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# Interactions between adiponectin, visfatin, and omentin in subcutaneous and visceral adipose tissues and serum, and correlations with clinical and peripheral metabolic factors

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ABSTRACT

Adiponectin, visfatin, and omentin are adipokines involved in insulin sensitivity. This study aimed to determine interactions between these adipokines in subcutaneous and visceral fat and in serum, and their associations with clinical factors. Adiponectin was present at the highest levels in subcutaneous and visceral fat and serum. Subcutaneous adiponectin showed positive correlations with serum adiponectin and the quantitative insulin sensitivity check index (QUICKI). Serum adiponectin correlated positively with QUICKI and serum omentin-1 but negatively with body weight, BMI, and homeostasis model assessment of insulin resistance (HOMA-IR). Subcutaneous omentin correlated positively with QUICKI but negatively with waist and hip circumferences. Serum omentin-1 correlated positively with QUICKI but negatively with body weight, BMI, waist and hip circumferences, weight gain, and HOMA-IR. Serum visfatin correlated positively with serum omentin-1 and negatively with weight gain. Serum peptide YY (PYY) levels were correlated positively with subcutaneous visfatin but negatively with visceral visfatin. Positive correlations were observed between subcutaneous expression of adiponectin, visfatin, and omentin and visceral expression of these genes. Multiple linear regression analysis showed that serum adiponectin was associated with BMI and QUICKI. Serum omentin-1 could be predicted from BMI, QUICKI, and weight gain. Weight gain, serum adiponectin, omentin-1, and DBP could be used to predict serum visfatin. In conclusion, adiponectin and omentin from subcutaneous fat displayed correlations with decreased obesity and increased insulin sensitivity while visfatin showed an association with serum PYY and weight gain. The expressions of these adipokines were correlated within each type of fat but not between different fat depots.



## Introduction

Obesity, an excessive accumulation of adipose tissue, is one of the most serious global health problems with many adverse consequences including cardiovascular diseases, atherosclerosis, insulin resistance, and type 2 diabetes mellitus (type 2 DM) [3,5]. Adipose tissue is not only a site of energy storage [24], it is also a highly active and dynamic endocrine organ that synthesizes and secretes several adipose tissue hormones or adipokines including leptin, cytokines, adiponectin, complement components, plasminogen activator inhibitor-1, proteins of the renin-angiotensin system, and resistin [24]. Among these adipokines, adiponectin, visfatin, and omentin have previously been shown to be involved in obesity and insulin sensitivity or resistance [6,7,16–18,20,28,34,45]. This led us to study the expression of these adipokine genes, and their correlation with obesity status, levels of insulin sensitivity (quantitative insulin sensitivity check index – QUICKI), and levels of insulin resistance (homeostasis model assessment of insulin resistance – HOMA-IR) within one setting.

Adiponectin is a 30 kDa protein that was first described as adipocyte complement-related protein of 30 kDa (Acrp30) in 1995 [37] and then as a type of mRNA highly expressed in adipose tissue, termed apM1 (adipose most abundant gene transcript 1) in 1996 [29,31,37]. Serum adiponectin has previously been found to be reduced in obesity [17] and low serum concentrations are related to type 2 DM [20]. The expression of adiponectin from subcutaneous and visceral adipose tissues was found to be reduced in obese individuals compared with non-obese subjects [28]. However, another study found subcutaneous adiponectin expression was similar between obese and non-obese female subjects [27].

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Infusion of adiponectin into the blood stream has been shown to decrease blood glucose levels and increase insulin sensitivity [7,34]. In hepatocytes, adiponectin decreases gluconeogenesis [49] and it increases fatty acid oxidation in both skeletal muscle [47] and hepatocytes [43].

Another adipokine of interest, visfatin, was first discovered in 1994 and referred to as pre-B-cell colony-enhancing factor (PBEF) [35]. It was subsequently described as a nicotinamide phosphoribosyl transferase (Nampt) in 2001 [30] and was named visfatin in 2006, because it was found to be expressed mainly in visceral adipose tissue [18]. However, subsequent studies found similar expression of visfatin in subcutaneous and visceral adipose tissues [8] with one study finding higher expression in subcutaneous adipose tissues in both lean and obese groups [33]. Administration of recombinant visfatin was shown to decrease plasma glucose in a dose-dependent manner, both in insulin resistant and insulin deficient mice [18]. However, this finding has contradictory support; one study found that high visfatin levels in plasma were associated with type 2 DM [14] and another study claimed low visfatin levels were not related to insulin resistance [33]. Comparisons of serum levels of visfatin, as well as its expression in adipose tissue, between obese and non-obese individuals are still controversial [17,48]. Expression of subcutaneous visfatin [6] and serum visfatin [17] were found to be lower in obese individuals in some studies; however, other studies found higher visfatin serum levels [48] and similar gene expression between subcutaneous and visceral fat in obese compared with lean individuals [40]. Collectively, comparisons of blood levels of visfatin and its expression in adipose tissue in obese and non-obese people, as well as when comparing subcutaneous and visceral fat, have been inconclusive [6,40].

