

Avenanthramide supplementation attenuates eccentric exercise-inflicted blood inflammatory markers in women

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Abstract

Purpose Rigorous exercise is known to generate reactive oxygen species (ROS) and inflict inflammatory response. The present study investigated whether dietary supplementation of avenanthramides (AVA) in oats would increase antioxidant protection and reduce inflammation in humans after an acute bout of eccentric exercise.

Methods Young women (age 18–30 years, $N = 16$) were randomly divided into two groups in a double-blinded fashion, receiving two cookies made of oat flour providing 9.2 mg AVA (AVA) or 0.4 mg AVA (Control, C) each day for 8 weeks. Before and after the dietary regimen each group of subjects ran downhill (DR) on a treadmill at -9% grade for 1 h at a speed to elicit 75 % of maximal heart rate. Blood samples were collected at rest, immediately and 24 h post-DR.

Results Before dietary supplementation plasma creatine kinase activity and tumor necrosis factor (TNF)- α concentration were increased immediately after DR ($P < 0.05$), whereas neutrophil respiratory burst (NRB) was elevated 24 h post-DR ($P < 0.05$). CK and TNF- α response to DR was abolished during post-supplementation tests in both AVA and C groups, whereas NRB was blunted only in AVA

but not in C. Plasma interleukin-6 level and mononuclear cell nuclear factor (NF) κ B activity were not affected by DR either before or after dietary supplementation, but were lowered 24 h post-DR in AVA versus C ($P < 0.05$). Both groups increased plasma total antioxidant activity following 8-week dietary regimen ($P < 0.05$), whereas only AVA group increased resting plasma glutathione (GSH) concentration ($P < 0.05$), decreased glutathione disulfide response to DR, and lowered erythrocyte GSH peroxidase activity ($P < 0.05$).

Conclusions Our data of pre- and post-supplementation difference reflect an interaction between repeated measure effect of eccentric exercise and AVA in diet. Long-term AVA supplementation can attenuate blood inflammation markers, decrease ROS generation and NF κ B activation, and increased antioxidant capacity during an eccentric exercise bout.

Keywords Antioxidant · Avenanthramide · Cytokine · Exercise · Inflammation

Abbreviations

AVA	Avenanthramides
BMI	Body mass index
CK	Creatine kinase
CRP	C-reactive protein
DR	Downhill running
GSH	Glutathione
GSSG	Glutathione disulfide
GPx	Glutathione peroxidase
IL	Interleukin
(NF) κ B	Nuclear factor-kappaB
NRB	Neutrophil respiratory burst
ROS	Reactive oxygen species
SOD	Superoxide dismutase

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TAC Total antioxidant capacity
TNF- α Tumor necrosis factor- α

Introduction

With the demand for increasing physical activity to reduce risks of certain chronic diseases, maintaining proper antioxidant defense during exercise and sports is an important issue for musculoskeletal health. Overproduction of reactive oxygen species (ROS) not only causes subtle cellular damage to muscle but also increases the risk of systemic inflammatory response following rigorous physical exertion (Proske and Allen 2005; Ji et al. 2009). However, recent research indicates that supplementation of large doses of antioxidants of pharmaceutical source can be problematic as it interferes with intrinsic adaptive responses and sometimes abolishes the benefit of exercise (Ristow et al. 2009; Gomez-Cabrera et al. 2008). Thus, exercise and nutrition scientists are increasingly interested in phytochemicals that demonstrate antioxidant and anti-inflammatory properties for potential dietary supplementation.

Avenanthramides (AVA) are a group of diphenolic acids that are found only in oats (*Avena sativa*) (Collins 1989). Consisting of an anthranilic acid and a hydroxycinnamic acid derivative linked by a pseudo-peptide bond, these compounds play the role of intrinsic antioxidants in oats, and share structural similarity to the pharmaceutical antioxidant Tranilast (Isaji et al. 1998). Of all the AVA that have been identified, three stand out due to their abundance and have been labeled as AVA-A, -B, and -C, which differ by a single moiety on the hydroxycinnamic acid ring. All three AVA demonstrate clear antioxidant activity in vitro, with AVA-C being the most potent (Peterson et al. 2002). In vitro studies have shown that AVA have anti-inflammatory and anti-atherogenic effects of decreasing monocyte adhesion to human aortic endothelial cells (HAEC), as well as suppressing adhesion molecule and pro-inflammatory cytokine production (Liu et al. 2004). AVA-C displayed further anti-atherogenic potential by inhibiting vascular smooth muscle cell (SMC) proliferation and enhancing nitric oxide production in both SMC and HAEC, along with up-regulation of endothelial nitric oxide synthase and decreased nuclear factor (NF) κ B activity (Nie et al. 2006a, b). However, the aforementioned in vitro data were derived with AVA doses not possible to reach with human dietary studies; therefore, their implication to health benefit remains unclear.

