Vascular Pharmacology 79 (2016) 1–5

Contents lists available at ScienceDirect

Vascular Pharmacology

journal homepage: www.elsevier.com/locate/vph

Arterial stiffness and sedentary lifestyle: Role of oxidative stress

Gianfranco Lessiani [a,1,2], Francesca Santilli [a,1,2], Andrea Boccatonda [a,2], Pierpaolo Iodice [c,2], Rossella Liani [a,2], Romina Tripaldi [a,2], Raoul Saggini [b,2], Giovanni Davì [a,*,2]

[a] Internal Medicine and Center of Excellence on Aging, "G. d'Annunzio" University of Chieti, Italy
[b] Department of Neuroscience and Imaging, "G. d'Annunzio" University of Chieti, Italy
[c] Institute of Cognitive Sciences and Technologies, National Research Council, Rome, Italy

ARTICLE INFO

Article history:
Received 13 May 2015
Received in revised form 28 May 2015
Accepted 29 May 2015
Available online 2 June 2015

Chemical compounds:
8-iso-PGF2α
Cholesterol
Triglycerides

Keywords:
Exercise
Arterial stiffness
Oxidative stress
Isoprostanes

ABSTRACT

Sedentary lifestyle is a risk factor for the development of cardiovascular disease, and leads to a quantifiable impairment in vascular function and arterial wall stiffening. We tested the hypothesis of oxidative stress as a determinant of arterial stiffness (AS) in physically inactive subjects, and challenged the reversibility of these processes after the completion of an eight-week, high-intensity exercise training (ET).

AS was assessed before and after ET, measuring carotid to femoral pulse wave velocity (PWV) with a Vicorder device. At baseline and after ET, participants performed urine collection and underwent fasting blood sampling. Urinary 8-iso-$PGF_{2\alpha}$, an *in vivo* marker of lipid peroxidation, total, HDL and LDL cholesterol, and triglyceride concentrations were measured.

ET was associated with significantly reduced urinary 8-iso-$PGF_{2\alpha}$ ($p < 0.0001$) levels. PWV was significantly reduced after ET completion ($p < 0.0001$), and was directly related to urinary 8-iso-$PGF_{2\alpha}$ (Rho = 0.383, $p = 0.021$). After ET, cardiovascular fitness improved [peak oxygen consumption ($p < 0.0001$), peak heart rate ($p < 0.0001$)]. However, no improvement in lipid profile was observed, apart from a significant reduction of triglycerides ($p = 0.022$). PWV and triglycerides were significantly related (Rho = 0.466, $p = 0.005$) throughout the study period. PWV levels were also related to urinary 8-iso-$PGF_{2\alpha}$ in our previously sedentary subjects.

We conclude that regular physical exercise may be a natural antioxidant strategy, lowering oxidant stress and thereby the AS degree.

1. Introduction

Arterial stiffness (AS) is a major factor contributing to the development of cardiovascular disease (CVD). Increased AS determines an augmentation of central aortic pressure, thus causing systolic hypertension, left ventricular hypertrophy, and potentially impaired coronary perfusion [1–3]. AS is associated with a high risk of cardiovascular morbidity and of death [1,2]. Aging increases the risk of CVD[3] and is associated with stiffening of the large elastic arteries, mainly caused by changes including functional alterations of vascular smooth muscle tone and structural changes in the arterial wall. Such changes may be caused and/or sustained by the development of age-related oxidative stress and inflammation [3–6]. PWV is the "gold standard" method to estimate AS[7]. PWV establishes the time delay between pressure waves occurring at proximal and distal sites along the aorta (mainly the carotid and femoral arteries). The faster the pressure wave travels along the aorta, the greater the AS is [3,8]. Mean PWV in healthy, normotensive (systolic blood pressure [SBP] < 140 mm Hg) volunteers is 6.1 ± 1.4 m/s and significantly increases with age [9]. PWV is an independent predictor of cardiovascular events in patients with established CVD as well in healthy adults [10].

Isoprostanes are a family of bioactive compounds produced from arachidonic acid *via* a free radical-catalyzed mechanism of lipid peroxidation on cell membrane phospholipids or circulating low density lipoproteins (LDLs) [11]. Urinary levels of these compounds represent reliable and sensitive markers of *in vivo* lipid peroxidation [13].

