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Does High Volume of Exercise Training Increase Aseptic Vascular Inflammation in Male Athletes?

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Abstract

Aseptic vascular inflammation can be caused by high levels of various inflammatory and apoptotic factors such as tumor necrosis factor (TNFα), nitric oxide (NO), 3-nitrotyrosine (3-Nitro), and free and oxidized low-density lipoproteins (oxLDL) generated during intense exercise. Endothelial dysfunction resulting from enhanced inflammation has been implicated in cardiovascular disease (CVD). The purpose of the study was to observe the effects of high volume of exercise training on inflammatory mediators and their interaction with conventional CVD risk factors.

Blood samples were collected from highly-trained men ($n = 16$, 21.8 ± 4.0 years) as well as from nonactive men ($n = 20$, 21.1 ± 1.1 years). NO concentration did not differ between groups while TNFα, 3-Nitro, oxLDL, and CRP levels were significantly higher in athletes compared to nonathletes. TNFα reached even 7-fold higher level in athletes and was highly correlated with CVD risk factor such as TG, lipoproteins LDL and HDL as well as CRP. Approximately 50% of physically active men demonstrated a 20% increase in non-HDL caused by high levels of TC and LDL.

These findings suggest that athletes with a high exercise volume demonstrate increased levels of circulating biomarkers of vascular inflammation and may be more likely to have CVD.

Keywords

nitric oxide, nitrotyrosine, inflammation, non-HDL, sport training

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It is widely accepted that regular physical activity is beneficial for cardiovascular health. Frequent exercise significantly attenuates the atherosclerotic process by reducing inflammatory risk factors, retarding arterial wall ageing, delaying development of endothelial dysfunction, and preserving vascular function. Furthermore, physical activity reduces vascular oxidative stress, increases nitric oxide (NO) production via endothelial nitric oxide synthase (eNOS), modifies the lipid profile, inhibits the production of pro-inflammatory and pro-apoptotic cytokine TNFα (Durstine et al., 2001; Ribeiro, Alves, Duarte, & Oliveira, 2010; Urschel & Cicha, 2015) and decreases both oxidative stress and the circulating concentrations of endogenous inhibitors of NOS (Gomes, Casella-Filho, Chagas, & Tanus-Santos, 2008). The relationship between physical activity and cardiovascular disease (CVD) has been studied since 1960, when a postmortem study found a similar degree of coronary atherosclerosis in sedentary and active men (Spain & Bradess, 1960). In the last few years, there was debate about the dose–response relationship of exercise and CVD outcomes and whether high volume of exercise may accelerate endothelial dysfunction and atherosclerosis (Aengevaeren et al., 2017; Nystoriak & Bhatnagar, 2018). Prior observations shown significantly higher coronary artery calcification in marathon runners compared with the control subjects who

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were matched for both age and CVD risk factors (Möhlenkamp et al., 2008). This contrasts with other observational studies that found either no association or an inverse relationship between physical activity or fitness and atherosclerosis (Delaney et al., 2013; Sung et al., 2012). *It is still unknown how inflammation affects endothelial cells activity in highly-trained subjects.* In athletes, high concentration of TNFα can induce endothelial apoptosis through disturbance to the equilibrium between eNOS and iNOS activities, which results in pro-apoptotic NO activity. Finally, disturbances to vascular endothelial activity precede the development of arteriosclerosis (Föstermann, 2010; Zhang et al., 2009). The excess of NO reacts with the superoxide anion to produce peroxynitrite (ONOO) by 1000000-fold. ONOO, in turn, can "uncouple" eNOS to become a dysfunctional superoxide-generating enzyme that contributes to vascular oxidative stress. Oxidative stress and endothelial dysfunction can promote atherogenesis. Without superoxide, the formation of ONOO by NO reaction with oxygen is minimal. NO and superoxide do not even have to be produced within the same cell to form peroxynitrite because NO can readily move through membranes and between cells (Föstermann, 2010). Athletes have demonstrated significantly higher level of reactive oxygen and nitrogen species as well as TNFα and other pro-inflammatory cytokines (Borges et al., 2013; Main, Dawson, Grove, Landers, & Goodman, 2009, Marin et al. 2011; Ostrowski, Rohde, Asp, Schjerling, & Pedersen, 1999; Reinke et al., 2009; Zembron-Lacny, Slowinska-Lisowska, & Ziemba, 2010), which can serve as predictors of overtraining-induced inflammation (Main et al., 2009; Smith, 2004). Survey research involving endurance athletes who completed a training monocycle reported a rate of overtraining syndrome of approximately 10% (range 7%–21%; Raglin & Wilson, 2000).