The ability of AVA to reduce exercise-induced ROS and oxidative damage has been reported sparsely in the literature. Ji et al. (2003) showed that rats fed AVA-C at the dose of 0.1 g/kg for 50 days displayed lower ROS generation and lipid peroxidation in the soleus muscle after an acute bout of treadmill running. Furthermore, AVA-supplemented

rats showed higher levels of antioxidant enzyme activities in the muscle, heart, liver and kidney. It is important to note that after rats were orally gavaged a mixture of synthetic AVA, all three fractions of AVA appeared in significant quantity in the plasma, heart, liver and skeletal muscle during a 48 h period, indicating AVA are bioavailable to tissues (Koenig et al. 2011). The above studies have provided some promise that dietary supplementation of AVA could be a potential protector against muscle oxidative stress and inflammatory response during and following rigorous muscle contraction. However, no study to date has examined the potential anti-oxidant and anti-inflammatory effect of AVA in human subjects.

Thus, the purpose of the present study is to examine whether long-term dietary supplementation of an oat product rich in AVA content could enhance blood antioxidant capacity and reduce blood inflammatory markers after a downhill running (DR) protocol in young female subjects. We tested the following hypotheses: (1) In healthy human subjects, high concentration of AVA supplementation would significantly decrease DR-induced muscle damage and subsequent inflammatory responses in blood circulation; (2) the protective effects of AVA supplementation are due to its ability to enhance blood antioxidant defense and to inhibit NF κ B activation in the immune-active blood cells.

Materials and methods

Subjects

Young women aged 18–30 years were recruited from the Madison, Wisconsin, community. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Institutional Review Board of University of Wisconsin-Madison Health Science (protocol number H-2007-0078-2).

Informed consent was obtained from all individual participants included in the study. The subjects also completed a Health History Survey to ensure that they were eligible for the study and healthy enough to exercise. During the initial visit subjects were instructed to complete the International Physical Activity Questionnaire (IPAQ) and health history questionnaire. Major exclusion criteria were cardiovascular disease, diabetes, asthma, smoking or other tobacco use, drinking alcohol in excess of 5 drinks per week, use of nutraceuticals (e.g., St. John's Wort), blood pressure medication, non-steroidal anti-inflammatory drugs (NSAIDs), anticoagulants, antidiabetic and hypoglycemic

drugs. Those subjects who had been routinely engaged in high level of physical training were also excluded. Subjects were told to refrain from heavy exercise with heart rate over 120 BPM. A diary was provided to each subject to register any physical activity that exceeds the above level. We found that exercise habits during the course of the study did not differ between treatment groups over time.

Dietary supplementation

The subjects were randomly assigned to one of following two groups ($N = 8$ per group) receiving either a high dose of AVA supplementation in the diet, or a low dose of AVA. Other than this difference, the groups were treated exactly the same in a double-blind fashion. Both dietary groups of subjects received cookies made of oat flours with known AVA concentration, provided courtesy of Ceapro Inc. (Edmonton, AB, Canada). AVA concentrations were verified using high-performance liquid chromatography (HPLC) in our laboratory (see below). The high-AVA oat flour contained 190 mg/kg, and the low-AVA control group flour contained 8 mg/kg. We used this line of oat as control because it contained the lowest amount of AVA concentration among all oat lines available, but contained identical levels of starch, lipid, protein and other nutrients such as tocopherols, flavonoids and phenolic acids, as the high-AVA oat. The recipe for each type of cookie was identical except in the type of flour used. Each cookie contained 30 g flour (AVA or Control), 5.91 mL unsweetened apple sauce (Surefine, USA), 12.32 mL artificial sweetener (Natrastaste Gold, NY), 0.616 mL baking soda, and 0.0308 mL table salt, with a total of 125 kcal per cookie. They were baked in a low temperature oven (121° C) for 15 min to ensure that AVA was not broken down or produced by the oat during the process (Dimberg et al. 2001). Final AVA concentration in the high- and low-AVA cookie was 4.6 mg/cookie (9.2 mg/day) and 0.2 mg/cookie (0.4 mg/day), respectively. Subjects were instructed to consume two cookies per day: one in the morning with breakfast and one in the evening with dinner. All subjects were instructed to maintain their normal dietary habit and not to take oat products throughout the experimental period. They were also asked to keep a record of any drug or antioxidant intake, which was periodically checked by the study administrator. No such violation was found for any of the subjects.

Exercise protocol

Before and after the 8-week dietary regimen, each subject performed a DR consisted of four bouts of 15 min of treadmill running at -9% grade, separated by three sessions of 5 min rest. Subject wore a heart rate monitor

and the treadmill speed was manually adjusted at 5-min intervals to keep the heart rate at 75 % of its maximum (220—age). Three blood samples were taken prior to DR at the resting state, immediately after DR, and 24 h after DR. All blood samples were taken in the post-absorptive state at 7:00–10:00 am. Subjects were told to terminate cookie consumption at least 24 h prior to the visit when the resting blood sample was taken. In order to control for the potential effect of hormone levels, DR was performed between days 7 and 10 of the participant's menstrual cycle, determined by self-report.

