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Physical exercise reduces synthesis of ADMA, SDMA, and L-Arg

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1. ABSTRACT

Increased levels of asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA) and low plasma level of L-arginine (L-ARG) are all conditions likely to decrease nitric oxide (NO) production. Aim of this study is to evaluate ADMA, SDMA, and L-ARG plasmatic levels before and after physical exercise in patients with coronary artery disease (CAD). We studied 30 patient with mean age 52 ± 4.5 years. After inclusion in the study, before the execution of physical exercise, heparinized blood sample was drawn from an indwelling arterial line for determination of ADMA, L-ARG and SDMA (baseline values). Subsequently a blood sample was drawn after the physical exercise. The mean plasma concentrations of ADMA (0.68 0.06 vs 0.48 ± 0.05 mol/L) and SDMA (0.45 ± 0.03 vs 0.30 ± 0.03 ìmol/L) were significantly lower after physical exercise in comparison to baseline value, while L-ARG mean levels were increased (44.20 ± 10.5 vs 74.13 ± 11.2 ìmol/L). Physical exercise has a beneficial effect by reducing plasmatic ADMA and SDMA levels, and increasing L-ARG substrate for endothelial NO.

2. INTRODUCTION

Endothelial dysfunction represents the earliest stage in the atherosclerotic process and contributes to the pathogenesis of acute cardio-cerebrovascular syndromes by predisposing to plaque rupture and intravascular thrombosis (1). Nitric oxide (NO) is a potent endothelium-derived vasodilator that plays a critical role in maintaining vascular homeostasis through its anti-atherogenic, anti-inflammatory, and anti-thrombotic effects on the vascular wall. NO is produced from L-arginine (L-ARG) by a family of NO synthases (2). Three distinct isoforms of nitric oxide synthase (NOS), derived from separate genes, exist: neural NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS) (3).

Asymmetric dimethylarginine (ADMA), a naturally occurring amino-acid produced by methylation of arginine residues, inhibits the NOS activity, leading to the derangement of vasoprotective and vasodilatory effect of NO (4). ADMA is degraded by the isoenzymes dimethyldiarginine hydrolase (DDAH-1 and -2), which hydrolyzes it to l-citrulline and methylamine. DDAH-1 is primarily found in tissues expressing the neuronal

form of NOS (nNOS), while DDAH-2 is mostly expressed in tissues also expressing the endothelial form of NOS (eNOS). Elevated ADMA levels cause eNOS uncoupling, a mechanism which leads to decreased NO bioavailability and increased production of hydrogen peroxide. Many published studies have shown a strong associations between raised ADMA levels and cardiovascular risk factors, endothelial dysfunction, atherosclerosis, hyperlipidemia, and cardiovascular mortality (5-8).

Plasma ADMA levels are also related to the severity of peripheral arterial disease. In 2003 Lu and co-workers reported that an elevated plasma concentration of ADMA indicates an increased risk of developing restenosis after elective coronary angioplasty in 153 patients with stable coronary artery disease (CAD) during a median follow-up of 16 months (9). Regular physical activity is associated with favorable modification of cardiovascular risk factors such as hypertension, diabetes, obesity, and hypercholesterolemia (10,11). Large epidemiological studies in healthy subjects and in patients with documented cardiovascular disease (CVD) demonstrated an inverse, graded, independent, and robust association between fitness status and mortality (12,13).

Aim of this study is to evaluate ADMA, SDMA, and L-ARG plasmatic levels before and after physical exercise in patients with coronary artery disease (CAD).

2. SUBJECTS AND METHODS

2.1. Study design

Between July until December 2011 thirty consecutive patients with diagnosis of CAD admitted to the Cardiology Unit of San Camillo De Lellis Hospital (Manfredonia, Italy) were invited to participate in this study. Inclusion criteria for the CAD group were left ventricular ejection fraction (LVEF) of ≥ 55%, with normal dietary intake, and no contraindications for treadmill physical exercise test. Exclusion criteria were acute cardiac decompensation within the previous 7 days or acute coronary syndrome, age under 18 or above 75 years, impaired hepatic function (prothrombin time > 1.5 times the upper limit of normal or alanine aminotransferase -ALT- > 2.5 times the upper limit of normal). Stable CAD was defined as a common disease due to the obstruction of the coronary arteries by atheromatous plaque. Written informed consent was obtained from all subjects who participated in the study. The study was approved by the Ethical Committee of the San Camillo de Lellis Hospital. Written informed consent was obtained from all subjects.

