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Serum concentrations of L-arginine and L-homoarginine in male patients with intermittent claudication: A cross-sectional and prospective investigation in the CAVASIC Study

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ABSTRACT

Background: High serum concentrations of L-arginine and L-homoarginine increase nitric oxide (NO) availability and thereby improve endothelial function. Information about the association of these markers with peripheral arterial disease (PAD) and related outcomes is sparse.
Methods: L-arginine, its metabolites and L-homoarginine were analyzed in the CAVASIC Study including 232 male patients diagnosed with intermittent claudication and 246 age- and diabetes-matched controls. After the baseline investigation PAD patients were prospectively followed (median 7 years). The association of these markers with symptomatic PAD at baseline, incident cardiovascular events and all-cause mortality was assessed.
Results: At baseline each increase of ln-L-homoarginine and L-arginine by one standard deviation was associated with symptomatic PAD: OR = 0.75, 95%CI 0.59–0.96, P = 0.02 and OR = 1.36, 95%CI 1.07–1.73, P = 0.01, respectively (both models adjusted for ln-CRP, GFR, HDL cholesterol, and current smoking). Only L-arginine remained significant after additional adjustment for ln-NT-proBNP and hs-cTnT: OR = 1.49, P = 0.002. In the Cox regression analysis elevated ln-L-homoarginine significantly reduced the risk to die (n = 38) even independent from ln-NT-proBNP and hs-cTnT: HR = 0.59, 95%CI 0.41–0.84, P = 0.004. L-arginine was significantly predicting incident cardiovascular events (n = 65): HR = 1.68, 95%CI 1.35–2.10, P < 0.001.
Conclusions: This study in male patients with intermittent claudication and age- and diabetes-matched controls showed an association of L-homoarginine and L-arginine with PAD. During follow-up, L-arginine was associated with incident cardiovascular events probably due to its primary role in NO metabolism and impact on endothelial integrity. L-homoarginine was related to all-cause mortality implying a broader role in metabolic processes besides endothelial function.

1. Introduction

The progressive occlusion of the large peripheral arteries is a common consequence of atherosclerosis leading to peripheral arterial disease (PAD). PAD affects around 16% of the general population aged over 55 years and is steadily increasing [1]. PAD

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patients have a high risk for cardiovascular and cerebrovascular events [2]. Endothelial cell dysfunction and a disturbed nitric oxide (NO) metabolism are important in the development of PAD and subsequently contribute to the long-term clinical outcome in these patients [3]. Therefore biomarkers that indicate such processes are needed.

L-arginine is the substrate for producing the endogenous vasodilator NO by nitric oxide synthase (NOS) [4]. This enzyme may also form NO from L-homoarginine hence showing a lower catalytic efficiency than for L-arginine [5]. L-arginine concentrations were not associated with cardiovascular outcomes and mortality in advanced PAD, patients undergoing coronary angiography (CAG) and the general population [6-10].

L-homoarginine is formed from lysine by arginine glycine amidinotransferase (AGAT) and is mainly produced in the kidney [5]. It has been shown to increase NO production by inhibiting either arginase or protein arginine methyltransferases (PRMTs) [11]. Thus, increased L-homoarginine concentrations might exert a positive effect on endothelial function [12]. Low concentrations of L-homoarginine have been related to progression of kidney disease [13]. Recent studies reported low L-homoarginine concentrations to be a risk marker for cardiovascular events and all-cause mortality in the general population and coronary heart as well as kidney disease patients [14-20].

Ornithine, formed from L-arginine by arginase as part of the urea cycle, can be transformed into citrulline by ornithine transcarbamylase. Ornithine competes with L-arginine for cellular uptake and therefore might reduce L-arginine bioavailability [21]. Citrulline is generated during NO production and further converted into L-arginine by argininosuccinate synthase and lyase [22]. Ornithine and citrulline have been evaluated in patients with coronary artery disease (CAD) [6].

The dimethylarginines asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) are endogenous analogues of L-arginine formed by PRMTs [11]. Increased ADMA plasma concentrations have been associated with an increased risk for total mortality and incident cardiovascular events in PAD and end-stage renal disease (ESRD) [8,23,24]. Studies on the association of SDMA with cardiovascular disease outcomes or mortality brought conflicting results [24,25].