Blood sample collection and preparation

Mixed venous blood was drawn from an antecubital vein into 4 EDTA-coated Vacutainer tubes (7 mL each). One tube of whole blood was immediately centrifuged at $500\times g$ at 4° C for glutathione assay. The remaining blood volume was gently pipetted over two layers of density gradient (Histopaque and Ficoll-Paque) for isolation of blood cells. After centrifugation at $500\times g$ for 30 min at 20 °C, plasma was removed by aspiration and frozen at $-80\text{ }^{\circ}\text{C}$. A band of monocytes was then removed by aspiration, washed with phosphate buffered saline (PBS), and frozen at $-80\text{ }^{\circ}\text{C}$. The remaining fluid was removed and washed with ice cold PBS to attain neutrophils. Packed erythrocytes were removed and stored immediately at $-80\text{ }^{\circ}\text{C}$.

Biological measurements

Elisa

Plasma IL-1B, IL-6, TNF α , and CRP were analyzed in duplicate via commercially available ELISA kits (eBioscience, Read-Set-Go! ELISA. San Diego, CA). All samples were measured in duplicate using 96-well plates coated with various capture antibodies.

Nuclear factor-kappaB binding to DNA was measured by ELISA in nuclear extracts of mononuclear cells using antibody against p65 (eBioscience InstantOne ELISA. San Diego, CA).

High-performance liquid chromatography (HPLC)

Plasma concentration of reduced (GSH) and oxidized glutathione (GSSG) were measured by HPLC (Shimadzu Scientific, Columbia, MD) equipped with a UV–VIS detector set at 365 nm wavelength (Ji and Fu 1992). AVA concentrations in the oat cookie were measured using HPLC equipped with a Supelco C-18 column and a UV–VIS detector set at 330 nm absorption wavelength according to Chen et al. (2004) with some modifications (Koenig et al. 2014).

Table 1 Information of human subjects

	Age (year)	Height (m)	Pre-supplementation		Post-supplementation	
			Body weight (kg)	BMI	Body weight (kg)	BMI
AVA ($N = 8$)	22.1 ± 0.76	1.58 ± 0.02	59.32 ± 1.78	23.9 ± 0.89	59.2 ± 1.6	23.9 ± 0.81
Control ($N = 8$)	22.4 ± 0.68	1.59 ± 0.01	59.09 ± 1.67	23.3 ± 0.72	59.3 ± 1.8	23.4 ± 0.79

Data are mean ± SEM

AVA avenanthramide, *BMI* body mass index defined by the ratio of body weight (kg) divided by height (m²)

Neutrophil respiratory burst assay

Neutrophils respiratory burst activity was measured using a procedure according to Benbarek et al. (1996) with modifications. Three concentrations of phorbol myristate acetate (PMA) were used to evaluate the effects of maximal (160 µmol/L), moderate (16 µmol/L), and minimal (1.6 µmol/L) stimulation to the cells. A total of 1×10^6 neutrophils were used in each trial. The respiratory burst chemiluminescence was tracked for 30 min by a luminometer (Turner Biosystems, Madison, WI).

Spectrophotometric assays

Plasma total antioxidant capacity (TAC) was measured by spectrophotometer by monitoring the attenuation of 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) oxidation at 734 nm according to Re et al. (1999). A solution of 7 mmol/L ABTS and 2.45 mmol/L aluminum potassium sulfate (APS) was made immediately before the conducting of the assay and kept in the dark. An aliquot of 100 µL plasma was added to a final volume of 1 mL with ABTS/APS solution. The cuvette was mixed by inversion and then incubated at 37 °C for 5 min. The cuvettes were then read against a Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) standard curve using a spectrophotometer (Shimadzu UV160).

Plasma creatine kinase (CK) activity was measured as a marker of eccentric muscle damage according to the procedure of Tanzer and Gilvarg (1959). The CK reaction was coupled to NADH conversion to NAD by lactate dehydrogenase (LDH), which, along with pyruvate kinase (PK), phosphoenol pyruvate (PEP), and NADH, was present in the reaction mixture. The decrease in NADH concentration was tracked using a spectrophotometer. Erythrocyte superoxide dismutase (SOD) activity was measured spectrophotometrically by tracking the decrease in auto-oxidation of epinephrine to adrenochrome according to Sun and Zigman (1978). Epinephrine autoxidation rate was measured at 320 nm for 3 min in the presence of an aliquot of erythrocyte lysate. The slope of the linear portion of the absorption graph was used to determine

SOD activity by determining the percent inhibition of epinephrine autoxidation via comparison to the blank. Erythrocyte glutathione peroxidase (GPx) activity was measured by monitoring the change in NADPH concentration in a system with excess GSH and glutathione reductase (GR) in the presence of H₂O₂ (Flohe and Gunzler 1984). SOD and GPx activities were normalized to hemoglobin concentration.

