Altered Levels of Fatty Acids and Inflammatory and Metabolic Mediators in Epicardial Adipose Tissue in Patients With Systolic Heart Failure

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ABSTRACT

Background: Adipose tissue has endocrine properties, secreting a wide range of mediators into the circulation, including factors involved in cardiovascular disease. However, little is known about the potential role of adipose tissue in heart failure (HF), and the aim of this study was to investigate epicardial (EAT) and subcutaneous (SAT) adipose tissue in HF patients.

Methods and Results: Thirty patients with systolic HF and 30 patients with normal systolic function undergoing thoracic surgery were included in the study. Plasma was sampled and examined with the use of enzyme-linked immunosorbent assays, whereas SAT and EAT biopsies were collected and examined by means of reverse-transcription polymerase chain reaction and gas chromatography. Significantly higher expressions of mRNA encoding interleukin-6, adrenomedullin, peroxisome proliferator−activated receptor α, and fatty acid (FA)−binding protein 3, as well as higher levels of monounsaturated FA and palmitoleic acid, were seen in the EAT of HF patients, whereas the levels of docosahexaenoic acid were lower. Palmitoleic acid levels in EAT were correlated with 2 parameters of cardiac remodeling: increasing left ventricular end-diastolic diameter and N-terminal pro−B-type natriuretic peptide.

Conclusions: Our results demonstrate adipose tissue depot−specific alterations of synthesis of FA and inflammatory and metabolic mediators in systolic HF patients. EAT may be a source of increased circulatory and myocardial levels of these mediators through endocrine actions. (*J Cardiac Fail 2015;21:916−923*)

Key Words: Heart failure, adipose tissue, fatty acids, palmitoleic acid.

Adipose tissue is a multicellular tissue consisting of adipocytes, stromal vascular cells, and various immune cells with functions far beyond that of a mere depot of lipids. It produces a wide range of mediators, including factors known to be involved in development of cardiovascular disease (eg, adipokines, inflammatory cytokines, and growth factors).[1] The specific localization of adipose tissue is recognized as having distinct characteristics, and epicardial adipose tissue (EAT) is a visceral fat depot of growing interest in relation to myocardial disease. There is no separating fascia between EAT and the myocardium, and the 2 tissues share the same microcirculation.[2] Under pathologic circumstances, EAT can locally affect the heart through vasocrine (cell-to-cell communication by microcirculation)

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or paracrine secretion of mediators.[3] In addition, EAT thickness reflects visceral adiposity rather than general obesity, and EAT volume has been shown to correlate with the occurrence of metabolic syndrome and coronary artery disease (CAD).[4]

To this end, little is known about the role of adipose tissue in the development of heart failure (HF). Earlier reports demonstrated that obesity is an independent risk factor for development of HF.[5,6] However, it has also been shown that a high body mass index (BMI) is associated with lower mortality in patients with existing HF,[7,8] and a few clinical studies have demonstrated an inverse relationship between EAT volume and increasing HF.[9,10] Still, the biochemical properties of different adipose depots in HF and their relation to disease severity remain unknown.

We have previously demonstrated that patients with HF are characterized by a certain fatty acid (FA) phenotype in plasma, and this may support a link between disturbed FA composition and progression of HF.[11] In the present exploratory study, our aim was to investigate whether production of FA and various mediators (ie, adipokines, inflammatory cytokines, metabolic mediators, and vasoactive peptides) in EAT and subcutaneous adipose tissue (SAT) is altered in patients with systolic HF and whether these mediators are associated with parameters of cardiac function.

Methods

Patients' Characteristics

Sixty patients hospitalized for elective cardiac surgery were included in the study. Thirty patients had left ventricular (LV) systolic dysfunction with LV ejection fraction (LVEF) $<40\%$, whereas 30 patients had normal LVEF ($>50\%$) and thereby served as control subjects with no sign of systolic HF. The 2 groups were equally balanced regarding CAD (as demonstrated with the use of angiography). None of the patients had significant comorbidity, and none were taking immunosuppressive drugs. Hip circumference was measured around the widest portion of the hip, and waist circumference was measured at the level of the umbilicus at the end of expiration with the subjects supine.

