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RESEARCH

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Vitamin C Status Is Related to Proinflammatory Responses and Impaired Vascular Endothelial Function in Healthy, College-Aged Lean and Obese Men

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ABSTRACT

Vitamin C supplementation has been suggested to reduce cardiovascular disease risk. However, no studies have examined the relationship between vitamin C status and vascular dysfunction in lean and obese individuals in the absence of supplementation. We examined whether vascular function is interrelated with vitamin C status and inflammation in healthy, college-aged lean and obese men with no history of dietary supplementation. A cross-sectional study was conducted during winter 2008 in lean and obese men aged 21±3 years (n=8/group). Brachial artery flow-mediated dilation (FMD) was measured to determine vascular endothelial function. Plasma antioxidants (vitamin C, vitamin E, and thiols), inflammatory proteins (C-reactive protein [CRP], myeloperoxidase [MPO], and cytokines), and cellular adhesion molecules were measured. Participants also completed 3-day food records on the days preceding their vascular testing. Group differences were evaluated by *t* tests, and correlation coefficients were determined by linear regression. FMD was 21% lower ($P<0.05$) in obese men. They also had 51% lower vitamin C intakes and 38% lower plasma vitamin C concentrations. Obese men had greater plasma concentrations of CRP, MPO, inflammatory cytokines, and cellular adhesion molecules. Participants' CRP and MPO were each inversely related ($P<0.05$) to FMD ($r=-0.528$ and -0.625) and plasma vitamin C ($r=-0.646$ and -0.701). These data suggest that low vitamin C status is associated with proinflammatory responses and impaired vascular function in lean and obese men. Additional study is warranted to determine whether improving dietary vitamin C intakes from food attenuate vascular dysfunction.

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Cardiovascular disease (CVD) is the leading cause of mortality among Americans (1). Obesity increases CVD risk (2) and contributes to ≥13% of CVD-related deaths (1). Two thirds of American adults are overweight or obese, and the underway obesity epidemic is expected to increase CVD-related mortality. Greater CVD risk in individuals with obesity is attributed partly to vascular endothelial dysfunction (3) and greater oxidative stress and inflammation (4,5). Vascular dysfunction is characterized by impaired vasodilatory responses, altered anti-inflammatory and anticoagulant activities, and dysregulation of vascular growth and remodeling, all of which contribute to CVD (6). Endothelial-dependent flow-mediated dilation (FMD), a measure of vascular function, can be measured non-invasively in the brachial artery by ultrasonography (7). Lower FMD responses are associated with obesity (8) and a greater incidence of future CVD-related events (9).

Oxidative stress, which is common in obesity, contributes to CVD risk (5). Oxidative stress arises from inadequate antioxidant defenses (10) and greater reactive oxygen species production (11) and could lead to increased inflammation. Indeed, atherosclerotic lesions from CVD patients have greater accumulation of myeloperoxidase (MPO) (12), a neutrophil-derived enzyme that induces vascular damage and is associated with future fatal CVD events (13). Importantly, MPO and C-reactive protein (CRP), which also independently predicts CVD risk (14), are elevated with obesity (15,16). The involvement of oxidative stress in CVD suggests that antioxidants, including vitamin C, may decrease CVD risk. However, the results of studies examining vitamin C status on CVD risk are equivocal. Some suggest that CVD risk is lower among those having greater vitamin C status (17) and

that high-dose vitamin C supplementation increases endothelial-dependent vasodilation in high-risk populations (18-21). In contrast, vitamin C supplementation (500 mg/day, >8 years) in men and women with or without CVD did not improve CVD-related endpoints (22,23). Thus, it remains unclear if vitamin C status, particularly that associated with normal intakes, affects CVD risk.

Most studies examining the relation among vitamin C and CVD risk involve high-dose supplementation (17). No studies have examined the relation between vitamin C status and vascular dysfunction in healthy, college-aged individuals under free-living conditions. Therefore, the objective of this study was to define whether vascular function is interrelated with vitamin C status and inflammation in healthy, college-aged lean and obese men having no recent history (>2-mo) of dietary nutrient supplementation.

