

The Effect of Detraining and Intensive Training on Asymmetric Dimethylarginine and Homocysteine: A Study of Cardiovascular Disease Risk Factors in Elite Young Athletes

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ABSTRACT The aim of the present study was to investigate the effects of a 12 week detraining after a 16 week multicomponent training program including strength, anaerobic and aerobic exercises on the main determinants of Asymmetric Dimethylarginine (ADMA), homocysteine (Hcy) and some physiological markers. For this purpose, twenty-three well-trained young wrestlers (14.9±1.1 yr, 46.6 ± 9.9- 49.4 ± 9.6kg, 18.7± 2.3- 19.1 ± 2.0kg/m²) volunteers were assigned into training (n = 24) and control (n =13) groups. ADMA and Hcy were significantly higher during training season in wrestlers compared to control (P < 0.05). However, ADMA and Hcy were not significantly in control group compared to detraining season. There was no significant difference in training season between detraining season in wrestlers (P>0.05).

INTRODUCTION

Despite the extensive literature and knowledge regarding the health benefits associated with exercise among children (Lloyd 2014), only a few studies have, to the best of our knowledge, examined the effects of detraining on Asymmetric Dimethylarginine (ADMA) and Homocysteine (Hcy) levels in elite athletes. Higher blood concentrations of ADMA and Hcy are known to be risk factors for cardiovascular diseases (CVDs) (Murakami et al. 2011). Regular physical activity represents an important aspect of ensuring good health and preventing diseases. Physical activity is also recognized as an effective means for reducing CVD risk, since it decreases the levels of chronic inflammation – which assumes a central role in atherogenic processes, insulin sensitivity, behavior and body composition (Böger et al. 2009; Leong et al. 2008; Kielstein et al. 2003). A three month detraining period is known to be sufficient for causing a return to initial state prior to training (Toulotte et al. 2006). However, there are currently no comprehensive studies regarding the effect on detraining on ADMA and Hcy levels among elite young wrestlers.

Increases in Hcy levels are associated with the development of thrombosis and atherosclerosis through a number of mechanisms (Vincent et al. 2006; Kothekar 2007). It is known that lifestyle changes, such as increases in the level of

physical activity, can improve micro vascular reactivity as well as endothelial function (Hamdy et al. 2003; Krcma et al. 2009). High volume and intensity resistance exercise can lead to damage and injury in muscles, especially when performed by unaccustomed persons (Clarkson and Hubal 2002; Proske and Morgan 2001). Ending a regular exercise routine triggers changes in amino acid and protein metabolism; hence, it is both necessary and important to understand how and to what extent ADMA and homocysteine levels can be affected by the detraining process. Detraining-associated cardiovascular changes need to be examined in parallel with changes in physical fitness, since the reversibility of detraining can vary among individuals; in addition, it is important to bear in mind that health factors are not only associated with physical fitness markers.

In this context, the aim of the current study was to investigate the effect of detraining on ADMA and homocysteine levels in elite young wrestlers.

MATERIAL AND METHODS

Subjects

All the subjects who participated in the present study were healthy young male wrestlers (n = 24) from the Wrestling Training Center (WTC) in Çorum, Turkey. However, only fourteen subjects completed the study. The subjects

did not have any ongoing diseases. The control group consisted of sedentary students (n=12). The study protocol was approved by the Ethics Committee of Kirikkale University, Faculty of Medicine Sciences in Turkey. The study procedures were conducted in accordance with the Helsinki Declaration. After the subjects were informed concerning the study's benefits and possible risks, they and their parents signed their written consent forms prior to participation in this study.

Measurement of Anthropometrics and Average Anaerobic Power

Body composition was assessed based on the participating subject's weight, and height. The body weight of the study participants were measured by utilizing digital scales (Seca 707; range 0.1-150 kg, Hanover, MD), and the measurements were recorded up to precision of 1 kg. Height was measured while the participants were barefoot and wearing light clothes, and the measurements were recorded to a precision of 0.1 cm. The body mass index (BMI) was calculated as a function of weight (kg) divided by the square of height (m²). The Lewis formula or nomogram (Fox and Mathews 1974) is a commonly used formula that only estimates average power, and is based on the modified falling body equation. The original formula uses kg.m.sec.⁻¹ as units. To convert these units to Watts, the factor of 9.81, as well as the standard unit for Power, needs to be added.

Testing Procedures

Blood samples were collected from the subjects during their competition (after the 9 month competition period) and detraining periods (which is 3 months after the competition period). Blood samples for the control group were collected at the midterm period, according to the time of taken blood samples of experimental group. Performance tests were performed at the same time both the competition (after the 9 months) and detraining periods (after the 3 months from the competition period). No performance tests were performed for the control group.