The earlier studies did not examine participants exposed to high volumes of exercise training for a prolonged period of time. Therefore, the question remains whether extreme exercise exposure accelerates the development of aseptic vascular inflammation. There were only a few observations concerning changes in markers of endothelial activity in elite male athletes following intense training periods or after completing their sports career. It is known that detraining period induces a rapid increase in total cholesterol (TC) and lipoprotein LDL levels, which elevates the risk of CVD (Petibois, Cassaigne, Gin, & Deleris, 2004). Maron et al. (2000) reported that 18.5% incidence of sudden death in young athletes (<35 years old) was related to vascular endothelial dysfunction and atherosclerotic coronary artery disease. According to Suarez-Mier, Aguilera, Mosquera, and Sánchez-de-León (2013), the most frequent sports associated with sudden death included cycling (29%), soccer (25.5%), running (8.9%), and gymnastics (6.5%). Approximately 70% of the cases were not related to some personal pathological antecedents or familial sudden deaths. De Van and Seals (2012) observed that masters endurance athletes demonstrated a more favorable arterial phenotype and a lower risk of CVD compared with untrained middle-aged and older adults. In contrast, masters athletes for whom training and competitive sport required primarily or exclusively intensive muscle resistance activities exhibited a less favorable arterial function-structure profile. The differences in arterial properties between masters athletes participating in endurance sports versus sports requiring resistance training can potentially be explained by differences in the intravascular mechanical forces generated during these activities. Agrotou et al. (2013) demonstrated that the type of anaerobic exercise, for example, weightlifting, was an important determinant of subclinical atherosclerosis such as intima-media thickness and flow-mediated dilation.

On the basis of the gathered data on endothelial dysfunction in athletes, the study was designed to evaluate the blood levels of pro-inflammatory and pro-apoptotic factors and their interaction with conventional CVD risk factors in men exposed to the high lifelong exercise volume.

Material and Methods

Subjects

Sixteen elite wrestlers, members of the national team, were observed during preparatory period for the new competition season (Table 1). They participated in a 14-day training camp at the National Olympic Sport Centre. Throughout the camp all athletes lived at the same accommodation and followed the same training schedule and diet. Daily energetic value of food offered on the menu did not exceed 5,200 kcal and the protein dose varied from 1.6 to 1.8 g/kg of body mass. During the camp, the wrestlers consumed an isotonic sports drink Vitargo (osmolality 317 mOsm/kg H_2O) or plain water. The dehydration level was assessed by Osmocheck calibrated in mOsm/kg H_2O from 0 to 1500 mOsmols. None of the athletes demonstrated dehydration, that is, urine osmolality was <600 mOsmols. The training loads were demonstrated using TRENING/TREOB4 program prepared by the Department of Sport Theory at the University School of Physical Education Warsaw (Table 2).

Twenty healthy, untrained males with no history of CVD constituted a reference group (Table 1). At the time of the investigation, the study subjects did not take any nutrition supplements or medications that could interfere with nitro-oxidative evaluation. All the subjects were informed of the aim of the study and signed a written

Table 1. Anthropometrics and Body Composition (Mean ± *SD*).

