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MINI-FOCUS: ADIPOSE INFLAMMATION AND CARDIAC DISEASES

Reduced Adipose Tissue Inflammation Represents an Intermediate Cardiometabolic Phenotype in Obesity

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Objectives The purpose of this study was to determine whether obese individuals with reduced adipose tissue inflammation exhibit a more favorable cardiovascular risk profile.

Background Obesity is associated with a low-grade state of chronic inflammation that might be causally related to cardiometabolic disease.

Methods With immunohistochemistry, we categorized obese individuals dichotomously as having inflamed fat (n = 78) or noninflamed fat (n = 31) on the basis of the presence (+) or absence (−) of macrophage crown-like structures (CLS) in subcutaneous abdominal fat biopsy samples. We compared their metabolic, vascular, and adipose tissue characteristics with lean subjects (n = 17).

Results Inflamed CLS+ obese individuals displayed higher plasma insulin, homeostasis model assessment, triglycerides, glucose, blood pressure, high-sensitivity C-reactive protein, low-density lipoprotein cholesterol, lower high-density lipoprotein cholesterol, and brachial artery flow-mediated dilation compared with lean subjects ($p < 0.05$). Adipose messenger ribonucleic acid expression of inflammatory genes including CD68, leptin, matrix metalloproteinase-9, CD163, and CD8A were significantly greater and vascular endothelial growth factor was lower in the CLS+ group ($p < 0.05$). In contrast, obese subjects with noninflamed fat exhibited a mixed clinical phenotype with lower insulin resistance, reduced proatherogenic gene expression, and preserved vascular function as in lean subjects. In multiple linear regression adjusting for age and sex, CLS status (beta = −0.28, p = 0.008) and waist circumference (beta = −0.25, p = 0.03) were independent predictors of flow-mediated dilation.

Conclusions These findings lend support to the novel concept that factors in addition to absolute weight burden, such as qualitative features of adipose tissue, might be important determinants of cardiovascular disease. Therapeutic modulation of the adipose phenotype might represent a target for treatment in obesity. (J Am Coll Cardiol 2011;58:232–7) © 2011 by the American College of Cardiology Foundation

There is growing recognition that many overweight individuals maintain a relatively favorable cardiometabolic profile even in extreme obesity. Determinants of a metabolically healthier obese phenotype are poorly understood and likely multifactorial, relating to differences in body fat distribution, physical activity, and adipose metabolism (1). In addition, recent data suggest that inflammation of adipose tissue orchestrated by monocyte/macrophage infiltration and overproduction of proatherogenic cytokines might mediate metabolic and vascular disease in human obesity (2,3). Chronic activation of the immune system has been strongly implicated in the pathogenesis of obesity-associated disorders, including type 2 diabetes mellitus, cancer, and cardiovascular disease, and is a growing target of interest for therapeutic intervention (4).

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We and others have previously shown that proinflammatory changes in fat are linked to metabolic stress and endothelial dysfunction (2,5,6). The goals of the present study were to determine whether qualitative differences exist between the adipose tissue of obese versus normal

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weight individuals and to investigate whether overweight patients with reduced adipose inflammation are polarized toward a lean phenotype.

Methods

Study subjects. We enrolled overweight adult men and women (≥18 years of age) with a body mass index (BMI) of ≥25 kg/m^2 receiving care at the Boston Medical Center. We also recruited lean adults (BMI <25 kg/m^2) through advertisements to the general public. Subjects with unstable medical conditions or pregnancy were excluded. The study was approved by Boston Medical Center Institutional Review Board, and all subjects gave written informed consent. Blood pressure, heart rate, height, weight, BMI, and waist circumference (WC) were recorded for each subject, and all biochemical analyses were quantified from fasting blood samples.

Subcutaneous adipose tissue collection. From each subject, we collected abdominal subcutaneous adipose tissue via percutaneous needle biopsy technique or directly harvested during gastric bypass surgery, as previously described (2,7). All subjects were in a fasting state for ≥12 h before biopsy. Each subject provided a single biopsy specimen from the subcutaneous region for analysis.