A sedentary lifestyle has been identified as a risk factor for the development of CVD[12] and leads to quantifiable impairment in vascular function and arterial wall stiffening, as a result of increased oxidative stress, favoring endothelial dysfunction [13]. Exercise training (ET) may delay the development of arterial stiffness

Abbreviations: AS, arterial stiffness; ET, exercise training; PWV, pulse wave velocity; CVD, cardiovascular disease; SBP, systolic blood pressure; NO, nitric oxide; eNOS, endothelial nitric oxide synthase; ROS, reactive oxygen species; NADPH, nicotinamide adenine dinucleotide phosphate; SOD, superoxide dismutase; GSH, glutathione; MI, myocardial infarction; NSAIDs, nonsteroidal anti-inflammatory drugs.

* Corresponding author at: Center of Excellence on Aging, "G. d'Annunzio" University Foundation, Via Luigi Polacchi, 13, 66013 Chieti, Italy.
E-mail address: gdavi@unich.it (G. Davì).

[1] Gianfranco Lessiani and Francesca Santilli equally contributed to this work.
[2] Each author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

http://dx.doi.org/10.1016/j.vph.2015.05.017
1537-1891/

by increasing shear stress and augmenting nitric oxide (NO) bioavailability [14].

Several studies have documented increased shear stress to upregulate endothelial nitric oxide synthase (eNOS) activity in cell culture, animal or human studies [15]. Extended periods of ET also has an impact on the generation of reactive oxygen species (ROS) [15], by lowering the expression of nicotinamide adenine dinucleotide (phosphate) (NAD(P)H) oxidase and stimulating radical scavenging systems that include copper/zinc-containing superoxide dismutase (SOD), extracellular SOD, glutathione (GSH) peroxidase and GSH levels [15]. Thus, aerobic ET decreases oxidative stress by increasing the efficiency of the antioxidant system, and finally improving endothelial dysfunction [15,16].

In a previous study in sedentary subjects [17], aimed at assessing the effects of high-amount, high-intensity exercise on *in vivo* platelet activation, we reported a significant reduction of the F_2-isoprostane8-iso-$PGF_{2\alpha}$, after completion of the exercise program. We also unraveled that modulation of oxidative stress might be a major determinant of the "antiplatelet effect" of aerobic exercise. Indeed aerobic activity is considered to be an effective component of CVD prevention [12]. While there is compelling evidence of beneficial effects of various exercise modalities on endothelial function [16] and oxidative stress [17–23], their impact on PWV is more controversial [24,25]. Therefore, the main objective of this study was to test the hypothesis of a significant association between evaluation of *in vivo* oxidative stress and the degree of arterial stiffness in sedentary subjects, and to challenge their parallel reversibility after 8 weeks of high-amount, high-intensity ET. As a secondary objective, we aimed at assessing ET effects on cardiovascular fitness and lipid profile.

2. Materials and methods

2.1. Patient selection

Informed consent was obtained from each patient and the study protocol conformed with the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by the institutional Human Research Committee. After giving written, informed consent, 18 subjects (12 males), median age 54 (48–66) years, were enrolled in the study. The clinical characteristics of participating subjects are summarized in Table 1. Subjects were enrolled if they reported a sedentary lifestyle (regular aerobic exercise <3 times/week and for <20 min (min)/session, with a sedentary occupation) and their baseline HDL cholesterol concentration was <50 mg/dL (1.0 mmol/l). All subjects were classified at low or intermediate risk by the Framingham Risk Score (FRS) at the time of study entry. Exclusion criteria included obesity (BMI >30 kg/m^2), a diagnosis of diabetes mellitus, poorly-controlled hypertension or hypercholesterolemia, pregnancy, impaired liver or renal function, previous vascular events (myocardial infarction (MI), stroke), or other medical conditions that would preclude vigorous exercise, treatment with non-steroidalanti-inflammatory drugs (NSAIDs), anticoagulants or antiplatelet drugs, and statins.

