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Adverse effects of long-term weight gain on microvascular endothelial function

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ABSTRACT

Background: Endothelial dysfunction is the first stage of the atherosclerotic cascade, and independently associated with cardiovascular events. We evaluated the associations of longitudinal changes in weight, waist circumference, body fat percentage and lean mass index with changes in endothelial function.
Methods: 521 community-based subjects who belonged to hypertensive sibships and had no history of myocardial infarction or stroke had their anthropometric measures and endothelial function assessed a mean of 8.5 years apart. Endothelial function was assessed with brachial artery ultrasound, yielding measures of flow-mediated dilation and reactive hyperemia. We used multivariable linear regression with generalised estimating equations to assess the associations of longitudinal changes (Δ) in anthropometric measures with Δ flow-mediated dilation and reactive hyperemia, adjusting for potential confounders.
Results: Mean $\pm$ standard deviation age was 57.6 ± 8.7 years, 58% were women, and 72% were hypertensive. Most (84%) were overweight or obese at baseline. At end of follow-up, flow-mediated dilation and reactive hyperemia increased by 1.9 ± 7.6% and 51.2 ± 605.8% on average, respectively. In multivariable linear regression, changes in anthropometric measures were not associated with changes in flow-mediated dilation. However, Δ weight ($\beta \pm$ SE: -9.00 ± 2.35), Δ waist circumference (-6.78 ± 2.21) and Δ body fat percentage (-19.72 ± 5.62, $P < 0.0001$ for each) were inversely associated with Δ reactive hyperemia. Δ lean mass index was not associated with Δ reactive hyperemia.
Conclusions: Long-term increases in weight, waist circumference and body fat percentage are associated with progressive worsening of microvascular endothelial function, but not conduit vessel endothelial function, in subjects without a history of cardiovascular events, independently of risk factors.

Introduction

The social, economic, and human costs of cardiovascular (CV) disease continue to escalate. However, despite scientific advances in the past decades, the number of effective CV therapies and viable therapeutic targets remains limited. Importantly, once the atherosclerotic process commences, containing its progression is difficult, and reversing established structural abnormalities is improbable. For these reasons, to contain the health and economic burden of CV diseases, there is a critical need to enhance early detection of individuals at risk in order to enhance primary prevention strategies, arrest the pathogenic process, and improve health and outcomes.

With the goals of understanding CV pathogenesis and identifying targets for early detection and prevention of CV disease, scientists have often turned their attention to the vascular endothelium. The endothelium is a highly active structure capable of producing several substances that modulate vascular tone and reactivity, and inhibit cell proliferation and thrombogenesis [1]. Thus, it is essential for maintaining vascular health [1]; and it is now well established that adverse alterations in endothelial function represent the first abnormalities in the atherogenic cascade [2]. The role of the endothelium on CV diseases has been further corroborated by longitudinal clinical studies, which have highlighted the association endothelial dysfunction with future development of myocardial infarction and stroke independently of ageing and conventional CV risk factors [3]. Thus, measures of endothelial dys-

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function represent a viable target for early detection of individuals at higher CV risk, and for identifying environmental and heritable factors that may contribute to the initiation of the atherogenic cascade.

Obesity is a known risk factor for CV disease [4,5], and cross-sectionally associated with endothelial dysfunction [6]. Acutely, purposeful weight gain leads to impairment of endothelial function [7]. However, whether non-purposeful weight gain detrimentally affects endothelial function in free-living individuals remains unknown. This is the knowledge gap that we sought to address in the present study. We hypothesised that longitudinal increases in measures of total and central obesity would be associated with worsening of endothelial function. To this end, we studied a community-based cohort to evaluate whether changes in weight, waist circumference (WC), body fat percentage (BF%) and lean mass were associated with worsening in conduit artery and microvascular endothelial function over time.

Subjects, materials and methods

Study participants and baseline characteristics

The study was approved by the Mayo Clinic's Institutional Review Board and participants gave informed consent. The cohort consisted of 557 community-based non-Hispanic white participants from the Genetic Epidemiology Network of Arteriopathy (GENOA) study who had their endothelial function assessed on 2 separate occasions (baseline: between January 2003 and December 2008; follow up: between October 2009 and December 2011). The GENOA study is a cohort study of hypertensive sibships from Olmsted County, MN, USA. We excluded 36 participants with a history of myocardial infarction or stroke, leaving 521 participants for the present analyses.

On the day of each study visit, participants met with the study coordinator and completed a comprehensive questionnaire that included demographic and medical information. Methods for assessment of vital signs and baseline laboratory tests are described in the *Supplemental Methods*.