Blood Samples

All blood samples were obtained at the same time. The blood samples were prepared and

stored as necessary at -80°C until evaluation and analysis. For each study participant, three blood samples were collected during the Testing session of the Control Group, the Testing Session of the Training Season, and 12 weeks after the Testing Session of the Detraining Season.

Homocysteine Assessments

Assessment of homocysteine levels in the sample was performed by first reducing the compound. The albumin-bound and oxidized homocysteine was then combined with a fluorescent probe. High molecular substances were removed through precipitation. The sample was cooled to 2-8°C, centrifuged, and then injected into an HPLC. The isocratic separation via HPLC at 30°C using a reversed phase column. One run lasts 5 minutes. The chromatograms were recorded using a fluorescence detector. The quantification was performed by the delivered plasma calibrator. Concentration was calculated using the "internal standard method" by integration of the peak areas and peak heights.

(Treatment of the HPLC column: Following analysis, the column should be washed with 15 ml aqua bidest (1 ml/min) and stored in 50% methanol in aqua bidest (ca. 15 ml, flow 0.7 ml/min). Prior to use, the system should also be equilibrated with ca. 30 ml Elu.)

The following formulas were used for calculations:

$$\text{Conc. Sample } (\mu\text{mol/l}) = \frac{\text{Peak area patient} \cdot \text{conc. calibrator } (\mu\text{mol/l})}{\text{Peak area IS patient}} \cdot F$$

$$F = \frac{\text{Peak area IS of the calibrator}}{\text{Peak area homocysteine of the calibrator}}$$

ADMA as an Endothelial Damage Marker

ADMA measurements were performed using HPLC in accordance with the method described by Chen et al. (1997), and developed by Cakir et al. (2004) and Avci et al. (2008). In this method, 20 mg 5-sulfosalicylic acid was added to 1 ml serum. The mixture was then left in an ice bath for 10 minutes. The mixture was then centrifuged at 2000 g for 10 min to remove the precipitating protein. Ten μ l of the supernatant was filtered through a filter with 0.2 μ m-sized pores, and then mixed with 100 μ l of a derivatization reagent, prepared by dissolving 10 mg *p*-phthalaldehyde in 0.5 ml methanol, 2 ml 0.4 M borate buffer (pH10.0), and 30 μ l 2-mercaptoethanol. The

mixture was then injected into the HPL Csystem. ADMA separation was performed using a 150 × 4 mm Nova-Pak C₁₈ column, with 5 μm particle size (Waters, Millipore Corp., Milford, Massachusetts, USA). The mobile phase of this column consisted of 50 mM sodium acetate (pH 6.8), methanol and tetrahydrofurane (A; 82/17/1, B; 22/77/1 [v/v/v], respectively), and the flow rate of this mobile phase was maintained at 1.0 ml/min. Quantification was performed using the peak areas detected by the fluorescent detector (excitations, 338 nm; emission, 425 nm). Coefficients of variation for the intra- and inter-assay ADMA assessments were determined as 2.8 percent and 4.5 percent, respectively.

Training Protocol

The training season lasted for nine months, and was divided into four different periods. These periods included: (1) *The first period*, which involved basic preparation for a period of two months with aerobic, strength, technique and tactic exercises, and with the average HR being maintained at 70 percent-85 percent of its maximum level in all activities; (2) *The second period*, which involved general preparation for a period of four months with aerobic, strength, technique, tactic, coordination and balance exercises, and with the average HR being maintained at 80 percent-90 percent of its maximum level in all activities; (3) *The third period*, which involved specific preparations for one month

with aerobic-anaerobic power, strength, technique, tactic, coordination, balance, reaction, and wrestling workout exercises, and with the average HR being maintained at 90 percent-100 percent of its maximum level in all activities; and (4) *The last period*, which involved competitions for two months, as well as technical and tactical training, preparation for matches and official matches, and the average HR being maintained at 90 percent-100 percent of its maximum level in all activities. Trainings were performed 6 days a week, and lasted for 1.5 hours.

Statistical Analysis

Data analysis was performed using analysis of variance (ANOVA) and post hoc least significant difference (LSD) tests with the SPSS version 22.0 statistical package program (SPSS Inc., USA). A *p* value < 0.05 was considered as statistically significant. The standard deviation of the mean was shown together with the relevant mean. To determine whether the data followed a normal frequency distribution, evaluation of the coefficients of skewness and kurtosis (Paz-Gonzalez et al. 2000) were deemed sufficient.