	Athletes $n = 16$	Nonathletes $n = 20$	*p* value
Age yr	21.8 ± 4.0	21.1 ± 1.1	.414
Height cm	173.8 ± 9.4	183.5 ± 7.8	<.05
Weight kg	75.9 ± 14.9	77.1 ± 7.2	.914
BMI kg/m^2	24.9 ± 2.3	23.0 ± 1.4	.056
%FM	12.9 ± 3.5	19.8 ± 2.7	<.001
FM kg	10.0 ± 4.3	14.8 ± 2.4	<.001
FFM kg	66.0 ± 11.9	59.7 ± 4.2	.084

Note. BMI = body mass index; FM = fat mass; FFM = fat-free mass.

Table 2. Sport Training Protocol During Preparatory Period Before New Season.

Type of training	Training load %
Endurance training: team games, marches and cross-country running, cross-country skiing, acrobatic exercises, climbing at ropes, pull ups, exercises with partner	53
Directed training: intervals, toss from knees, back suplex, reverse waist, turns	9
Special/wrestling training: elevation from the low position, keys, trolleys, throws with different amplitude of movement, gym	38

consent to participate in the project. The protocol of the study was approved by the ethics committee at Medical University Poznan (N^o 550/11), in accordance with the Helsinki Declaration.

Body Composition

Body mass (BM) and body composition (fat-free mass FFM and fat mass FM) were estimated using Tanita Body Composition Analyser MC-980 (Japan) calibrated prior to each test session in accordance with the manufacturer's guidelines. Duplicate measures were taken with the participant in a standing position; the average value was used for the final analysis. The recurrence of measurement amounted to 98%. The measurements were taken between 7.00 and 8.00 a.m. before blood sampling.

Blood Sampling

Peripheral venous blood was taken using S-Monovette tubes (Sarstedt, Austria) between 7.00 and 8.00 a.m. after 15 min of rest (and an overnight sleep). Within 20 min, they were centrifuged at 3000 g and +4°C for 10 min. Aliquots of serum were stored at −80°C.

Inflammatory and Apoptotic Mediators

Serum nitric oxide (NO) and marker of NO bioavailability (3-nitrotyrosine, 3-Nitro) concentrations were determined by enzyme immunoassay methods using the Oxis Research kits (USA). NO and 3-Nitro detection limits were 0.5 μmol/L and 2 nmol/L, respectively. Tumor necrosis factor α (TNFα) level was determined by means of the R&D Systems kit (USA), and its detection limit was 0.038 pg/ml. Oxidized low-density lipoprotein (oxLDL) and C-reactive protein (CRP) concentrations were determined using commercial kits from EIAab Science kit (China) and DRG International (USA). The detection limits for oxLDL and CRP were 0.312 ng/ml and 0.001 mg/L, respectively. All samples were analyzed in duplicate or triplicate in a single assay to avoid inter-assay variability. The average intra-assay coefficient of variation for the kits was <10%.

Lipoprotein-Lipid Profile

Total cholesterol (TC), high-density lipoproteins (HDL), and low-density lipoproteins (LDL) as well as triglycerides (TG) were determined by professional laboratory company Diagnostyka (Poland, ISO 15189). The non-HDL cholesterol was calculated by subtracting HDL from total cholesterol concentration.

Statistical Analysis

Statistical calculations were performed using the statistical software Statistica 13.2 (StatSoft Inc., Tulsa, OK, USA). All data were tested for distribution normality using the Shapiro–Wilk test. The values of W for biochemical markers were close to one; therefore, statistical

Table 3. Inflammatory and Apoptotic Mediators (Mean ± *SD*).

	Athletes $n = 16$	Nonathletes $n = 20$	p value
TNFα pg/ml	5.35 ± 1.34	0.75 ± 0.11	<.001
NO µmol/L	15.35 ± 3.47	13.15 ± 0.71	<.01
3-Nitro nmol/L	44.00 ± 3.24	34.94 ± 2.15	<.001
oxLDL ng/ml	3.26 ± 0.86	2.11 ± 0.96	<.01
CRP mg/L	1.07 ± 0.31	0.13 ± 0.11	<.001

Note. TNFα = tumor necrosis factor α, NO = nitric oxide, 3-Nitro = nitrotyrosine, ox-LDL = oxidized low-density lipoprotein, CRP = C-reactive protein.