Anthropometric assessment

Height, weight and WC were obtained at baseline and during the follow-up visit. Height was measured with a stadiometer, weight was measured with an electronic scale, and WC was measured with a measuring tape at the level of the umbilicus. Participants were classified as being centrally obese if their WC was >102 cm in men and >88 cm in women [8]. Body mass index (BMI) was calculated as weight (in kg)/height (in m)^2. Patients were classified as having underweight, normal, overweight or obese BMI according to World Health Organization (WHO) criteria [8]. BF% was calculated using the formula: $(1.20 \times \text{BMI}) + (0.23 \times \text{Age}) - (10.8 \times \text{sex}) - 5.4$; where sex = 1 for men and 0 for women. This validated formula provides accurate measures of body composition, with prediction errors that are compatible to those obtained from direct measures of body fat such as skinfold thickness of bioelectrical impedance [9]. Lean mass index (LMI) was calculated as $\text{BMI} \times (1 - \text{BF\%})$ as previously described [10].

Assessment of endothelial function

Endothelial function was assessed non-invasively during the baseline and follow-up visits using brachial artery ultrasound, a technique that yields measures of conduit artery endothelial (flow-mediated dilation, FMD) and microvascular (reactive hyperemia, RH) endothelial function. Participants were asked to fast and refrain from smoking, ingesting caffeine, or taking anti-hypertensive medications for 12 h prior to the study visit. Brachial artery ultrasound was performed by trained technicians while participants were laying supine in a dark, quiet room with controlled temperature, according to published guidelines [11] and described in detail in the *Supplemental Methods*. FMD was calculated as $[(\text{mBAD} - \text{rBAD})/\text{rBAD}] \times 100$, where mBAD is the maximum brachial artery diameter during hyperemia following cuff deflation, and rBAD is the resting brachial artery diameter. RH was calculated as a $[(\text{dFlow} - \text{rFlow})/\text{rFlow}] \times 100$, where dFlow is hyperemic post-deflation brachial artery flow and rFlow is resting brachial artery flow.

Biomarkers

To further elucidate mechanisms underlying the link between weight and fat gain on endothelial function, we also measured circulating levels of adipocytokines (leptin, resistin and adiponectin) and inflammatory markers [C-reactive protein (CRP), homocysteine, intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), E-selectin, P-selectin, homocysteine, interleukin-6 (IL-6) and interleukin-18 (IL-18)] at baseline. Details about biomarker measurement, performance and quality control are summarised in the *Supplemental Methods*.

Statistical methods

Continuous variables were reported as mean ± standard deviation (SD) for normally distributed variables, and as median (interquartile range, IQR) for skewed variables. Nominal variables were reported as n and %. Longitudinal changes (Δ) in weight, BMI, WC, BF, LMI, FMD and RH were calculated by subtracting values obtained at the baseline visit from the values obtained at the follow-up visit.

We used multivariable linear regression models adjusted for sex, baseline age, blood pressure, renal function, lipids, baseline FMD or RH, follow-up time, history of hypertension, diabetes and smoking, and use of statins and anti-hypertensives to assess the associations of Δ weight, Δ BMI, Δ WC, Δ BF% and Δ LMI (independent variables, separate models for each anthropometric measure) with Δ FMD and Δ RH (dependent variables). Only covariates significantly associated with the dependent variable were included in the final models, but baseline age, sex and follow-up time were forced into all models. In all models, we tested interactions terms for changes in all anthropometric measures with age and sex; and for Δ BMI × Δ WC. To aid in interpretation, we also performed multivariable logistic regression models to predict FMD increase (Δ FMD < 0) and RH increase (Δ RH < 0).

To determine whether adipocytokines and inflammatory markers mediated associations of Δ anthropometric measures with worsening of endothelial function, we followed the principles outlined by Baron and Kenny [12] (see *Supplemental Methods*).

All regression analyses were performed using generalised estimating equations to account for the familial relatedness of the participants. All analyses were performed using SPSS vs. 22 (IBM Corp., Armonk, New York, U.S.A.), and a 2-tailed P-value ≤ 0.05 was considered statistically significant.

Results

Participant characteristics are summarised in Table 1. After a mean follow-up of 8.5 ± 1.0 years, 54% of participants gained weight, which was predominantly due to a gain in BF% despite a decline in LMI (Table 1). At the end of follow-up, 41% of participants experienced a decline in FMD (magnitude of decline: −4.8 ± 4.7%), and 46% experienced a decline in RH (magnitude of decline: −441.5 ± 382.7%); although the mean values for these

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