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Increased expression of the adipocytokine omentin in the epicardial adipose tissue of coronary artery disease patients

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ABSTRACT

Background and aims: Omentin, an adipocytokine secreted by visceral adipose tissue, protects against obesity-linked cardiovascular complications. However, little is known about its role in epicardial adipose tissue (EAT) and coronary artery disease (CAD). We investigated the expression of omentin in EAT from CAD subjects.

Methods: EAT, subcutaneous adipose tissue (SCAT), and plasma samples were collected from CAD (n = 15; 23.3 ± 3.1 kg/m^2) and non-CAD patients (n = 10; 20.8 ± 3.9 kg/m^2). Omentin mRNA expression was measured using real-time PCR, while plasma concentrations were measured using an ELISA. EAT volume was determined with 64-slice computed tomography.

Results: Omentin expression in EAT and EAT volume were higher in CAD patients compared with controls (2.49 ± 2.6 *vs.* 0.85 ± 0.3, $p = 0.002$ and 113 ± 58 ml *vs.* 92.4 ± 30 ml, $p = 0.045$, respectively). Omentin expression in SCAT was similar between CAD and control patients (1.37 ± 0.84 *vs.* 1.07 ± 0.55, $p = 0.267$). Plasma omentin levels were lower in CAD patients compared with controls (343 ± 158 ng/ml *vs.* 751 ± 579 ng/ml, $p = 0.025$), and were negatively associated with the expression of omentin in EAT, in patients with CAD ($\beta = -0.78$, $p = 0.049$). On the other hand, there was no association between omentin in EAT and clinical variables in patients with non-CAD.

Conclusions: Omentin expression increases in the EAT of non-obese CAD patients, despite a decrease in plasma levels, suggesting that omentin may play a role in the pathogenesis of CAD.

1. Introduction

Adipose tissue is recognized as an active endocrine organ system that secretes adipocytokines responsible for both local and systemic regulation of numerous metabolic and inflammatory processes [1]. The circulating concentrations of many adipocytokines have been found to be increased in overweight individuals with higher amounts of visceral fat [2]. In fact, the dysregulated secretion of adipocytokines appears to trigger obesity-associated chronic inflammation and contributes to the development of cardiovascular atherosclerosis [3]. This link has been suggested to be related primarily to the adipose tissue surrounding arteries, including perivascular adipose tissue, periadventitial adipose tissue, and epicardial adipose tissue (EAT). For example, perivascular adipose tissue has been shown to play a direct role in the pathogenesis of vascular disease acceleration [4], while inflammation in periadventitial adipose tissue induces various cytokines to infiltrate atherosclerosis lesions, which enhances neointima formation and plaque vulnerability [5,6]. Furthermore, EAT, the adipose tissue

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surrounding the heart, is deposited under the visceral layer of the pericardium and is thought to serve as a source of adipocytokines [7]. Given its close proximity, it is not surprising that excessive EAT is thought to be directly responsible for the inflammation of adjacent coronary arteries and appears to contribute to the pathogenesis of atherosclerosis [8–11].

Omentin, also known as intelectin-1, is a recently identified adipocytokine, whose expression is abundantly detected in human visceral adipose tissue [12,13]. Omentin is also detectable in human plasma and appears to be downregulated in obese subjects and those with type 2 diabetes [14,15]. Moreover, low levels of plasma omentin have been associated with insulin resistance and endothelial dysfunction [16,17]. Thus, it is conceivable that circulating omentin may also be associated with obesity-linked coronary artery disease (CAD).

Previous research indicates that omentin is predominantly found in EAT compared with internal mammary artery periadventitial and subcutaneous fat [18]. However, the functional significance of omentin expression in these tissues is not fully understood. Furthermore, the relationship between omentin expression in EAT and CAD is also largely unexplored. In the present study, we have investigated the expression of omentin in EAT and subcutaneous adipose tissue (SCAT) in CAD patients and compared them to non-CAD patients in order to determine any potential associations between omentin and CAD pathogenesis.