Table 4. Relationships Between Tumor Necrosis Factor α (TNFα), 3-Nitrotyrosine (3-Nitro), Oxidized Low-Density Lipoprotein LDL (oxLDL), C-Reactive Protein (CRP), Triglycerides (TG), Low-Density Lipoprotein (LDL), High-Density Lipoprotein (HDL), Non-HDL and Atherogenic Coefficient (AC).

	3-Nitro nmol/L	oxLDL ng/ml	CRP mg/L	TG mg/dl	LDL mg/dl	HDL mg/dl	Non-HDL mg/dl
TNFα pg/ml	$r = 0.833$ $p < .001$	$r = 0.459$ $p < .01$	$r = 0.856$ $p < .001$	$r = 0.834$ $p < .001$	$r = 0.433$ $p < .05$	$r = -0.197$ $p > .05$	$r = 0.481$ $p < .01$

significances were assessed using one-way analysis of variances (ANOVA) and post-hoc test (HSD Tukey). Associations among measured parameters were analyzed using Pearson's linear regression (coefficient, r). Statistical significance was set at $p < .05$. Results are expressed as mean and standard deviation ($x \pm SD$).

Results

The study comprised 60 athletes and 20 nonathletes, and was concerned with the effects of sports activity on inflammatory and apoptotic factors and their interaction with conventional CVD risk factors.

Body Composition

All subjects demonstrated their body weight and body mass index at similar levels. Significant differences were found in the percentage of fat content (FM%) and fat mass (FM) which were lower by 30% in the athletes. The fat-free mass (FFM) was slightly elevated in the athletes (Table 1).

Inflammatory and Apoptotic Mediators

The concentrations of TNFα, NO, 3-Nitro, oxLDL, and CRP were significantly higher in athletes than nonathletes. TNFα reached even a 7-fold higher level in the athletes and was highly correlated with conventional CVD risk factor, with the exception of HDL (Table 4). Although NO generation was enhanced in the athletes, its bioavailability was reduced, which was demonstrated by high concentration of 3-Nitro. A relationship between 3-Nitro and lipoprotein profile (Figure 1) was observed, which indicates that 3-Nitro could be a new CVD risk factor as it was previously confirmed in the study by Bencsik et al. (2015) (Table 3).

Lipoprotein-Lipid Profile

TG and HDL concentrations were found to be at similar levels in all subjects; however, non-HDL was significantly higher in the athletes than nonathletes. The high levels of TC and LDL were observed in 56% (>200 mg/dl) of the athletes and 44% (>130 mg/dl) of the nonathletes, respectively. Finally, non-HDL exceeded the level of 145 mg/dl in 56% of the athletes (Table 5).

Discussion

Vascular inflammation is an early marker of endothelial dysfunction prior to the development of structural changes and clinical symptoms. It contributes to the progression of atherosclerosis and increases the risk of coronary events. Over the past year, studies have demonstrated the significance of inflammation in endothelial apoptosis. In athletes, endothelial apoptosis can be induced by the binding of TNFα to one or more of the extracellular receptors of the tumor necrosis factor receptor (TNFRs) superfamily located on the surface of macrophages, T lymphocytes, and endothelial cells. The binding of this ligand to its receptors ultimately leads to the activation of