RESULTS

The characteristics of the study participants are provided in Table 1. Some of the physiological and strength values in control group and wrestlers are presented in Table 2. Some routine

Table 1: Pre- and post-detraining characteristics of the study participants

	Controls ^a (n:24)	Training Season ^a (9 th Month)(n:24)	Detraining Season ^b (12 th Month)(n:24)	Sig.
Age (year)	14.54 ± 0.52	14.92 ± 1.14	14.92 ± 1.14	NS
Height (cm)	1.57 ± 0.05	1.57 ± 0.08	1.59 ± 0.09	NS
Weight (kg)	51.09 ± 8.40	46.64 ± 9.93	49.35 ± 9.60	NS
BMI (kg/m ²)	20.64 ± 3.03	18.69 ± 2.34	19.11 ± 2.02	NS

Compared to Training Season^a and Detraining^b *p*<0.05. NS= Not significant

Table 2: Aerobic endurance, anaerobic power and medicine ball throwing (MBT) strength values in pre and post detraining

Parameters	Training Season ^a (9 th Month)(n:23)	Detraining Season ^b 12 th Month)(n:23)	Sig.
Aerobic endurance (ml/kg/min)	53.50 ± 2.7	48.20 ± 2.8	0.00
Anaerobic power(Watts)	424.38 ± 122.98	422.46 ± 112.29	NS
MBT Strength (meter)	7.30 ± 1.54	6.04 ± 1.27	0.00

Compared to Training Season^a and Detraining^b, significant *p*<0.05^{*}. NS= Not significant

biochemical characteristics of training and detraining athletes before and after a 12-week lifestyle modification are shown Table 3. And the marker of cardiovascular disease evaluated in athletes' training, detraining and controls groups before and after a 12-week lifestyle modification are presented in Table 4.

Demographic features of the subjects groups were similar regarding age, height and BMI. There was no significant difference in age, height and BMI between groups ($p>0.05$) (Table 1).

The values of aerobic endurance (VO_{2max}) of training season (53.50 ± 2.7 ml.kg.min⁻¹) were higher than from detraining period (48.20 ± 2.8 ml.kg.min⁻¹) ($p<0.05$). MBT strength performance values were significantly higher (7.30 ± 1.54 meter) than group of detraining Period (6.04 ± 1.27 meter) ($p<0.05$). There was no significant difference in anaerobic power between training season (424.38 ± 122.98 watts) and wrestlers detraining period (422.46 ± 112.29 watts) ($p>0.05$) (Table 2).

There was no significant difference in triglycerides (mg/dl) and HDL – cholesterol (mg/dl) of control (78.36 ± 10.38 ; 58.27 ± 7.8), training season (80.20 ± 9.25 ; 62.16 ± 7.84) and detraining period groups (83.42 ± 10.29 ; 58.78 ± 6.78) respectively ($p>0.05$).

LDL-cholesterol (mg/dl) and total cholesterol (mg/dl) levels were significantly increased in the training season (101.25 ± 12.50 ; 158.04 ± 20.97),

and detraining period (103.14 ± 14.67 ; 164.71 ± 24.33) groups compared the control group (81.45 ± 10.74 ; 129.63 ± 19.08) (respectively, $p<0.05$) (Table 3.).

Comparisons between the training and detraining periods, as well as comparisons between the study and control groups, showed that there were statistically significant differences in ADMA and homocysteine levels. An increase and variability was observed in the levels for the wrestlers. Significantly higher ADMA and Hcy levels were observed during the training season among the wrestlers in comparison to the control ($P<0.05$). On the other hand, ADMA and Hcy levels were not significantly different between the control group and the study group's detraining season. Significant differences were not observed between the training season and detraining period of the wrestlers ($P>0.05$) (Table 4).

DISCUSSION

The aim of the current study was to examine among elite young wrestlers the effect of a 12-week detraining period following a nine month competition season on certain indicators of muscle strength, anaerobic power, aerobic performance, and certain metabolic indexes (such as lipid profile, inflammatory cytokines, and endothelial function).