2.2. Patients

At study entry, medical history, physical condition, and medication of patients were recorded. Details of clinical CAD diagnoses were evaluated by review of hospital records. Of thirty patients included in the study 20 underwent percutaneous coronary angioplasty (PTCA) with stenting of one or more coronary arteries and 10 underwent coronary artery by-pass graft (CABG). After inclusion in the study, before the execution of physical exercise, heparinized blood sample was drawn from an indwelling arterial line for determination of ADMA, L-ARG and SDMA at baseline. Subsequently a blood sample was drawn after the physical exercise. Simultaneously, laboratory parameters indicating renal function (creatinine, urea) and hepatic function (aspartate aminotransferase (AST), ALT and complete haematocytometer exams were determined before physical exercise.

2.3. Sample collection, storage and preparation

Blood samples were collected in polypropylene tubes containing EDTA 1 mM. Samples were stored in an ice box prior to centrifugation at 3000g for 10 min at 4°C. 200µl aliquots of plasma were transferred into a Eppendorf tubes. Plasma samples were either used for immediate extraction or stored in the dark at -80°C until analysis was performed.

2.4. Biochemical analysis

The concentration of ADMA, SDMA and L-ARG were determined by high-performance liquid chromatography (HPLC) (14). In brief, solid-phase extraction on polymeric cation-exchange columns was perform and after addition of monomethylarginine as the internal standard. After derivatization with ortho-

phtaldialdehyde reagent containing 3-mercaptopropionic acid, analytes were separated by isocratic reversed-phase HPLC with fluorescence detection.

2.5. Physical exercise

The exercise protocol was structured according to the guidelines for CAD management. The session was divided into aerobic conditioning, muscle strengthening and increasing joint flexibility activities. Aerobic conditioning consisted of walking on a treadmill for 10minutes; the physical exercise was monitored using a heart rate (HR) and ECG monitor. Thus, strain intensity (speed, in km/h) was gradually adjusted to maintain the target of the reached distance (3000 meters). Relaxation and elongation exercises were conducted before and after the session, including stretching to increase the flexibility of large muscle-joint groups.

2.6. Statistical analysis

Results were expressed as mean ± SD. Data were analysed by using SPSS statistical software (version 15.0 for Windows; SPSS Inc., Chicago). For each baseline characteristic, the mean value or the corresponding percent of study participants was calculated. The significance of changes in ADMA, SDMA and L-ARG was examined using the paired Student t-test. A two-tailed p value <0.05 was considered significant.

3. RESULTS

3.1. Characteristic of subjects

Personal data (sex, age) and clinical details (body mass index, ejection fraction, HR, blood pressure, liver and renal function, and complete haematocytometer exams) of population study are summarized in Table 1. We studied thirty CAD patients (14 males, 16 females) with mean age 52 ± 4.5 years.

The mean plasma concentrations of ADMA (0.68 ± 0.06 vs 0.48 ± 0.05 µmol/L) and SDMA (0.45 ± 0.03 vs 0.30 ± 0.03 µmol/L) were significantly lower after physical exercise in comparison to baseline values (pre-physical exercise), while L-ARG mean levels were increased (44.20 ± 10.5 vs 74.13 ± 11.2 µmol/L).) (see Table 2).

4. DISCUSSION

To date CVD remains one of the leading causes of morbidity and mortality worldwide (15). In stable CAD, exercise training has well-documented the positive effects on arterial endothelial function. Regular physical exercise training partially corrects endothelial dysfunction in CAD and leads to an economization of left ventricular function (16,17).

