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Femoral artery diameter and arteriogenic cytokines in healthy women

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ABSTRACT

Animal studies have identified monocyte chemoattractive protein-1 (MCP-1) and vascular endothelial growth factor (VEGF) as critical mediators of arterial diameter enlargement in response to chronic increases in blood flow (arteriogenesis). Furthermore, cellular studies have shown that the shear stresses resulting from increased blood flow stimulate synthesis of MCP-1, which in turn stimulates synthesis of VEGF. The purpose of this study was to determine if these mechanisms are evident in healthy women. Resting femoral artery diameter and blood flow, lean leg mass, MCP-1 and VEGF concentrations, and aerobic capacity were measured in 34 healthy women along with plasma concentrations of lipids associated with cardiovascular disease risk. Femoral artery diameter was independently related to metabolically active (lean) leg mass (b=0.41, P=0.008) and aerobic capacity (b=0.45, P=0.004). Plasma MCP-1 correlated negatively with the ratio of femoral artery diameter to lean leg mass (b=−0.42, P=0.009) and positively with serum triglycerides (b=0.46, P=0.005). Plasma VEGF exhibited similar correlations and strongly correlated with MCP-1 (R=0.92, P<0.0001). The results indicate that circulating MCP-1 and VEGF concentrations are associated with both arteriogenic and atherogenic stimuli in healthy women.

1. Introduction

Arteries adapt to changes in the chronic metabolic requirements of the tissues they perfuse through structural modifications that lead to changes in arterial lumen diameter. Common femoral artery diameter correlated significantly with quadriceps mass in a study of seven men and one woman (Radengran and Saltin, 2000). In a larger cross-sectional study of 108 men, femoral artery diameter was 7% greater in exercise-trained men than in sedentary men (Dinenno et al., 2001). This same study showed that three months of walking exercise by previously sedentary men increased their femoral diameter by 9%. In contrast, deconditioning of 16 men via 52 days of bed rest reduced femoral artery diameter by 17% (Bleeker et al., 2005).

A prevalent experimental model for studying the mechanisms responsible for such arterial remodeling, termed arteriogenesis (Heil and Schaper, 2004), involves occluding the femoral arteries of laboratory animals and analyzing the collateral vessels that take on increased blood flow. Among the early responses is increased gene expression for monocyte chemoattractant protein-1 (MCP-1) by endothelial cells (Buschmann et al., 2003). MCP-1 recruits circulating monocytes into the arterial vessel wall by virtue of its chemotactic properties and by upregulating adhesion molecule expression (Mantovani et al., 2003). These monocytes differentiate into macrophages and are postulated to clear space for vessel enlargement by breaking down existing extracellular matrix through the release of proteolytic enzymes and by phagocytosis (Heil and Schaper, 2004). Arteriogenesis was significantly enhanced in animals receiving local infusion of MCP-1 protein (Ito et al., 1997), and reduced in animals with selective deletions of the genes for either MCP-1 (Voskuil et al., 2004) or its receptor (Heil et al., 2004). Macrophages also promote proliferation of vascular endothelial and smooth muscle cells by secreting mitogenic growth factors such as vascular endothelial growth factor (VEGF) (Heil and Schaper, 2004). Treating animals with a VEGF receptor antagonist during femoral artery occlusion completely blocked collateral artery enlargement (Lloyd et al., 2004). MCP-1 not only promotes monocyte recruitment, but also stimulates VEGF synthesis (Parenti et al., 2004).

Gene expression for MCP-1 is induced in human endothelial cells by laminar fluid shear stress in vitro (Shyy et al., 1993), and a changing shear rate exerts a more potent stimulus for expression than a steady shear rate (Bao et al., 1998). Thus, the stresses imposed upon a vessel by increased blood flow trigger the synthesis and release of factor(s) that stimulate enlargement of the vessel.

The purpose of this study was to determine if evidence for these mechanisms could be observed in healthy human subjects. Specifically, we hypothesized that circulating concentrations of MCP-1 and

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VEGF would be high in women who had high femoral artery shear rates resulting from small femoral artery diameters, relative to their lean leg mass.

In addition, elevated MCP-1 concentrations have been associated with vascular disease (Hoogeveen et al., 2005; Martinovic et al., 2005). Cardiovascular risk factors such as leptin (Bouloumie et al., 1999) and triglycerides (Norata et al., 2007) stimulate human vascular endothelial cells to synthesize MCP-1 in vitro. Therefore, we also sought to determine if the endogenous concentrations these factors were related to circulating MCP-1 or VEGF in our subjects. These studies were intended to determine if the arteriogenic mechanisms identified in cellular and animal experimentation are applicable to arterial diameter homeostasis in human beings.

2. Methods

2.1. Subjects

Women ranging in age from 25 to 40 years were screened initially by telephone to exclude for self-reported cardiovascular, inflammatory, and neuromuscular diseases, as well as smoking. Subjects brought into the laboratory for testing were excluded for hypertension (arterial blood pressure >140/90 mm Hg), diabetes (fasting plasma glucose concentrations >125), or lipid concentrations exceeding the National Cholesterol Education Program — Adult Treatment Program III "borderline high" cutoffs (Expert panel on detection evaluation and treatment of high blood cholesterol in adults, 2001).