Statistical analysis

Data were shown as mean ± SEM and analyzed using the Planned Comparison method. A three-way repeated measures ANOVA was first conducted using R (version 2.14.1) statistical software. The three main factors are (a) post- versus pre-AVA supplementation, (b) timing with respect to DR test, and (c) high-AVA versus low-AVA supplementation. The standard error of estimate of the ANOVA was used to complete a priori planned comparisons. Significance for each comparison was set at $P < 0.05$.

Results

Participant data

The age, height, weight, and body mass index (BMI) of the study participants are displayed in Table 1. There were no significant differences between groups for any of the parameters measured. Body weight and BMI were unchanged following dietary supplementation.

Muscle damage caused by Down-hill running

During the pre-supplementation trials, plasma CK activity increased sharply ($P < 0.05$) after DR and returned to baseline 24 h post-DR (Fig. 1). After 8-week dietary supplementation, CK response to DR was abolished in both high- and low-AVA groups. Furthermore, post-DR CK levels were significantly lower after supplementation compared to pre-supplementation. However, there was no AVA dose difference at any time point.

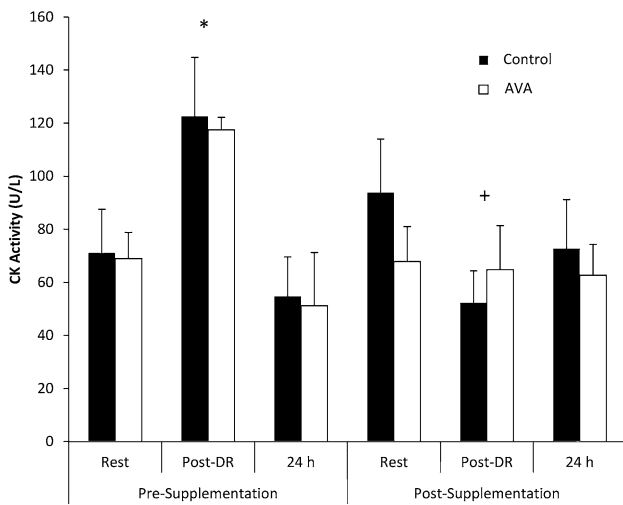


Fig. 1 Plasma CK activity in young women. Data are mean ± SEM (*N* = 6). **P* < 0.05, Post-DR versus Rest. +*P* < 0.05, Post- versus pre-supplementation in post-DR regardless of AVA dose

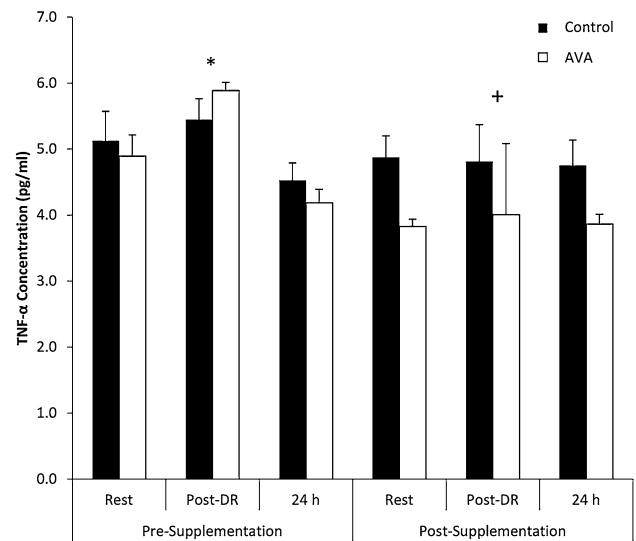


Fig. 3 Plasma tumor necrosis factor (TNF)-α concentration in young women. Data are mean ± SEM (*N* = 8). **P* < 0.05, Post-DR versus rest. +*P* < 0.05, post- versus pre-supplementation in post-DR regardless of AVA dose

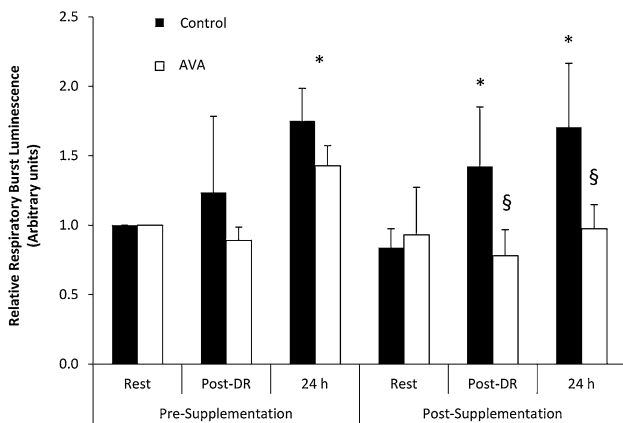


Fig. 2 Neutrophil respiratory burst activity in young women. Data are mean ± SEM (*N* = 8), normalized to pre-supplementation rest value. **P* < 0.05, 24 h post-DR versus rest. §*P* < 0.05, high-AVA versus low-AVA in post-DR or 24 h post-DR