Training interruptions for reasons such as injury, illness, holidays, the postseason break

Table 3: Some routine biochemical characteristics of training and detraining athlete before and after a 12-week lifestyle modification

Parameters	Controls ^a (n:24)	Training Season ^a (9 th Month)(n:24)	Detraining Season ^b (12 th Month)(n:24)	Sig.
Triglycerides (mg/dl)	78.36± 10.38	80.20± 9.25	83.42± 10.29	NS
LDL-cholesterol (mg/dl)	81.45± 10.74*	101.25± 12.50*	103.14± 14.67*	(a-b) (a-c)
HDL-cholesterol (mg/dl)	58.27± 7.8	62.16± 7.84	58.78± 6.78	NS
Total cholesterol (mg/dl)	129.63± 19.08	158.04± 20.97	164.71± 24.33	(a-b) (a-c)

Compared to control^a, Training Season^b and Detraining^c significant $p<0.05$. NS= Not significant

Table 4: Marker of cardiovascular disease evaluated in athletes training, detraining and controls groups before and after a 12-week lifestyle modification

Marker of cardiovascular disease (Markers of damage)	Wrestling exercise				Sig.
	Controls ^a (n:23)	Training Season ^a (6 Month)(n:23)	Detraining Season ^b (9. Month)(n:23)		
ADMA (̑mol/l)	0.68± 0.16*	0.91± 0.35*	0.88± 0.20		a-b
Homocysteine (̑mol/l)	12.74± 4.44*	15.97± 3.78*	15.66± 4.09		a-b

ADMA (̑mol/l); Homocysteine (̑mol/l); * $P<0.05$, compared to control^a, Training Season^b and Detraining^c

or other causes occur in all sports. Most studies until now have specifically investigated the effect of training methods and periods, while paying relatively limited attention to detraining-related effects. Intense training routines can be associated with short-term immunosuppression and higher infection risk among athletes. In this study, the researchers attempt to compare the effects of detraining on ADMA and Hcy levels on young wrestlers. Previous studies (Herrman et al. 2003; Borrione et al. 2008) had demonstrated that plasma ADMA levels are positively correlated with serum Hcy levels. The findings of this study were in agreement with this observation. It was previously reported that the effects of detraining in young adults reached significant levels within 2 weeks following the end of a cardio-respiratory fitness training program (Green and Crouse 1993). Plasma ADMA levels decrease and increase in parallel with the Hcy levels (Siervo and Bluck 2012). The researchers' results were also in agreement with these findings.

In this study, the training period serum ADMA and Hcy levels among young wrestlers were determined as 0.91 ± 0.35 and 15.97 ± 3.78 , respectively. The researchers identified higher serum ADMA and Hcy levels in wrestler who experienced intense training seasons. The significance of elevated homocysteine (Hcy) as an indicator for cardiovascular disease (CVD) risk continues to be a subject of debate (Wald et al. 2002; Fruchart et al. 2004; Kaul et al. 2006). However, physical exercise has a beneficial effect by reducing plasmatic ADMA and SDMA levels and increasing L-ARG substrate for endothelial NO (Riccioni et al. 2015). Selecting suitable intensity, duration and frequency levels, as well as the proper type of exercise, is of importance, given that intense exercise can potentially cause damage to blood vessels. Detraining time and exercise intensity need to be considered as well. Nevertheless, the relationship between the intensity of exercise and endothelial functions in humans has still not been fully clarified (Abraham et al. 1997). A number of data from certain studies have demonstrated that the cardiorespiratory parameters of sedentary ex-athletes (lengthy detraining period) and controls are not significantly different (Unt et al. 2008).

ADMA, which is synthesized through the methylation of arginine, functions as an endogenous nitric oxide synthase inhibitor. Homocys-

teine and ADMA are believed to induce negative vascular effects by impairing endothelial, nitric oxide-dependent functions. ADMA is nowadays defined as a cardiovascular risk factor (Lentz et al. 2003; Vallance et al. 2004). ADMA is a predictor of coronary heart disease, with an increase in plasma free ADMA concentrations of 0.1 $\mu\text{mol/L}$ being associated with an odds ratio of 2.61 for (Schulze et al. 2006). Patients with diabetes mellitus (Lin 2002), hypercholesterolemia (Engler 2004), chronic renal failure (Kari et al. 1997), hypertension (Goonasekera 2000), and pulmonary hypertension (Gorenflo et al. 2001) also tend to exhibit high ADMA levels. Low Hcy levels among individuals who are physically active may be accounted for by better vitamin consumption. Folic acid and vitamins B6 and B12 are important cofactors for the enzyme metabolism of Hcy (König et al. 2003). A recent publication suggested that Hcy levels higher than 10 mmol/L may necessitate treatment among patients higher risk patients (Stanger 2003). Furthermore, a previous study reports that Hcy levels of 12.1 mmol/L are associated with significant risk for cardiovascular diseases (Leong et al. 2005). Based on both of these studies, the Hcy levels in this current study appears to be somewhat high for the control (12.74 ± 4.44), and the training (15.97 ± 3.78) and detraining seasons (15.66 ± 4.09). Former athletes who had ended their lifestyle of intense physical activity exhibited mean Hcy levels above 12.0 mmol/L (Unt et al. 2008). It is well-known that physical exercise and activity are essential for good health as well as the prevention of diseases. Physical exercise plays a particularly important role in decreasing the occurrence of CVDs – mainly through the reduction of chronic inflammation, which plays a crucial role in the atherogenic processes, and in disorders associated with blood pressure, body composition, and insulin sensitivity (Venta et al. 2009; Borrione et al. 2008). According to Venta et al. (2009) the increase in Hcy in parallel to the increasing level of exercise can be explained by three tentative mechanisms: (1) the increase in the formation of free radicals due to increased exercise; (2) the increase in the formation of the methylated forms of creatinine and acetylcholine due to increased exercise; and (3) the increase in protein breakdown, and hence free amino acid formation, due to increased exercise (Borrione et al. 2008). A limited number of cross-sectional studies have attempted to in-