The total amount of testing per subject was anticipated to be burdensome in a single session; therefore subjects were studied on 2 days within a 7-day span. One visit included measurements of blood pressure, self-reported physical activity (Baecke questionnaire), collection of a resting, fasting venous blood sample, and determination of peak oxygen consumption during a progressive exercise test. On the other visit, femoral artery diameter and blood velocity were determined along with body composition. Complete vascular measurements were obtained from 17 subjects recruited and tested at the University of Georgia in Athens and 17 subjects at the Medical College of Georgia in Augusta. All procedures were approved by the Institutional Review Board at The University of Georgia and the Human Assurance Committee at the Medical College of Georgia. All subjects were informed of the procedures involved in the project and gave written informed consent before undergoing testing. Factors influencing blood pressure in these subjects were also studied and are described in a separate report (Sabatier et al., 2008).

2.2. Cardiorespiratory fitness

Peak aerobic capacity was measured by open-circuit spirometry (Davis et al., 2002) on friction-braked Monark cycle ergometers. The fitness test began with 4 min of cycling without resistance, followed by an increase of 15 W every minute. Heart rate (EKG) and blood pressure (auscultation) were assessed during each stage and subjects provided subjective ratings of perceived exertion (RPE) during every other stage of the test (Borg, 1982). Peak oxygen consumption (VO_2 peak) was taken as the maximal 30-second average. Oxygen consumption was calculated from measures of pulmonary ventilation and mixed expired oxygen and carbon dioxide using Sensormedics Vmax metabolic carts (Yorba Linda, CA).

2.3. Body composition

Body composition was measured using whole-body dual-energy X-ray absorptiometry (DEXA). The Hologic QDR 4500 W Elite was used at Augusta, and Hologic Delphi A (Bedford, MA) was used at Athens. One investigator at each site performed all scans and analyzed all data. Each scan was compartmentalized and analyzed using Hologic whole-body software for bone mineral density, fat-free soft tissue and percent body fat. Inter-machine variability for DEXA scanners by the same manufacturer, with respect to percent body fat, has previously been found to be minimal (−1.7±1.0%)(Tataranni et al., 1996). Furthermore, a soft-tissue phantom was used to calibrate the DEXA scanner at both sites, as previously recommended (Paton et al., 1995).

2.4. Resting femoral artery diameter, blood flow, and shear rate

Subjects rested quietly in the supine position for 15 min before data acquisition began. One sonographer performed all tests for this study. B-mode imaging was used to visualize the artery 1–5 cm distal to the femoral bifurcation using a LogiQ 400CL ultrasound machine (General Electric, Rainbow City, AL) equipped with a 7–13 MHz linear-array transducer. B-mode images were recorded for off-line diameter measurements during diastole. Resting blood velocity was assessed using pulsed Doppler ultrasound recorded in the longitudinal view using an insonation angle between 45° and 60°. The velocity gate was set to include the entire lumen area. Time averaged maximum velocity (TAMAX) was auto calculated every cardiac cycle by the GE 400CL advanced vascular program. TAMAX values were acquired over five minutes of quiet rest and saved directly to a computer using specially coded optical character recognition software (NI LabVIEW 6i, Austin, TX), allowing data acquisition on a beat-by-beat basis. Arterial diameter was measured manually in three images by applying straight lines conforming to the wall-lumen interface of two-dimensional images captured during data collection. Blood flow (mL/min) was calculated as the product of arterial cross sectional area (π(diameter/2)^2) times (velocity*60 s/min). Shear rate (blood velocity÷diameter) was used as an estimate of shear stress. Day-to-day reproducibility for resting diameter, blood velocity, blood flow, and shear rate was evaluated between 2 testing days in 15 subjects (9 women, 6 men; 21±1 yr of age) who were not part of the study. The coefficients of variation for diameter, blood velocity, blood flow, and resting shear rate measurements were 1.2%, 21.1%, 22.7%, and 20.3%, respectively.

2.5. Plasma and serum measurements

Blood samples (30 cc) were obtained by antecubital venipuncture after a 12-hour fast. Samples were collected in EDTA-treated vacuum tubes for plasma and untreated tubes for serum. After centrifugation, the separated plasma and serum were stored at −70 °C until assay. Plasma glucose, triglyceride, cholesterol and lipoprotein concentrations were measured by enzyme-coupled colorimetric reactions on a Vitros DT60 II analyzer (Ortho-Clinical Diagnostics, Raritan NJ).

Plasma MCP-1 and VEGF concentrations were determined with a multiplex immunoassay (Cytometric Bead Array, BD Biosciences, San Diego, CA). The sensitivity for MCP-1 was 2 pg/ml with intra- and inter-assay variabilities of 6 and 9%, respectively. For VEGF, sensitivity was 10 pg/ml with intra- and inter-assay variabilities of 8 and 7%, respectively.

Serum leptin concentrations were determined by a standard ELISA protocol using antibodies and standards from R&D Systems (Minneapolis, MN) as described previously (Salkeld et al., 2001). The detection limit was 0.06 ng/ml, inter-assay variability was 5% and intra-assay variability was 2%. Total nitric oxide metabolites (nitrates plus nitrites) were measured with an assay kit based on the Griess reaction (KGE001, R & D Systems). The detection limit was 0.25 µmol/L, inter-assay variability was 4% and intra-assay variability was 2%. Serum estradiol and progesterone concentrations were measured using ELISA kits from Diagnostic Systems Labs (Webster, TX). The estradiol assay (DSL-10-4300) had a sensitivity of 7 pg/ml with an intra- and inter-assay variability of 5 and 8%, respectively. The progesterone assay (DSL-10-3900) had a sensitivity of 0.13 ng/ml with an intra- and inter-assay variability of 7 and 5%, respectively.