Inflammatory markers

NRB activity immediately post-DR was not significantly different from the resting level, but showed an increase 24 h post-DR (*P* < 0.05) (Fig. 2). After supplementation, NRB activity was increased after DR and remained elevated 24 h post-DR in low-AVA group (*P* < 0.05). In high-AVA group NRB activity was not altered by DR, and the levels of post-DR and 24 h post-DR were lower than their counterparts during pre-supplementation trial (*P* < 0.05).

Plasma TNF-α concentration was increased after DR (*P* < 0.05), but returned to the resting level after 24 h during the pre-supplementation trials (Fig. 3). After

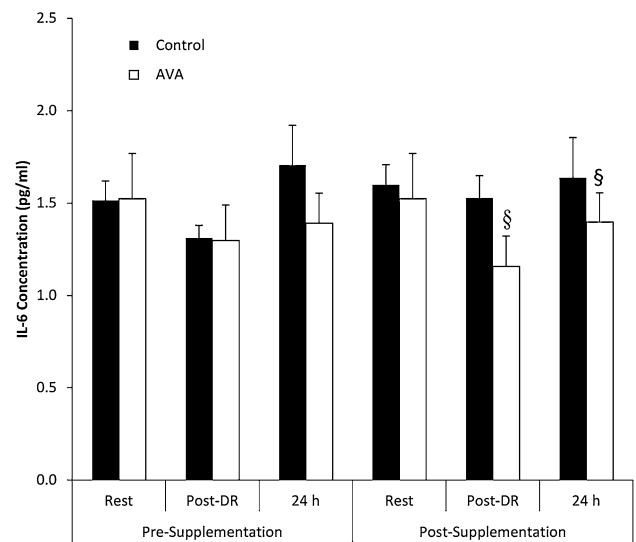


Fig. 4 Plasma interleukin (IL)-6 concentrations in young women. Data are mean ± SEM (*N* = 8). §*P* < 0.05, high-AVA versus low-AVA in post-DR or 24 h post-DR

dietary supplementation TNF-α concentration was no longer altered by DR regardless of AVA dose, whereas post-DR levels were lower compared to pre-supplementation values (*P* < 0.05). Also, there was a trend towards a lower TNF-α levels in high-AVA versus low-AVA group (0.05 < *P* < 0.1).

Plasma IL-6 concentration was not affected by DR during pre-supplementation trials (Fig. 4). However, after supplementation IL-6 level was significantly lower at post-DR

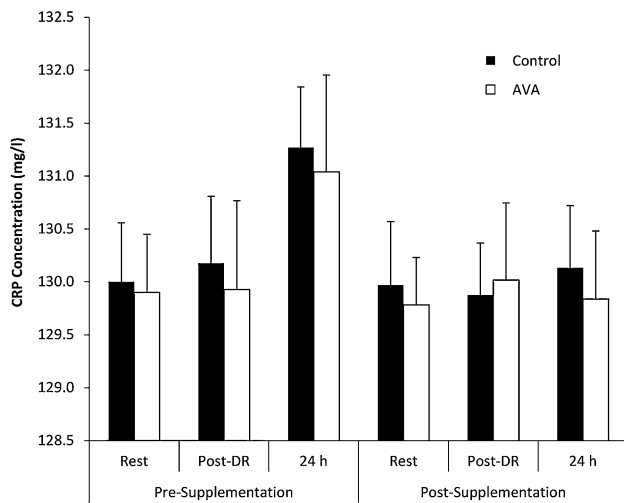


Fig. 5 Plasma C-reactive protein (CRP) concentrations in young women. Data are mean \pm SEM ($N = 8$)

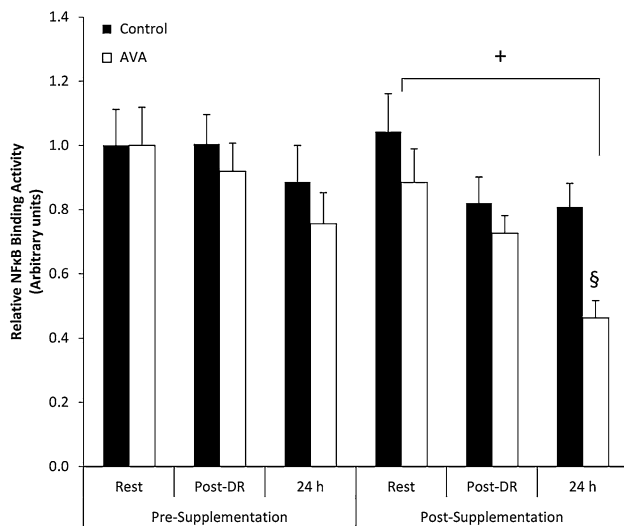


Fig. 6 Mononuclear cell NFκB binding activity in young women. Data are mean \pm SEM ($N = 8$), normalized to pre-supplementation rest value. $^+P < 0.05$, Post- versus pre-supplementation regardless of time. $^{\S}P < 0.05$, high-AVA versus low-AVA in 24 h post-DR