Adipose tissue histology and real-time polymerase chain reaction. Macrophages in adipose tissue were identified with cell-specific stains targeted to CD68 (DakoCytomation, Carpinteria, California). Obese subjects were dichotomously categorized, on the basis of the presence (+) or absence (−) of macrophage crown-like structures (CLS), as CLS+ if any adipose tissue macrophage clusters were present in any examined field (inflamed fat) or CLS− if clusters were absent (non-inflamed fat) as previously validated (2,8,9). All lean subjects were CLS−. For messenger ribonucleic acid (mRNA) expression analyses, samples were homogenized with a MagNa Lyser tissue homogenizer (Roche Applied Science, Indianapolis, Indiana). Quantitative real-time polymerase chain reactions were performed with a high-throughput instrument (BioMark, Fluidigm, San Francisco, California) with Gene Expression Assays (Applied Biosystems, Foster City, California) and DynamicArray chips (Fluidigm, San Francisco, California) (10).

Vascular studies. For each subject, brachial artery ultrasound studies were performed in a fasting state. Brachial vasomotor responses were examined with a noninvasive, standardized method of ultrasound with a Toshiba Powervision 6000 system (Toshiba, Tokyo, Japan), as previously described (2,11). Flow-mediated dilation (FMD) after a 5-min cuff occlusion in an upper arm position and nitroglycerin-mediated dilation of the brachial artery served as measures of endothelium-dependent and -independent dilation, respectively. Sublingual nitroglycerin (0.4 mg) was omitted if contraindicated or the subject declined.

Statistical analysis. Analyses were completed with SAS for Windows (version 9.1, SAS Institute, Inc., Cary, North Carolina). Data are presented as mean ± SD, median with interquartile range, or proportions (%). Categorical group differences were examined with the chi-square test or Fisher exact test as appropriate. Kolgomorov-Smirnov tests, histograms, and normal probability plots were used to determine whether continuous variables were normally distributed or skewed. Natural log transformation was applied only to continuous variables not meeting normality, which specifically were glucose, homeostasis model assessment (HOMA), insulin, triglycerides, as well as adipose expression of interleukin (IL)-8, catalase, peroxiredoxin-1, guanylate cyclase I alpha/beta, IL-6, and IL-1beta. The latter 4 genes did not reach normality despite transformation and were analyzed by nonparametric methods. Group differences for continuous variables were examined with analysis of variance with Tukey post-hoc analysis, and the Kruskal-Wallis test was used for skewed variables. Univariate associations between vascular parameters or HOMA and clinical data were examined in obese subjects with Pearson's correlation. Alternatively, Spearman's rank correlation was used for skewed data. Multiple linear regression was used to determine whether CLS status was independently associated with FMD. Univariate clinical correlates of FMD with significance level of $p < 0.1$ were included in the model. For all analyses, p value <0.05 was considered statistically significant.

Abbreviations and Acronyms

- **BMI** = body mass index
- **CLS** = crown-like structures
- **FMD** = flow-mediated dilation
- **HbA1C** = glycosylated hemoglobin A1C
- **HOMA** = homeostasis model assessment
- **hs-CRP** = high-sensitivity C-reactive protein
- **IL** = interleukin
- **MMP** = matrix metalloproteinase
- **mRNA** = messenger ribonucleic acid
- **WC** = waist circumference

Results

Clinical and histological data. A total of 109 obese subjects (mean age 42 ± 11 years, 86% women) and 17 lean individuals (mean age 33 ± 12 years, 77% women) completed this study. The clinical characteristics of all participants are displayed in Table 1. The majority of obese individuals (72%) demonstrated evidence of adipose inflammation characterized by tissue presence of macrophage crown-like structures in subcutaneous fat (CLS+, n = 78) as shown in Figure 1, which were absent in 28% of overweight subjects (CLS−, n = 31). In contrast, all lean subjects were noninflamed (CLS−). As expected, the lean group had significantly lower BMI, WC, plasma insulin, HOMA, LDL-C, glycosylated hemoglobin A1C (HbA1c), triglycerides, glucose, hs-CRP, hypertension, diabetes prevalence, medication use, and higher HDL-C levels compared with the CLS+ obese group ($p < 0.05$). However, despite the same degree of adiposity, sex distribution, and age range