However, information about the role of these markers in symptomatic PAD and their association with adverse outcomes is sparse and remains to be elucidated. Therefore the aim of this study was to investigate the association of L-arginine, its metabolites and L-homoarginine with 1) symptomatic PAD and 2) all-cause mortality as well as incident cardiovascular disease in PAD patients.

2. Materials and methods

2.1. Study participants and study design

The CAVASIC (Cardiovascular Disease in Intermittent Claudication) Study, a case-control study with a prospective follow-up investigation, was set up to identify cardiovascular risk factors in patients with intermittent claudication [26,27]. Patients and controls were recruited in 2 clinical centers: the Department of Vascular Surgery, Medical University of Innsbruck, and the 3rd Medical Department of Metabolic Diseases and Nephrology, Hietzing Hospital, Vienna, Austria (in the years 2002–2006). Neither patients nor controls presented with signs of acute illnesses or inflammatory processes at the time of enrollment.

Patients (n = 248) with a history of intermittent claudication (PAD IIa or IIb according to the criteria of Fontaine) were included regardless of any former treatment procedure (bypass surgery or intervention). Patients with presence of acute or critical limb ischemia (Fontaine III or IV), impaired kidney function with serum creatinine >133 μmol/L, malignancy, previous organ transplantation, and therapy with nicotinic acid or corticosteroids were excluded.

The control group (n = 251) was recruited by an invitation published in the local newspaper, matched for age and type 2 diabetes mellitus (T2DM). The same exclusion criteria were applied in cases and controls. Controls with symptomatic PAD were excluded but those with known cardiovascular disease (CVD) were allowed to participate.

All participants provided written informed consent and the examination protocol was approved by the local Ethics Committee of the participating study centers.

All data and analyses at baseline described in this manuscript are based on 478 participants (232 patients and 246 controls) with available serum samples of L-arginine, its metabolites and L-homoarginine. The design of the baseline investigation is briefly described in Fig. 1.

2.2. Baseline examination

A standardized interview was conducted to obtain information about demographic data, history and atherosclerosis risk profile of participants.

Further details on the study design, the baseline examination, laboratory measurements and statistical methods can be found in the Supplementary material.

2.3. Follow-up examination

All PAD patients (n = 256) of the CAVASIC Study, not only those patients who were originally matched to respective control individuals in the baseline case-control analysis, were followed prospectively for a median follow-up time of 7 years to gather data on clinical endpoints (fatal and non-fatal cardiovascular events) and all-cause mortality. At the end of the observation period, follow-up information was available in 255 patients. Face-to face interviews including follow-up examinations were performed (in 86%) and/or hospital charts were available (in 100%). Finally, the follow-up investigation for this particular project included 239 PAD patients with available L-homoarginine, L-arginine and metabolite values. For an overview of the follow-up part see Fig. 1.

2.4. Clinical endpoints

Clinical endpoints were defined as all-cause mortality (primary outcome) and incident fatal and non-fatal cardiovascular events (secondary outcome) between baseline and follow-up. Autopsy reports, medical reports, death certificates or information reported by general practitioners provided data on mortality and/or the verification of causes of death as well as of all further reported clinical events. Cardiovascular mortality was specified according to the ICD10 Codes I00-I99, death due to cancer based on ICD10 C00-D48 and infectious mortality by codes ICD10 A00-B99.

The extended definition of cardiovascular events included non-fatal myocardial infarction (MI) diagnosed by clinical symptoms, electrocardiography, and an increase in cardiac troponins. Additionally, medical records of coronary angiographies (CAGs) with or without subsequent percutaneous transluminal coronary angioplasty (PTCA) were available in all MI cases. Elective PTCA, aorto-coronary bypass, angiographically proven coronary stenosis ≥50%, ischemic cerebral infarction (diagnosed by clinical appearance, computer tomography and/or magnetic resonance imaging), transient ischemic attack, carotid endarterectomy (CEA) as well as cardiovascular death were also part of the extended definition.