The mechanisms responsible for the beneficial effects of exercise training on endothelial function are controversial. Exercise training has been variably reported to improve several risk factors for CVD, such as hypercholesterolemia, obesity, glycemic control, and hypertension, factors that are also associated with endothelial dysfunction (10,18). Some early studies of exercise training in humans suggested that the improvement in endothelial function observed was secondary to amelioration of these coincident risk factors (19). An alternate explanation is that repeated exposure of the vasculature to increased shear stress, a primary physiological stimulus to NO production, may explain up-regulation of the NO dilator system (20,21).

Thus, exercise training can increase NO bioavailability and convey benefits to vascular protection. The amount of NO available depends on several key factors involved in the NO pathway as the availability of the NO precursor molecule L-ARG, the activity of eNOS and NO degradation, which depends on the intrinsic half-life and the reaction with reactive oxygen specie (ROS) (22-24).

There are two compounds that can inhibit NOS, N-monomethyL-arginine (NMMA) and ADMA, which both reduce NO synthesis by competing with arginine for NOS binding (25,26). NO so generated may even scavenge overwhelming radicals, such as superoxide anion, thereby preventing tissue damage (3,27). Clinical studies have shown that severe physical exercise can lead to the generation of more free radicals

than the endogenous antioxidant systems can scavenge, whereas moderate intensity aerobic exercise improves endothelial function and reduces cardiovascular risk (28,29).

Elevated levels of ADMA inhibit NO synthesis and therefore impair endothelial function and thus promote atherosclerosis (30,31). Our results support a possible relationship between ADMA, SDMA, L-ARG levels and endothelial dysfunction in CAD patients and suggest that the metabolism of ADMA by dimethylaminohydrolase (DDAH) is likely an important regulatory mechanism in the human cardiovascular system. ADMA is a naturally occurring methylarginine that inhibits all three isoforms of NOS, and has been shown to inhibit eNOS *in vitro* and in the arterial bed of the human forearm. Inhibition of DDAH permits ADMA to accumulate, suppressing the synthesis of NO (32,33).

Recently it was found that SDMA also stimulates production of ROS in monocytes by acting on Ca^{2+} entry via store-operated Ca^{2+} channels. This proinflammatory effect, together with the indirect effects of SDMA on NO synthesis, and the relationship of SDMA to renal function are thought to be possible mechanisms by which SDMA and CVD may be linked (34). Although no evidence exists as yet that SDMA inhibits NO synthase, it may interfere with arginine transport into the cells and, therefore, NO production could be reduced indirectly as already shown by others (35-37). The high SDMA concentrations in renal patients with CAD could indicate such an increase in vascular tone (38,39). The consequences on the hemodynamics could be the same as reported for ADMA.

The results of our study demonstrate clearly that mild-moderate physical exercise represent an important protective factor of endothelial function in CAD subjects, with an important role in the pathophysiology of endothelial dysfunction. In fact, increased plasma ADMA, SDMA and lower L-ARG concentrations occur in a wide range of disease or risk factors in which cardiovascular events are increased and in some situations there is a clear relationship between ADMA level and morbidity/mortality. ADMA competes with l-ARG for binding to NOS and thus competitively antagonizes the enzyme's catalytic activity. Our results show that physical exercise result in a reduction in plasma levels of ADMA and SDMA and an increase in plasma levels of L-ARG. This is important because L-ARG is the substrate of eNOS, whose metabolism is crucial for the bioavailability of NO. Since endothelial dysfunction represents the crucial event in CVD, our data are interesting since they show how physical exercise may have a beneficial effect on CAD, since it helps to maintain endothelial function through a positive modulation of L-ARG compared to plasma concentrations of ADMA/SDMA.

Even our study present many limitations (measurement of other endothelium-dependent dilation substances, lack of a control group, and cardiopulmonary exercise testing or a measure of VO2 or METs, further studies are clearly warranted so that we can have a better understanding of the mechanisms of exercise as a preventive and therapeutic measure for the CVS. An additional benefit is that by so doing, we will better customize appropriate levels of physical training for individual patients. Our future goals will be to investigate whether physical exercise could lead to the reduction of ADMA and SDMA with a modulation in the expression and activity of the protein arginine N-methyltransferases (PRMT or PRMT-1-2) or DDAH-2 or if there is a relationship between physical exercise and the uptake of L-arginine.

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