The L-Arginine−Asymmetric Dimethylarginine Ratio Is Strongly Related to the Severity of Chronic Heart Failure. No Effects of Exercise Training

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ABSTRACT

Background: The aim of this study was to relate levels of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide (NO) synthase, L-arginine, the substrate for NO generation, and radical oxygen species (ROS) formation to severity of chronic heart failure. The effect of 4 months' group-based exercise training was further investigated.

Method and Results: Eighty patients, aged 45-85 years with New York Heart Association (NYHA) functional class II-IIIb, all on optimal medical treatment, were included. A 6-minute walking test and a bicycle exercise test were performed, and fasting blood samples were collected for determination of N-terminal pro−brain natriuretic peptide (NT-proBNP), L-arginine, ADMA, and ROS generation in circulating leukocytes. ADMA levels were significantly higher in patients in NYHA functional class III versus II ($P = .024$), and the L-arginine−ADMA ratio was significantly lower ($P = .005$). After adjustment for covariates, L-arginine−ADMA ratio was associated with 6-minute walking distance ($P = .004$), exercise capacity ($P = .026$), and inversely with NT-proBNP ($P = .015$). Stimulated levels of peroxynitrite on monocytes were inversely related to left ventricular ejection fraction ($P = .005$). No effect of 4 weeks' exercise training on the measured variables was obtained.

Conclusions: The strong relationship seen between L-arginine−ADMA ratio, ROS formation in leukocytes, and severity of chronic heart failure contributes to increased knowledge of endothelial dysfunction related to the NO pathway in such patients. (*J Cardiac Fail 2011;17:135−142*)

Key Words: Cardiac failure, endothelial dysfunction, asymmetric dimethylarginine, radical oxygen species, NT-proBNP.

There is considerable evidence that patients with chronic heart failure (CHF) have abnormal endothelial function.[1,2] Endothelial dysfunction may affect the cardiovascular system in different ways, such as impairment of peripheral perfusion, a possible adverse long-term effect on vascular remodeling, and reduced compliance of the failing left ventricle (LV) and consequent impaired LV dysfunction.[3]

Among different biomarkers of endothelial dysfunction, the amino acid asymmetric dimethylarginine (ADMA), an important endogenous competitive inhibitor of nitric oxide (NO) synthase (NOS), has been suggested to be of great importance owing to the important role of NO in the vasoreactive process.[4] ADMA is synthezised by methylation of proteins containing L-arginine, the substrate of NOS. ADMA has also been shown to increase oxidative stress by uncoupling of electron transport between NOS and L-arginine,[5] resulting in increased production of radical oxygen species (ROS). A direct inactivation of endothelial-derived NO by ROS has also been demonstrated,[6] indicating oxidative stress to affect the endothelium in different ways. Patients with CHF are reported to have increased formation of ROS, which may influence cardiac remodeling.[7] It has also been discussed that oxidative stress could be an

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Manuscript received April 12, 2010; revised manuscript received August 20, 2010; revised manuscript accepted September 24, 2010.

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Supported by grants from the Eastern Norwegian Health Authority, the Norwegian Foundation for Health and Rehabilitation, and the Stein Erik Hagen Foundation of Clinical Heart Research, Oslo, Norway.

See page 141 for disclosure information.

1071-9164/$ - see front matter

doi:10.1016/j.cardfail.2010.09.003

important determinant of exercise tolerance in CHF patients.[8]

Increased levels of ADMA have been shown to be associated with increased risk of coronary events in selected populations,[9–11] and it was recently reported that elevated levels of ADMA increased cardiovascular risk prediction in patients with heart failure.[12] Several studies support the view that the ratio between L-arginine and ADMA is important for the regulation of endothelial NOS activity,[13–15] but this has not been reported in relation to the heart failure syndrome.

Beneficial effects of physical training in patients with CHF along with improvement of endothelial function measured by flow-mediated vasodilation have been shown in some studies.[16] However, limited results exist regarding an effect on the endothelium assessed by ADMA or the L-arginine−ADMA ratio. There are also, to our knowledge, no data on the effect of exercise training on formation of ROS in heart failure patients. Recently, a standardized group-based high-intensity model for exercise training suitable for CHF patients was published.[17]

The objectives of the present investigation were, in a population of patients with CHF, to study the relationship between plasma levels of ADMA or the L-arginine−ADMA ratio and the severity of the disease, as assessed by functional measures and the N-terminal pro−brain natriuretic peptide (NT-proBNP). Furthermore, we wanted to investigate any influence of a standardized group-based high-intensity interval training for 4-5 months on the levels of ADMA and the L-arginine−ADMA ratio in this population, which was randomized to such intervention or to a control group. We have recently shown that the intervention program[17] significantly improved exercise capacity compared with control subjects in this population.[18]

In a subset of patients, we also studied the intracellular expression of different ROS in circulating leukocytes as related to both the severity of the disease and potential intervention effects.