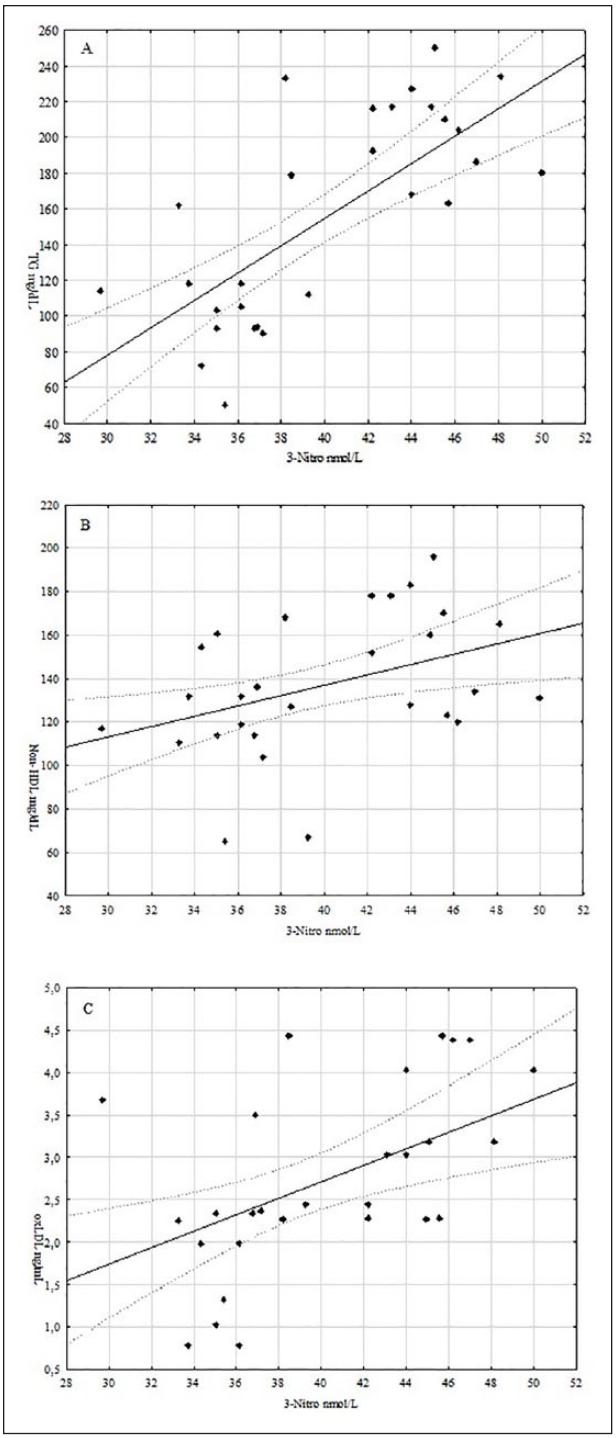

Figure 1. The significant positive relation between 3-Nitro and lipoprotein profile (A: Nitro-3/TG $r = 0.726$ $p < .001$; B: Nitro-3/non-HDL $r = 423$ $p < .01$; C: Nitro-3/oxLDL $r = 0.478$ $p < .01$).

a specific set of proteases crucial for the execution of apoptosis. TNFα plays a pivotal role in endothelial dysfunction. Direct evidence of TNFα-stimulated vascular dysfunction was provided by a study of intra-arterial TNFα administration in humans. In healthy volunteers, an acute local vascular inflammation was observed upon intra-arterial 30-min infusion of high-dose TNFα (80 or 240 ng/min). Administration of a lower TNFα dose (17 ng/min) for 60 min induced an increase in basal vascular resistance in healthy subjects which was blocked by pre-treatment with a NO synthase inhibitor. The authors concluded that the observed effects of TNFα were likely to be mediated by the reduced bioavailability of NO (Urschel & Cicha, 2015). TNFα decreases NO bioavailability by accumulation of the endogenous eNOS inhibitor ADMA and by enhanced removal of NO, for example via its reaction with superoxide, in which peroxynitrite is generated (Ito et al., 1999). In the present study, the athletes demonstrated elevated levels of TNFα and 3-Nitro which is a cytotoxic metabolite of NO. In all the subjects, serum 3-Nitro was positively correlated with TNFα and also with conventional CVD risk factors such as TG, non-HDL, and oxLDL. Earlier, there was observed the high TNFα level in wrestlers in every training period as well as the strong relation of TNFα with 3-Nitro (Zembron-Lacny, Ziemann, Zurek, & Hübner-Wozniak, 2017). It also confirms the study of Bjork, Jenkins, Witkowski and Hagberg (2012) that detection and quantification of 3-Nitro may be used as an indicator for the pathological processes in vascular endothelium.

