTF, VO₂, economy, [BLa]), except the effect of Lactaway on [BLa] at 95% PPO was unclear. The “best guesses” for the mean population effects (i.e., the sample mean differences) was 16% for TTF and between 5% and 24% for VO₂, economy, and blood lactate concentration (excluding [BLa] at 95% PPO). Figure 1A–D shows plots of the individual response to Lactaway supplementation on TTF, VO₂, economy, and blood lactate concentration.

**DISCUSSION**

It is generally accepted that intensive (muscle contraction) exercise results in fatigue that is associated with increased oxidative stress (Allen et al., 2008; Reid, 2008). The purpose of this study was to determine the effects of a new antioxidant-containing supplement (Lactaway) on endurance performance in
TABLE 1 Results for Each Variable Measured at 70 and 95% Peak Power Output (PPO) During Placebo and Supplement Trials (n = 9)

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>% PPO</th>
<th>Placebo (mean ± SD)</th>
<th>Lactaway (mean ± SD)</th>
<th>Mean difference (90% CL)†</th>
<th>‡Chance (%) that the true difference is . . .</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Substantially positive</td>
</tr>
<tr>
<td>Time to exhaustion (s)</td>
<td>95</td>
<td>494 ± 213</td>
<td>574 ± 265</td>
<td>80 (20, 140)</td>
<td>97.6 (very likely)</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>3699 ± 524</td>
<td>3508 ± 457</td>
<td>−190 (−339, −42)</td>
<td>95.5 (very likely)</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>4131 ± 755</td>
<td>3876 ± 593</td>
<td>−254 (−432, −76)</td>
<td>97.2 (very likely)</td>
</tr>
<tr>
<td>VO₂ (ml·min⁻¹)</td>
<td>70</td>
<td>60.8 ± 5.9</td>
<td>63.8 ± 7.2</td>
<td>3.0 (0.5, 5.5)</td>
<td>94.5 (likely)</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>73.1 ± 5.4</td>
<td>78.1 ± 7.2</td>
<td>5.0 (1.7, 8.3)</td>
<td>97.9 (very likely)</td>
</tr>
<tr>
<td>Economy (W·l⁻¹·min⁻¹)</td>
<td>70</td>
<td>79.7 ± 11.1</td>
<td>74.6 ± 11.3</td>
<td>−5.1 (−9.0, −1.2)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>115.1 ± 14.0</td>
<td>112.0 ± 17.2</td>
<td>−3.1 (−10.8, 4.5)</td>
<td>–</td>
</tr>
<tr>
<td>Vₑ (l·min⁻¹)</td>
<td>70</td>
<td>153 ± 14</td>
<td>149 ± 14</td>
<td>−4 (−6, −2)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>170 ± 9</td>
<td>171 ± 8</td>
<td>1 (−2, 4)</td>
<td>–</td>
</tr>
<tr>
<td>HR (b·min⁻¹)</td>
<td>70</td>
<td>4.1 ± 1.9</td>
<td>3.1 ± 2.0</td>
<td>−0.9 (−1.6, −0.3)</td>
<td>98.6 (very likely)</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>11.4 ± 2.8</td>
<td>11.5 ± 3.4</td>
<td>0.1 (−1.4, 1.7)</td>
<td>36.3 (possibly)</td>
</tr>
<tr>
<td>[BLa] (mM)</td>
<td>70</td>
<td>12.9 ± 1.1</td>
<td>11.9 ± 0.6</td>
<td>−1.1 (−1.6, −0.5)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>19.1 ± 1.1</td>
<td>17.3 ± 2.1</td>
<td>−1.8 (−2.7, −0.9)</td>
<td>–</td>
</tr>
</tbody>
</table>

¹Any discrepancies between this column and the previous two columns are due to rounding errors. ²Values are not given for some variables because the minimum worthwhile effect could not be discerned. 90% CL = 90% confidence limits for the mean difference; [BLa] = blood lactate concentration; HR = heart rate; PPO = peak power output; RPE = rating of perceived exertion; Vₑ = minute ventilation.
FIGURE 1 A–D. Profile plots of individual values ($n = 9$) for time to fatigue (A), oxygen uptake (VO$_2$) (B), economy (C), and blood lactate concentration (D) during exercise at 95% peak power output in the placebo and Lactaway trials. The large square symbols represent the mean values.
trained athletes. The main findings were that there were substantial improvements in time to exhaustion, VO$_2$, and economy at 70% and 95% PPO, and [BLa] at 70% PPO, when AO supplementation was consumed compared with a placebo. These improvements were unlikely or very unlikely to have been a result of a placebo effect as the chances of a substantial positive effect were $\geq 94.5\%$. Moreover, the chance that Lactaway has a deleterious effect on TTF and associated physiological responses was very small ($\leq 1.5\%$).