METHODS

Study Design

The study was approved by the Institutional Review Board at the University of Connecticut, and participants provided written consent before enrolling. Healthy, nonsmoking men (n=8 lean and n=8 obese) were recruited based on age (18 to 35 years), stable body mass (>2 months) and body mass index (calculated as kg/m^2) (18 to 25 or 27 to 40), not dietary supplement user (>2 months), fasting total cholesterol (<200 mg/dL [5.2 mmol/L]) and glucose (<100 mg/dL [5.556 mmol/L]), and resting blood pressure (<140/90 mm Hg). Men were specifically enrolled because they have lower FMD responses than women (24), which is partly attributed to differences in sex hormones, social habits (25), and greater inflammation (1). Participants had stable exercise patterns (<5 hours/week), were free of diabetes and other metabolic diseases, and did not use any medications. Waist circumference was determined at the umbilicus. Body density was estimated from skinfolds at seven sites (26) and fat mass was calculated using appropriate equations for race (27,28).

Participants visited the study center two times separated by ≥7 days. During each visit, fasting blood samples were collected, plasma was obtained by centrifugation, snap-frozen in liquid nitrogen, and stored at −80°C until analyzed. FMD was also measured during each visit to assess vascular function.

Dietary Analysis

Participants completed 3-day diet records before each visit and records were reviewed with participants for accuracy with a registered dietitian. Nutrient intakes were analyzed using Food Processor SQL (ESHA Research, Salem, OR) and were not different between visits ($P>0.05$). Thus, all analyses were performed using mean intakes of both visits.

FMD

Brachial artery FMD was assessed by ultrasonographic imaging (Acuson Corp, Elmwood Park, NJ) as described (29), with minor modification. Vascular function was measured following forearm occlusion because this was suggested to better reflect nitric oxide-mediated vascular dilation compared to upper arm occlusion (30). FMD was performed by the same technician and image analysis was assessed independently by two technicians in a blinded manner. FMD (%) was calculated by determining peak postocclusion vessel diameter relative to baseline diameter. Participants' FMD responses between the two visits were in agreement (coefficient of variation [CV] <8%) and were not different ($P>0.05$). Thus, FMD is reported as the mean response from both visits.

Clinical Chemistries

Plasma triglyceride, total cholesterol, and glucose levels were measured using clinical assays (Pointe Scientific, Canton, MI). Insulin was measured by ELISA (Diagnostic Systems Laboratories, Webster, TX). Homeostatic model of assessment (HOMA) was calculated from fasting glucose and insulin as described (31). Total nitrite/nitrate, an indirect index of nitric oxide, was measured spectrophotometrically (Cayman Chemical, Ann Arbor, MI). Intra-assay CV of these assays were 1.9% to 5.0%.

Inflammatory Markers

MPO and high-sensitivity CRP were measured by ELISA (BioCheck, Foster City, CA). Plasma interleukins (ILs; IL-6 and IL-10), tumor necrosis factor-α, soluble intracellular adhesion molecule-1 (ICAM-1), soluble vascular adhesion molecule-1, and soluble E-selectin (CV 3.0% to 9.1%) were measured using xMAP technology on a Luminex IS200 system (Austin, TX) with corresponding antibodies (Millipore, Danvers, MA).

Antioxidants

Total antioxidant status was measured using the oxygen radical absorbance capacity (32) and ferric reducing ability of plasma (FRAP) (33) assays as described (CV 2.6% to 8.5%). α- and γ-tocopherol as well as ascorbic acid and uric acid were measured by routine high-performance liquid chromatography-Coularray procedures (CV <3.8%) (34). γ-Carboxyethyl-hydroxychromanol (γ-CEHC), the metabolite of γ-tocopherol, was measured by high-performance liquid chromatography-Coularray (CV 3.9%) (35), with minor modifications. Briefly, methanolic plasma extracts were evaporated, reconstituted in water for enzymatic hydrolysis, and extracted with ethyl acetate. Extracts were evaporated, reconstituted in 70% methanol, and separated on a Phenomenex Luna C_{18} column (250×4.6 mm i.d., 5μ; Torrance, CA) and detected at potential settings of −100, −50, 200, and 350 mV. Plasma thiols, as cystine, cysteine, glutathione, and oxidized glutathione, were analyzed by high-performance liquid chromatography-fluorescence following derivitization with dansyl chloride, as described (CV 4.2% to 8.9%) (36).

Statistical Methods

A power calculation was performed using FMD responses of lean and obese individuals to calculate appropriate participant group sizes. Based on known decreases in