This work was approved by the Regional Committee for Medical and Health Research Ethics in the Southeastern Norway Regional Health Authority (Helse Sør-Øst). The study was conducted according to the Declaration of Helsinki. Informed written consents for participation were received from all individuals.

Echocardiography

The echocardiographic investigations were performed with the use of Vivid 7 (GE Healthcare, Horten, Norway) and analyzed with the use of commercially available software (Echopac, GE). With the use of conventional 2-dimensional echocardiography, we assessed LVEF ad modum Simpson in the apical 4-chamber and 2-chamber views. LV dimensions were measured as the largest internal systolic and diastolic diameters with the use of M-mode tracings in the parasternal long-axis view at the level of tip of the papillary muscles. Diastolic function was evaluated by measuring the ratio of peak early (E) diastolic mitral valve inflow velocities and tissue Doppler imaging–derived early diastolic myocardial velocities (e′) measured at the mitral annulus (mean of septal and lateral measurements) as recommended by the European Society of Cardiology.[12]

Blood Sampling Protocol

Before heparinization and surgery, blood was collected from a central venous catheter in the right jugular vein and drawn into chilled glass tubes containing EDTA (1 mg/mL blood), placed on ice, and centrifuged within 20 minutes at 4°C (2000*g* for 20 minutes). The plasma was immediately stored at −80°C until use and thawed only once.

Collection of Epicardial and Subcutaneous Adipose Tissue

Anesthesia was induced with diazepam, fentanyl, and pancuronium and maintained with fentanyl, midazolam, isoflurane (Alpharma, Oslo, Norway), and nitrous oxide, and the patients were given 4 mg/kg heparin. In all of the patients, the operative approach was through a median sternotomy. After skin incision, but before sternotomy, SAT was harvested. Subsequent to sternotomy but before cardiopulmonary bypass, EAT was harvested from the anterior surface of the heart adjacent to the right coronary artery before SAT was again harvested. Samples were quickly rinsed in saline solution and visible nonadipose tissues removed, immediately frozen in liquid nitrogen, and stored at −80°C.

Analysis of Total Levels and Composition of FA

Measurement of total levels (esterified and free FA) and composition of FA was performed after extracting lipids from the adipose tissue with the use of a mixture of chloroform and methanol. Extracts were transesterified with the use of BF_3-methanol. To remove neutral sterols and nonsaponifiable material, extracts of fatty acyl methyl esters were heated in 0.5 mol/L KOH in ethanol-water solution (9:1). Recovered FAs were reesterified with the use of BF_3-methanol. The methyl esters were quantified with the use of gas chromatography as previously described.[13]

Isolation of Total RNA, Reverse Transcription, and Real-Time Polymerase Chain Reaction Analysis

Total RNA was extracted with the use of acid-buffered phenol (Trizol; Invitrogen, San Diego, California), DNase treated, and purified with the use of RNeasy minicolumns (Qiagen, Hilden, Germany). cDNA was prepared with the use of the High-Capacity cDNA Archive Kit (Applied Biosystems, Foster City, California). Primer sequences were designed with the use of Primer Express software version 3.0 (Applied Biosystems). Sequence information can be provided on request. Quantitative real-time polymerase chain reaction (PCR) analysis was performed with the use of an ABI Prism 7300 sequence detector (Applied Biosystems) and Sybr Green assays (qPCR Master Mix for Sybr Green I; Eurogentec, Seraing, Belgium). β-Actin mRNA was used for normalization of target mRNA.

Enzyme-Linked Immunosorbent Assay Analysis

Plasma levels of adiponectin and visfatin were measured by means of enzyme-linked immunosorbent assays obtained from R&D Systems (Minneapolis, Minnesota) and Phoenix Pharmaceuticals (Burlingame, California), respectively.