investigate possible the negative relations between Hcy levels and physical exercise (Nygard et al. 1995; Hellgren et al. 2005; Kuo et al. 2005). It is known that physical exercise can, when performed regularly, decrease the risk for cardiovascular risk diseases. However, it is also observed that Hcy levels show considerable variation depending on the frequency and type of the exercises that are performed (Herrman et al. 2003; Randeve et al. 2002).

A number of researchers (Halbert et al. 1999; Kelley et al. 2004) have described that regular exercise display greater effectiveness in reducing lipid levels among hyperlipidemic individuals, while achieving significant changes through regular exercise is harder for people who already have normal lipid profiles. Furthermore, detraining can engender various metabolic changes. For example, a 7-day detraining period for endurance-trained men was shown to be associated with a 35 percent increase in fasting plasma triglyceride levels (with the majority of this increase stemming from a 50 percent rise in VLDL levels) and a 41 percent decrease in insulin sensitivity (Gill et al. 2003). Thompson et al. (1985), on the other hand, reported no significant changes for young individuals in the 6 week of their training in terms of their triglyceride and cholesterol concentrations and body fat mass levels. Total plasma Hcy levels were observed to exhibit an inverse relation with aerobic physical capacity, while self-reported physical activity data in METs suggested a negative correlation between these two parameters (Unt et al. 2008). Physical exercise and activity is essential for good health and the prevention of potential diseases and disorder. Long-term physical exercise and activity is known to have a positive impact on risk factors for CVD, such as glucose tolerance, blood lipid levels and obesity (Booth et al. 2000). Athletes who have no longer practice sports, or are in an extended period of detraining, constitute a subgroup of individuals who, owing to the sports activities they have performed at a very high level for many years, tend to have better physical health than most of the general population (Pihl et al. 1998). The lipid parameters the researchers evaluated (HDL-Triglycerides [mg/dl] and Total Cholesterol) indicated no statistically significant changes at any period of the study group. In this study, it was observed that cholesterol (mg/dl) and LDL cholesterol were significantly higher ($P < 0.05$) between groups.

CONCLUSION

ADMA and Hcy level were significantly higher (74.7 %, 79.7% respectively) during the training period of the wrestlers in comparison to the levels observed among the controls ($P < 0.05$; Table 3). On the other hand, ADMA and Hcy levels were not significantly different in the control group in comparison to the levels observed during the detraining period. There were no significant differences between the training and detraining seasons of the young wrestlers ($P > 0.05$). Heavy exercise as well as increased physical activity can be associated with increased concentrations of plasma ADMA and Hcy. This increase may, in turn, lead to a higher risk of CVD due to the excessive training load accumulated during trainings performed within the frame of the competition season. Due to the limited number of studies concerning the effect of detraining on the ADMA and Hcy levels, further studies are necessary to elucidate and understand young athletes' ADMA and Hcy responses to detraining. Furthermore, the relationship between ADMA and Hcy levels have not been investigated in individual sports such as wrestling, which is a sport in which periods of intermittent activity and intense anaerobic activity alternate. There is also a lack of information concerning the optimal Hcy levels and the relationship between ADMA and Hcy level with various risk factors for cardiovascular diseases (including the lifestyle of wrestler children). In conclusion, further studies are require to examine the interrelations between higher physical activity, ADMA and Hcy levels, and cardiovascular risk factors.

RECOMMENDATIONS

Coaches should pay much more attention to the loading intensity in young athletes. Different loading intensity should be examined in terms of cardiovascular disease risk. Future studies should also be done on different markers. Besides the beneficial aspects of the sport, the loss of overtraining should be investigated.

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