Table 1
Clinical characteristics of study subjects at baseline.

Variable	Baseline*
n, (%)	18
Age, years	54 (48–66)
Male gender, n (%)	12 (66.7)
BMI, kg/m^2	25.5 (24.4–27.5)
Obesity, n (%)	3 (16.7)
Hypertension, n (%)	8 (44.4)
Diabetes, n (%)	0 (0)
Smoking, n (%)	3 (16.7)
Fasting plasma glucose, mg/dl	89 (81–97)
AST, mg/dl	18.0 (16.0–23.7)
ALT, mg/dl	20.5 (17.2–24.5)
Smoke, n (%)	3 (16.7)
Rate of adherence, %	95.3 (90.6–99.8)
ACE-inhibitors, n (%)	3 (16.7)
ARBs, n (%)	4 (22.2)
Diuretics, n (%)	2 (11.1)
Beta-blockers, n (%)	1 (5.5)
Calcium channel blockers, n (%)	2 (11.1)
Statins, n (%)	1 (5.5)
PUFA, n (%)	0 (0)
PPI, n (%)	1 (5.5)

AST, aspartate transaminase; ALT, alanine transaminase; ACE, angiotensin-converting-enzyme; ARBs, angiotensin receptor blockers; PUFA, polyunsaturated fatty acids; PPI, proton pump inhibitors.

* Continuous variables are reported as median [interquartile range (IQR)].

2.2. Training program

Each participant completed an eight-week standardized aerobic training program. The exercise training involved 2 sessions per week of supervised exercise on a cycle ergometer (Monark 915E, Vansbro, Sweden). The exercise prescriptions in the exercise group was high-amount, high-intensity exercise for 55 min per session, the caloric equivalent of jogging approximately 20 miles (32.0 km) per week at 60% to 75% of peak oxygen consumption. During an initial period of one month the amount and intensity of exercise were gradually increased, followed by 8 weeks at the appropriate exercise prescription. Participants started at 55% of their baseline VO_2 max for 45 min per session, and progressed in intensity or duration every week according to a standardized protocol, until achieving the standards scheduled for the training program (55 min at 75% of baseline VO_2 max). The subjects performed a maximal incremental ramp test, consisting of 3 min at rest and 5 min of priming exercise at 50 W, followed by a continuous increase in the workload by 20 W/min until exhaustion. The accepted criteria for maximal effort were: respiratory exchange ratio >1.1, and heart rate >90% of the predicted maximum based on age. Breath-by-breath (B-by-B) VO_2 and carbon dioxide output (VCO_2) were measured continuously at the mouth (Quark b2, Cosmed, Rome, Italy). Peak VO_2 (mL/min/kg) was defined as the average of maximum 30-s attained VO_2 at the end of the exercise period. Analyzers and the respiratory flow transducer were calibrated following the manufacturer's instructions before each experimental run. All exercise sessions were verified by directly supervised by a physician under heart rate monitoring (Polar Electro, Kempele, Finland) that recorded data. Daily energy consumption was monitored with a metabolic armband (Sensewear Pro3, Pittsburgh (PA), USA). Nutrient intakes were determined at baseline and at the end of the study. To minimize the confounding effects of weight loss, participants were counseled to maintain body weight, which was ethically justified by the short time frame of the study. Thus, we suggested not to alter their health habits and to continue their usual eating pattern, physical activity outside of the study, alcohol and tobacco use. Data would be excluded from the analysis for subjects whose weight varied by more than 5% from baseline to the end of the study.

2.3. Assays

At baseline (before the run-in period) and after the 8-week training program, the participants were instructed to perform an overnight urine collection, and underwent fasting blood sampling the following morning. Plasma, serum, and urine were stored in aliquots at −20 °C until used for the various analyses. Urinary 8-iso-$PGF_{2\alpha}$ was measured by a previously described radioimmunoassay [26]. Measurements of urinary 8-iso-$PGF_{2\alpha}$ by this radioimmunoassay has been validated using different antisera and by comparison with gas chromatography/mass spectrometry, as detailed elsewhere [26]. Total, HDL and LDL cholesterol, and triglyceride concentrations were measured as previously described [27].