The endothelial dysfunction is characterized by a chronic alteration of inflammatory function and markers of inflammation and the innate immune response, including CRP. The exact biological actions of CRP are yet to be established but its levels predict the risk of systemic inflammation in overtraining (Fatouros et al., 2006). The observed 8-fold increase in CRP concentration in athletes indicates the presence of low-grade inflammation but does not prove overtraining syndrome. Waskiewicz et al. (2012) and Rubio-Arias et al. (2019) demonstrated the high levels of markers associated with inflammation and cardiac damage after ultra-endurance race. Souglis et al. (2015) observed a high plasma CRP level for 5 days after a soccer match. Jackman et al. (2018) suggested that CRP facilitated the observation of real changes in physiological response following resistance exercise and detection of incomplete muscle recovery. However, the usefulness of CRP in sport diagnostics requires further studies in athletes exposed to high volume of exercise training.

The long-term high level of TNFα as well as adverse changes in lipid profile elevate the risk of coronary artery disease (Angeli, Minetto, Dovio, & Paccotti, 2004; Arakawa et al., 2016; Maron et al., 2000; Petibois et al., 2004; Urschel and Cicha, 2015). This risk was not observed in healthy subjects with normal lipid profile engaged in exercise of moderate intensity (Koutroumpi, Dimopoulos, Psarra, Kyprianou, & Nanas, 2012;

Table 5. Lipoprotein-Lipid Profile (mean ± *SD*).

	Athletes $n = 16$	Nonathletes $n = 20$	*p* value
TG mg/dl	101 ± 45	105 ± 25	.966
TC mg/dl	199 ± 34	179 ± 26	.078
HDL mg/dl	51 ± 13	56 ± 13	.947
LDL mg/dl	126 ± 32	102 ± 23	.056
non-HDL mg/dl	149 ± 33	123 ± 22	<.05

Note. TG = triglycerides, TC = total cholesterol, HDL = high-density lipoproteins, LDL = low-density lipoproteins.

Möhlenkamp et al., 2009; Volaklis, Tokmakidis, & Halle, 2013). An intensive exercise with strength elements is an important determinant of subclinical atherosclerosis (Agrotou et al., 2013). Identification of risk factors such as elevated LDL, low HDL, hypertension, diabetes mellitus, and a family history of premature CVD are very important in athletes, especially after their sports career. In asymptomatic athletes with moderate to high risk, screening with exercise testing is a challenging task.

The primary target of lipid-lowering therapy in adults is to reduce non-HDL level because it contains all the lipids and lipoproteins considered to be atherogenic. From a practical standpoint, the assessment of non-HDL-C in athletes should be preferred because it can be assessed in the nonfasting blood tests, no additional costs are incurred as it is calculated as the difference between TC and HDL-C, and it offers well-documented benefits (Kelley and Kelley, 2012). In this study, the TG and lipoproteins except for non-HDL were at similar levels both in active and nonactive men. The high level of non-HDL was caused by high levels of TC and LDL in approximately 50% of athletes. The available literature lacks the information describing non-HDL changes in athletes, which prevents decisive conclusions on how chronic exercise affects CVD processes.

Conclusions

Athletes with a high lifelong exercise volume demonstrate increased levels of circulating biomarkers of vascular inflammation and may be more likely to have CVD. Therefore, screening of athletes who may have substantial asymptomatic coronary artery disease is a challenging task. However, future studies unraveling the inflammatory mechanisms leading to higher risk CVD in very active athletes are warranted.

Declaration of Conflicting Interests

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