and 24 h post-DR in high-AVA group than low-AVA group ($P < 0.05$, AVA-DR interaction). Plasma IL-1 β showed no significant change in response to DR or AVA supplementation (not shown). Although CRP levels appeared to be elevated in 24 h post-DR prior to AVA supplementation, the change was not significant due to high variation (Fig. 5).

Mononuclear cell NFκB activation

Nuclear factor-kappaB binding did not change significantly in response to DR before or after dietary regimen (Fig. 6). ANOVA revealed a main effect that NFκB binding was

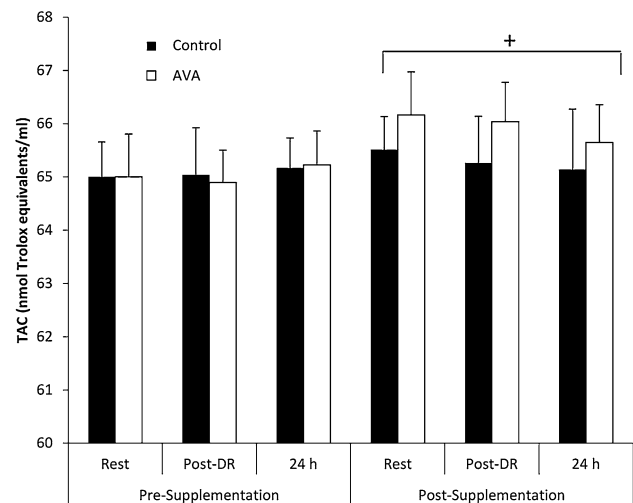


Fig. 7 Plasma total antioxidant capacity (TAC) in young women. Data are mean \pm SEM ($N = 8$). $^+P < 0.05$, post- versus pre-supplementation regardless of time or AVA dose

reduced after supplementation compared to before supplementation ($P < 0.05$). Post-hoc test showed that NFκB binding was lower in AVA versus C at 24 h post-DR ($P < 0.05$).

Plasma antioxidant capacity

Total antioxidant capacity was not affected by DR before oat supplementation, but elevated significantly after the dietary treatment regardless AVA dose ($P < 0.05$; main effect) (Fig. 7).

Erythrocyte SOD activity was not affected by DR or diet. Erythrocyte GPx activity showed no change following DR and 24 h post-DR before supplementation. However, after dietary supplementation GPx activity was lowered in high-AVA compared to low-AVA group and the difference was significant immediately post-DR ($P < 0.05$).

Plasma GSH concentration was not altered immediately after DR, but significantly elevated 24 h post-DR both before and after dietary supplementation ($P < 0.05$, Table 2). Following supplementation, resting plasma GSH was significantly higher in high- versus low-AVA group ($P < 0.05$). During pre-supplementation trials plasma GSSG concentration was increased above resting levels immediately after DR ($P < 0.05$). This change was again observed in the low-AVA group after supplementation, but not in the high-AVA group. The GSH:GSSG ratio was elevated above resting levels at 24 h post-DR ($P < 0.05$) in both AVA groups before supplementation, but after supplementation GSH:GSSG was increased only in high-AVA group ($P < 0.05$).

Table 2 Erythrocyte antioxidant enzyme activity and plasma glutathione status in young women

