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Thrombospondin 1 as a novel biological marker of obesity and metabolic syndrome

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ABSTRACT

Context. Thrombospondin 1 (THBS1 or TSP-1) is an adipose-derived matricellular protein, which has recently been highlighted as a potential mediator of insulin resistance and adipose inflammation in obesity.

Objective. In this study, we aimed to determine the clinical significance of THBS1 as a novel biological marker of visceral obesity, metabolic syndrome, and diabetes.

Methods. The *THBS1* mRNA level was quantified with real-time PCR in human adipose tissues obtained from 16 non-obese subjects. The relationships between serum THBS1 level and obesity/diabetes traits as well as the diagnostic components of metabolic syndrome were assessed in 164 normal-weight or overweight/obese subjects (78 males and 86 females; mean age, 50.4; mean BMI, 29.8) with analysis of covariance (ANCOVA) and regression analyses.

Results. *THBS1* was predominantly expressed in visceral adipose tissues relative to subcutaneous adipose tissues ($P < 0.001$). The visceral *THBS1* expression was positively associated with the body mass index (BMI; $\gamma_s = 0.54$, $P = 0.033$). ANCOVA demonstrated that the THBS1 level is associated with abdominal obesity ($P < 0.001$), hyperglycemia ($P = 0.02$), and hypertension ($P = 0.04$). Multivariable regression analysis suggested an association between serum THBS1 and fasting plasma glucose levels. The associations between serum THBS1 levels and obesity/diabetes traits were found preferentially in women (BMI, $\gamma_s = 0.30$, $P = 0.05$; FPG, $\gamma_s = 0.26$, $P = 0.016$). Subanalyses demonstrated that the association with obesity traits was

Abbreviations: THBS1, thrombospondin 1; BMI, body mass index; WC, waist circumference; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; IRI, immunoreactive insulin; HOMA-IR, homeostasis model assessment of insulin resistance; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, γ-glutamyl transpeptidase; hsCRP, high sensitivity C-reactive protein; FSH, follicle-stimulating hormone; ECM, extracellular matrix; TGF-β, transforming growth factor-β.

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predominantly found in premenopausal women (BMI, $\gamma_s = 0.41$, $P = 0.007$), whereas the association with diabetes traits was predominant in postmenopausal women (HbA1c, $\gamma_s = 0.38$, $P = 0.01$). During medical weight reduction treatment, the change in the serum THBS1 level was associated with the change in BMI and HbA1c in pre- and postmenopausal women, respectively.

Conclusions. Serum THBS1 is a useful biological marker of obesity and metabolic syndrome in Japanese subjects, particularly in women. THBS1 may act as a critical circulating factor that couples obesity with metabolic syndrome and diabetes in humans.

1. Introduction

Thrombospondin 1 (THBS1 or TSP-1) is a multifunctional glycoprotein released from various types of cell, including platelets, macrophages, and adipocytes [1–3]. The THBS1 precursor (MW = 145 kDa) is assembled into a disulfide-linked homotrimer and secreted into the extracellular milieu [4]. THBS1 exerts its diverse biological effects through binding to extracellular matrix (ECM) proteins and cell surface receptors, thereby regulating cell–cell and cell–matrix interactions [5]. THBS1 is also known to regulate the activation of transforming growth factor-β1 (TGF-β1) [6–8]. THBS1 interacts with a cohort of target molecules through its multiple functional domains and participates in a wide range of physiological and pathological processes such as tissue remodeling, wound healing, angiogenesis, and inflammation [6].

Recent findings suggest a causal role played by adipose-derived THBS1 in the pathogenesis of insulin resistance and adipose tissue inflammation [9–11]. In a mouse model, THBS1 was highly expressed in visceral adipose tissues, and the serum THBS1 protein level increased in response to high-fat diet challenge [10]. Targeted disruption of *Thbs1* in mice ameliorated diet-induced insulin resistance, adipose tissue inflammation, and muscle fibrosis [10]. In humans, adipose *THBS1* expression was increased in obese and insulin-resistant individuals [3,12].

We hypothesized that circulating THBS1 may serve as a novel biological marker of metabolic syndrome and adipose tissue inflammation associated with human obesity. While the link between adipose *THBS1* expression and obesity in humans has been demonstrated by others [3], the significance of serum THBS1 as a biological marker of human obesity, diabetes, and metabolic syndrome has not been fully examined to date. We determined the clinical significance of the serum THBS1 level in defining the complex phenotypes of human obesity, diabetes, and metabolic syndrome. Moreover, we assessed the fat depot-dependent expression of *THBS1* in Japanese subjects, whose body composition may differ from that of Caucasians and African-Americans [13,14].

2. Methods

2.1. Human Subjects

2.1.1. *THBS1 Gene Expression in Adipose Tissues*

Paired samples of visceral (omental) and subcutaneous adipose tissues were obtained from 16 patients (11 males and 5 females; mean age, 69.1 years; mean body mass index [BMI], 22.8 kg/m^2) who underwent abdominal surgery. Samples were frozen in liquid nitrogen immediately after resection and stored at −80 °C for RNA extraction. The study protocol was approved by the human research ethics committee of Kyoto Medical Center, and written informed consent forms were obtained from all participants.

2.1.2. *Correlation Analyses of THBS1 Levels in Circulation*

A total of 164 Japanese obese patients and non-obese volunteers (78 males and 86 females; mean age, 50.4 years; mean BMI, 29.8 kg/m^2) were consecutively enrolled at the National Hospital Organization Kyoto Medical Center. Blood samples were collected from the antecubital vein in the morning after a 12-h fast. The study protocol was approved by the human research ethics committee of Kyoto Medical Center and all participants agreed to the study by providing signed documents of informed consent.

2.2. Quantitative Real-Time PCR

Total RNA was isolated from adipose tissue samples with the RNeasy Lipid Tissue Mini Kit (QIAGEN), and reverse transcribed to cDNA using the High-Capacity RNA-to-cDNA Kit (Life Technologies). Gene expression was quantitated using the Power SYBR Green PCR Master Mix and ABI PRISM 7000 Sequence Detection System (Applied Biosystems). The respective gene expression is shown as the relative ratio to *36B4* (*Rplp0*) expression, which was used as an internal reference for normalization. The sequences of the primers were as follows: *36B4*, 5′-AGCCCAGAACACTGGTCTC-3′ and 5′-ACTCAGGATTTC AATGGTGCC-3′; *THBS1*, 5′-TCAGGACCCATCTATGATAAAACC TA-3′ and 5′-TCAGGTCAGAGAAGAACACCATTTC-3′ [3]; *IL-6*, 5′-AAATGCCAGCCTGCTGACGAAG-3′ and 5′-AACAACAATCTG AGGTGCCCATGCTAC-3′ [15].

2.3. Data Collection and Laboratory Assay Methods

The BMI, waist circumference (WC), systolic blood pressure (SBP), and diastolic blood pressure (DBP) were measured as described previously [16]. The fat distribution was measured with the dual bioelectrical impedance analysis (Dual-BIA) method (Omron Healthcare Corporation) [17]. Serum levels of fasting plasma glucose (FPG), hemoglobin A1c (HbA1c), immunoreactive insulin (IRI), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transpeptidase (γ-GTP), leptin, adiponectin, and high sensitivity C-reactive protein (hsCRP) were determined according to standard procedures [16]. The homeostasis model assessment of insulin resistance (HOMA-IR) was used as an index of insulin resistance [18]. The human THBS1 concentration in serum was determined with an enzyme-linked immunosorbent assay using