2. Materials and methods

2.1. Study population

Adipose samples and peripheral blood were obtained from 15 patients who underwent elective coronary artery bypass graft surgery (CAD group) and 10 patients who underwent elective surgery for aortic or mitral valve replacement (non-CAD group) at Chubu Rosai Hospital between November 2011 and January 2013. We excluded patients with acute coronary syndrome, acute decompensated heart failure, and hemodialysis. The protocol of this study was approved by the institutional review boards of the Chubu Rosai Hospital and written informed consent was obtained from each patient.

2.2. Adipose samples

Adipose samples were obtained after the opening the thorax, prior to heparinization and cardiopulmonary bypass. EAT biopsy samples were taken near the proximal left coronary artery at the base of the heart, and SCAT samples were taken from the subcutaneous fat on the sternum. Adipose samples were isolated from any attached connective tissue and superficial blood vessels, bisected, and stored separately at −80 °C.

2.3. Analysis of omentin gene expression in adipose tissue

The total mRNA was extracted from the EAT and SCAT samples using an RNA isolation kit (Qiagen, Valencia, CA, USA) according to the manufacture's protocol. We measured the expression of omentin mRNA using real-time PCR. Quantitative PCR analysis was performed with a BioRad real-time PCR detection system (TOYOBO, Osaka, Japan). Gene expression was normalized to that of glyceraldehyde 3-phosphate dehydrogenase.

2.4. Plasma analysis and measurement of plasma omentin levels

Peripheral venous blood samples were collected from each patient for chemical analysis after an overnight fast. Plasma omentin levels were determined with an omentin enzyme-linked immunosorbent assay (ELISA) kit (Bio Vendor, NC, USA). The intra-assay and inter-assay coefficients of variation of this kit were 4.1% and 4.8%, respectively.

2.5. CT image analysis of epicardial fat

The volume of EAT was determined on the basis of cardiac CT scanning by two experienced analysts. EAT volume was defined as the total amount of adipose tissue between the surface of the heart and the visceral layer of the pericardium. The methods of measurement of EAT volume have been described in our previous study [19]. Interobserver variability for the quantification of EAT volume was less than 5.0%.

2.6. SYNTAX score and Gensini scoring system

The SYNTAX score reflects a comprehensive anatomical assessment, with higher scores indicating more complex coronary disease [20]. To assess the severity of cardiac ischemia, we used the Gensini scoring system [21]. In this system, the coronary artery score equals the sum of all segment scores (where each segment score equals the segment weighting factor multiplied by the severity score).

2.7. Statistical analysis

Categorical and continuous variables are presented as the number (percentage) of patients, means ± SD and median with interquartile. Differences between mean values for two groups (CAD and non-CAD) were evaluated with the Student's unpaired *t*-test or the Mann-Whitney *U* test. Mann-Whitney test was performed in case of non-normal distribution, and Student's *t*-test was performed in case of normal distribution according to the Shapiro test. The potential correlation of omentin expression in adipose tissue with other clinical parameters was examined with Spearman's correlation coefficient by rank test. Multivariate regression analysis adjusted age and gender was performed to assess the relationship between omentin expression and clinical parameters. A p value less than 0.05 was considered statistically significant. All statistical analysis was performed with the use of StatView for Windows software (Abacus Concepts, Berkeley, CA).

3. Results

3.1. Clinical characteristics

The clinical characteristics of the patients included in this study are shown in Table 1. The mean age of CAD patients was significantly lower than that of non-CAD patients. 10 (66%) CAD patients and 7 (70%) non-CAD patients were male, but this difference was not significant. The average body mass index (BMI) was 23.3 ± 3.1 kg/m^2 for the CAD group and 20.8 ± 3.9 kg/m^2 for the non-CAD group. In terms of the number of coronary artery bypass grafts, 3 subjects received 4 bypass grafts, 5 received 3 bypass grafts, 6 received 2 bypass grafts, and 1 received 1 bypass graft.

The clinical data of the study population are shown in Table 2. Patients with CAD were found to have a higher volume of EAT and increased plasma low-density lipoprotein (LDL) cholesterol levels, but decreased plasma omentin levels compared to non-CAD patients. Furthermore, the increased EAT volume appeared to be related to the decreased plasma omentin levels, but this relationship was not found to be significant ($r = -0.30$, $p = 0.284$) (Supplementary Table 1).