	SOD (U/mg Hb)		GPX (U/mg Hb)		GSH (μmol/L)		GSSG (μmol/L)		GSH: GSSG	
	Control	AVA	Control	AVA	Control	AVA	Control	AVA	Control	AVA
Pre-supplementation										
Rest	312 ± 26.6	311 ± 18.6	388 ± 31.7	382 ± 61.0	5.52 ± 0.09	5.53 ± 0.09	0.49 ± 0.04	0.48 ± 0.04	11.9 ± 0.07	11.3 ± 0.16
Post-DR	240 ± 38.7	288 ± 22.9	340 ± 26.4	366 ± 23.6	6.05 ± 0.08	5.85 ± 0.08	0.53 ± 0.02*	0.51 ± 0.03	11.5 ± 0.08	11.7 ± 0.17
24 h Post-DR	292 ± 20.7	308 ± 22.2	358 ± 65.0	290 ± 57.4	6.54 ± 0.09*	6.58 ± 0.09*	0.50 ± 0.04	0.48 ± 0.04	13.1 ± 0.08*	13.8 ± 0.18*
Post-supplementation										
Rest	291 ± 27.8	288 ± 29.6	370 ± 31.2	270 ± 104.2	5.38 ± 0.09	5.88 ± 0.08§	0.48 ± 0.04	0.48 ± 0.03	11.2 ± 0.06	12.2 ± 0.13
Post-DR	285 ± 30.6	319 ± 19.1	331 ± 31.7	190 ± 54.8§	5.60 ± 0.08	5.90 ± 0.08	0.52 ± 0.02*	0.48 ± 0.02	10.7 ± 0.07	12.3 ± 0.15
24 h post-DR	304 ± 16.9	235 ± 36.8	345 ± 73.7	283 ± 60.4	5.97 ± 0.06*	6.06 ± 0.06*	0.51 ± 0.03	0.46 ± 0.02	11.8 ± 0.09	13.2 ± 0.21**§

Data are mean ± SEM (N = 8)

SOD superoxide dismutase, Gpx glutathione peroxidase, GSH reduced glutathione, GSSG glutathione disulfide, DR downhill running (see text for details)

* P < 0.05, Post-DR or 24 h post-DR versus rest

§ P < 0.05, AVA versus control group

Discussion

Several previous studies have revealed AVA as an inhibitor for inflammatory cytokine and chemokine production and cell proliferation in vitro (Liu et al. 2004; Nie et al. 2006a, b; Guo et al. 2008; Sur et al. 2008). We hypothesized that human subjects engaged in eccentric exercise or exercise containing eccentric component could benefit from dietary oat supplementation for some protection. Our data partially supported our hypothesis, but were complicated by a well-documented repeated measure effect of eccentric exercise that also predicts an attenuation of stress markers when two exercise testing protocols are repeated between a few months. Thus, given the current experimental design, this observed protection may reflect an interaction between the antioxidant and anti-inflammatory effect of AVA and the repeated measure effect.

The repeated measure effect was evidenced by the finding that plasma CK activity, a classic muscle damage marker, was elevated by DR but blunted after subjects had consumed two oat cookies daily for 8 weeks regardless of AVA concentration. In addition, DR-inflicted plasma TNF-α level was also blunted by the oat supplementation regimen in both high-AVA and Control group, again suggesting a possible repeated measure effect (Fig. 3). However, since oats contain several antioxidants unrelated to AVA, such as phenolic acids, flavonoids and tocopherols, it cannot be ruled out that these antioxidants exerted an inhibitory effect on the production of this pro-inflammatory cytokine.

Avenanthramides supplementation at high concentration reduced ROS production during neutrophil respiratory burst, a hallmark response after neutrophil infiltration into damaged muscle fibers. While NRB was elevated by 80 % at 24 h post DR prior to supplementation, this effect was completely abolished after high-AVA supplementation, whereas Control group still showed elevated NRB (Fig. 2). Furthermore, NFκB binding was significantly decreased by oat supplementation, and high-AVA group showed significantly lower binding than the Control group (Fig. 6). These data were consistent with the findings of Sur et al. (2008) showing that an AVA concentration of 1 part per billion was sufficient to reduce the breakdown of IκB from NFκB complex in cultured keratinocytes and provided evidence that this mechanism might be effective in vivo.

One of the most significant consequences of NFκB signaling is the production of pro-inflammatory cytokines such as TNF-α, IL-1, -6, and -8 with their DNA containing consensus NFκB binding sites. Elevated plasma TNF-α concentration and enhanced TNF-α gene expression in skeletal muscle have been reported following DR in rats (Liao et al. 2010) and in human (Nieman et al. 2001; Pedersen et al. 2001; Hamada et al. 2005). Pro-inflammatory function of

TNF- α is at least in part attributed to its ability of elevating cellular reactive oxygen species (ROS) generation (Li et al. 1999). Specifically, TNF- α has been shown to stimulate mitochondrial electron transport chain (ETC) to produce superoxide radical (O_2^-) and H_2O_2 , and to increase member-borne NADPH oxidase activity (Li et al. 2005). ROS can activate NF κ B, mitogen-activated protein kinase (MAPK), and the heat shock response, which regulate the expression of other pro-inflammatory cytokines such as IL-1 and IL-6 forming a self-propelled vicious cycle. Elevated plasma TNF- α also underlie the etiology of pathogenesises such as chronic infection, congestive heart failure, and cancer cachexia (Costelli et al. 1993). Our observation that plasma TNF- α concentration was decreased after the 8-week dietary regimen appeared consistent with the *in vitro* data reported by Guo et al. (2008) that AVA could inhibit IL-1 β -induced TNF- α expression in endothelial cells. However, caution must be exercised to suggest that AVA supplementation inhibits TNF- α production *in vivo*, because repeated eccentric exercise is known to reduce certain blood stress markers. Another possible interpretation is that oat antioxidants unrelated to AVA might have exerted an inhibitory effect on blood TNF- α .