A number of studies already have been conducted that have shown no performance benefits following consumption of diets containing higher levels of AOs (for summary see Peake et al., 2007). Comparison between studies is difficult, however, due to differences in the performance task performed, the type of diet or supplement used, and the duration of the intervention period. Pycnogenol demonstrates a strong AO effect with the flavonoid component significantly contributing to cellular defense against oxidative stress (Packer et al., 1999). Strenuous exercise induces a large oxidative stress response that is associated with acute fatigue manifestation (Allen et al., 2008; Reid, 2008). This oxidative stress-induced fatigue, however, could be blunted by preexercise consumption of AOs (Allen et al., 2008; Medved et al., 2004a; 2004b; Pavlovic, 1999; Reid, 2008). Hence, the results of the present study support this hypothesis, but they are unique in that it appears that a single dose of Lactaway containing pycnogenol is sufficient to enhance endurance performance.

It is worth noting that two other investigations have shown a similar performance improvement in a similar high-intensity exercise task (90% VO$_2$max) to that used in the present study when subjects consumed the AO N-acetyl cysteine (NAC) infused continuously during the exercise task, and this was associated with changes in plasma K$^+$ and glutathione concentration (Medved et al., 2004a; 2004b). Another study by the same group confirmed that NAC infusion during exercise improved K$^+$ regulation and fatigue resistance (McKenna et al., 2006). These authors concluded that NAC infusion could attenuate the ROS effect on skeletal muscle Na$^+$-K$^+$-ATPase activity. Whilst the data from the current study support these findings in terms of physical performance, it is unknown whether Lactaway consumed during exercise exhibits a similar response in terms of improved ionic regulation or increased AO capacity. Future studies are required to investigate these mechanisms.

During intense exercise the rate of blood flow is affected by increased blood viscosity (Windberger, Bartholovitsch, Plasenzotti, Korak, & Heinze, 2003). With increased blood viscosity, there is a narrowing of the microvessels, which may occlude as a consequence of inflexible red blood cells. Pycnogenol also has been shown to improve red blood cell membrane fluidity and prevent oxidative stress-induced hemolysis (Sivonová, et al., 2004). Such physiological changes may be important for improved physical performance and could have been a mechanism for the performance improvement.
observed in this study. This study was conducted in vitro, however, and in contracting skeletal muscle that may not have the same peripheral adaptations compared with muscle at rest. Additional work is required to examine further the hematological responses to supplementation with Lactaway and how these may affect endurance performance.

In this study the blood lactate concentration during submaximal (but not maximal) exercise was substantially lower when Lactaway was consumed prior to the exercise task. Previous studies have shown that by stimulating endothelial nitric oxide production, pycnogenol has been shown to relax constricted arteries in a dose-dependant relationship (Liu et al., 2004). As a consequence, vasodilation is significantly enhanced (Fitzpatrick, Bing, Rohdewald, 1998; Liu et al., 2004). Two previous studies also have demonstrated that pycnogenol significantly increases this vasodilatory response, which may improve micro-circulation and potentially lactic acid removal in muscle (Kohama, 2004; Wang et al., 1999). It is possible that the pycnogenol dosage was sufficient to promote increased lactate elimination and subsequent metabolism effecting blood lactate balance. Indeed, despite there being no substantial effect of the supplement on lactate concentration during exercise at 95% PPO, it appears there was considerable between-subject variation (Figure 1D), indicating that the supplement may have had differing effects on lactate production and elimination. Blood lactate concentration is a crude measure of lactate balance between lactate production and elimination. Hence the specifics of the adaptations observed in this study could well be due to a number of mechanisms including increasing muscle blood flow or upregulation of lactate transport mechanisms (Juel et al., 2004; Mourtzakis, González-Alonso, Graham, & Saltin, 2004).

In conclusion, this preliminary study indicates that a supplement (Lactaway) containing pycnogenol is able to improve endurance performance in trained athletes. Additional experiments are required to confirm these results, examine the optimal timing and dose amount of this supplement, as well as to establish the physiological mechanisms that explain the increased time to exhaustion during intense endurance exercise.

REFERENCES