Materials and Methods

Study Population and Design

The study population comprised 80 patients, aged 45−85 years (mean 70 years), 21% female, with CHF, New York Heart Association (NYHA) function class II-IIIb, and left ventricular ejection fraction (LVEF) <40% as evaluated by echocardiography or >40% with signs of diastolic dysfunction, all included from the medical outpatient clinic at Oslo University Hospital Ulleval, Oslo, Norway. Exclusion criteria were acute myocardial infarction within the preceding 4 weeks, untreated serious arrhythmias, serious malignancy expected to impede compliance, obstructive pulmonary disease thought to affect the physical capacity more than the heart failure, and patients with good physical capacity (6-minute walking test [6MWT] >550 m or bicycle exercise test >110 W). The etiologies of CHF in the included population were ischemic heart disease (69%), idiopathic dilated cardiomyopathy (18%), and hypertensive heart failure (13%).

The patients fulfilling the inclusion criteria were randomized in accordance with a computerized block-randomization list by use of consecutively numbered sealed envelopes to the exercise intervention program or to a control group for 4 months, and they were followed further for a total of 12 months. All patients were on individual optimal medical treatment throughout the study.

The study complied with the Declaration of Helsinki, and approval was given from the regional Ethical Committee. Each of the patients gave written informed consent to participate.

Intervention/Training Program

Exercise training was the main element in the intervention group and this was based on a standardized group-based high-intensity aerobic interval-training program, previously described in detail.[17] In brief, the program was conducted twice a week in groups of 6-10 patients in which the patients performed in accordance with their individual capacity, all under supervision from a physiotherapist and with a heart failure study nurse present. The training lasted ~1 hour and included 3 intervals of high intensity and 2 intervals with moderate intensity. The intensity was estimated by use of the Borg Scale.[19] Compliance was recorded by logging of every appearance. Patients randomized to the control group were followed clinically by their general physician. In addition, they were followed up at 4 and 12 months for blood sampling, 6MWT, and a bicycle exercise test.

Physical Performance

A standardized 6MWT was performed under supervision of an individual blinded for the randomization groups at baseline and after 4 months' intervention and after 12 months' follow-up. The distance in meters was recorded.

A maximal bicycle exercise test was performed after 4 and 12 months on an electrically braked cycle ergometer (Ergometrics 800; Ergoline, Bitz, Germany). A continuous model starting out at 30 W for both men and women with an increase of 10 W/min was used. The test was limited by exhaustion, typical chest pain, or other indications as judged by the medical doctor, which was blinded to the randomized groups. The Borg scale was used for subjective recordings of physical effort. Physical performance was recorded as watts to exhaustion.

Blood Sampling

Venous blood samples were collected in fasting condition between 8 and 10 a.m. at randomization (baseline) and after 4 and 12 months. In addition to routine clinical chemistry analyses, EDTA plasma (0.34 mol/L EDTA-K3) was prepared for determination of NT-proBNP, ADMA, and oxidized low-density lipoprotein (oxLDL). Serum was prepared for nitrate/nitrite (NOx) and thiobarbituric acid−reacting substances (TBARs) analyses. Plasma and serum were kept frozen at −80°C for batch analysis.

In addition, citrated blood (0.129 mol/L in dilution 1:10) was collected and placed on ice for 10-15 minutes for determination of ROS in leukocytes in a subset of patients (n = 25) at baseline and after 4 months' intervention.

Laboratory Methods

NT-proBNP was determined with Elecsys proBNP sandwich immunoassay on an Elecsys 2010 (Roche Diagnotics, Indianapolis, Indiana, USA). The interassay coefficient of variation was 7%. TBARs were determined as previously described.[20] Oxidized LDL was measured with an enzyme-linked immunosorbent assay method (Mercodia, Uppsala, Sweden). NOx was analyzed using the Total Nitric Oxide Assay kit (R&D Systems Europe,