The source of elevated plasma TNF- α could not be confirmed at present but skeletal muscle has been considered a main production site when it performs demanding eccentric contraction (Liao et al. 2010; Nieman et al. 2001). A second source of TNF- α could be the plasma membrane of endothelial cells wherein NADPH oxidase complex is activated to produce ROS, and hence stimulates TNF- α expression via NF κ B signaling (Frey et al. 2002). It is conceivable that inhibiting NF κ B may represent a major cellular mechanism by which AVA reduces TNF- α production from both muscle cells and blood vessels in the damaging site.

After dietary oat supplementation, high-AVA group showed significantly lower IL-6 levels immediately and 24 h post-DR. IL-6 is considered a pleiotropic cytokine linked to not only pro- but also anti-inflammatory responses, such as suppressing TNF- α and IL-1 β secretion in cultured monocytes (Tilg et al. 1994). Our finding that AVA reduces plasma IL-6 suggests that plasma inflammatory stimulus to IL-6 production was attenuated by AVA. However, plasma IL-1 β concentration was unaltered by DR, which was consistent with several other reported studies (Bruunsgaard et al. 1997; Hirose et al. 2004). Taken together, our data provided new evidence that long-term dietary intake of AVA at the given dose can attenuate selective pro-inflammatory cytokine production caused by eccentric muscular contraction. However, whether or not people chronically consuming large dose of AVA in diet would suffer from potential negative effects, as has been reported with high dose of antioxidant supplementation, remains unknown.

Previous research has provided strong evidence that high dose of antioxidant supplementation may interfere with cellular redox signaling thereby compromising training adaptation of mitochondrial and antioxidant enzymes (Ristow et al. 2009; Gomez-Cabrera et al. 2008). In the current study, although plasma TAC was significantly elevated by high-AVA consumption, there was no sign of adverse effect resulting from dietary AVA supplementation. Erythrocyte SOD activity was not affected whereas GPx activity was decreased immediately post-DR. The main function of GPx in the red cell is to defend against H_2O_2 formed from SOD-catalyzed O_2^- dismutation, and its activity is one of the highest among all body tissues. A reduction of GPx activity might indicate a lower H_2O_2 production or lipid peroxide formation from lipid membrane of the red cell.

Eccentric exercise has been shown to induce disturbances to muscle glutathione homeostasis due to ROS generation and oxidation of GSH to GSSG, and increased export of GSSG into the blood circulation (Zembron-Lacny et al. 2010; Goldfarb et al. 2011). In the present study, resting plasma GSH content was elevated whereas GSSG response to DR was blunted in high-AVA group, which also showed a higher GSH:GSSG ratio 24 h post-DR. These protective effects caused by AVA consumption have never been reported and the reason is unknown. Since glutamyl-cysteine ligase (GCL), the rate-limiting step of GSH synthesis, is strongly inhibited by TNF- α (Rahman et al. 1999), it is possible that reduced plasma TNF- α level seen after AVA supplementation could lessen GCL inhibition and hence allowed for a greater hepatic GSH output during DR.

Previous animal and clinical studies indicated that AVA is bioavailable to body tissues after being orally ingested. For example, when synthetic AVA (6 mg/100 g body weight) was gavage-fed to rats, a maximum combined plasma AVA (a, b and c) concentration (C_{max}) of 5.5 nmol/L was reached at 2 h post ingestion and an accumulation of AVA in liver, heart and skeletal muscle was observed at 2–12 h (Koenig et al. 2011). In human, after 60 and 120 mg of AVA enriched mixture was consumed, C_{max} of total plasma AVA was reported to be 168 and 560 nmol/L, respectively, at 2 h and elevated AVA in plasma lasted for 10 h (Chen et al. 2007). The AVA dose in the current study at 9.2 mg/day was considerably lower compared with previous human or animal studies based on unit of body weight. However, high-AVA supplementation still increased plasma TAC and reduced selective inflammation and oxidative stress markers by DR, rendering promises for the efficacy of AVA supplementation.

In summary, our data indicate that a diet containing high daily dose of AVA may provide some protection to woman subjects who participate in eccentric muscle contraction that elicits an inflammatory response. Based on the current

experimental design, some of the protective effects, such as reduced plasma CK activity and dose-unrelated TNF- α reduction, could result from the repeated measure effect, an adaptive muscle response previously documented. However, other effects such as DR-induced reduction of neutrophil respiratory burst, NF κ B activation, plasma IL-6 concentration, and erythrocyte GPx activity, as well as a more reduced plasma glutathione status, were only observed in young women supplemented with high-AVA. These specific antioxidant and anti-inflammatory properties of AVA demonstrated in this study may have potential merit to consider AVA as a dietary supplement.

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