The third adipokine of interest, omentin, was first described as intelectin-1 in 2001 [41] and named "omentin" in 2004 because it was found to be highly expressed in omentum [45]. Visceral omentin mRNA expression was shown to be reduced in obesity [16], insulin resistance, and type 2 DM [10]. Serum omentin-1 levels were found to be significantly reduced in obese compared with lean individuals in one study [16] but were similar between these groups in another study [17]. In human subcutaneous and visceral adipocytes, omentin enhances insulin-stimulated glucose transport and Akt phosphorylation [45] suggesting that omentin may improve insulin sensitivity.

Regarding insulin sensitivity, a previous study has shown that fasting peptide YY (PYY) levels were positively associated with glucose infusion rate and negatively correlated with insulin resistance in humans [9]. Serum PYY levels were determined in the current study because of this association with insulin sensitivity. PYY is one of the gut peptide hormones that inhibits food intake and decreases body weight by binding to neuropeptide Y 2 receptors, which are the inhibitory receptors of NPY orexigenic signaling in the hypothalamus [11,22].

Collectively, adiponectin, visfatin, and omentin are three adipokines associated with insulin resistance; however comparisons of their gene expressions in subcutaneous and visceral adipose tissues, and of serum levels of these hormones between obese and non-obese individuals, have yielded inconclusive results. Moreover, interactions and comparisons among these three adipokines in terms of their expression and how this correlates with their blood levels, and with related clinical parameters, have not yet been determined in a single setting. The aims of this study were (1) to determine gene expression and serum levels of these three adipokines overall, and in obese and non-obese subjects, (2) to determine correlations of adiponectin, visfatin, omentin mRNA expression with their serum levels, with serum PYY, and with clinically relevant factors, and (3) to determine interactions between these adipokines in subcutaneous and visceral adipose tissues. Knowledge regarding the differential expression of these adipokines specific to each depot, or differences in obese and non-obese subjects and correlations between genes and blood levels of adipokines and other clinical parameters, may further reveal the roles of these hormones in regulating obesity and obesity-related conditions. The outcomes of this study will lead to new strategies in preventing or treating obesity by enhancing or reducing expression of key adipokine genes in specific adipose tissue depots.

## Materials and methods

### *Subjects*

The study protocol was approved by the Siriraj Institutional Review Board (Si.423/2013) of the Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand. Informed consent was given prior to the study by a total of 35 Thai female patients who underwent abdominal surgery. Appropriate BMI classifications for Asian people were applied [2]. The recruited subjects were classified as either lean ($n = 2$; body mass index (BMI) < 18.5 kg/m$^2$), normal weight ($n = 11$; BMI 18.5–22.9 kg/m$^2$), overweight ($n = 2$; BMI 23–24.9 kg/m$^2$), or obese ($n = 20$; BMI > 25 kg/m$^2$); individuals in the normal weight and lean groups were collectively identified as the non-obese group. Comparisons were made between obese and non-obese groups. Exclusion criteria for the study were subjects undergoing endocrine therapy (e.g., steroids, hormone replacement therapy, thyroxine), pregnancy, lactation, traumatic operation, malignancy diseases, operations related to endocrine diseases, severe abdominal inflammation, or menopause. In this study, only female subjects were recruited because most male patients who underwent open abdominal surgery were cancer patients or patients requiring emergency operations. For each female subject, the menstrual cycle phase could not be controlled because most of the patients underwent abdominal surgery for myoma uteri displaying irregular menstruation.

### *Demographic details and anthropometric measurements*

The patients were requested to provide demographic data including age, body weight, BMI, waist circumference (WC), hip circumference (HC), and waist to hip circumference ratio (WHR). WC was measured at the level of the umbilicus with silent breathing and HC was measured at the inter-trochanteric girth according to the WHO guideline [1] in standing position. WHR was obtained from WC divided by HC.

### *Tissue and blood collection*

All fasting blood samples were collected in the morning between 8.00 and 9.00 am before intravenous cannulation prior to the operation. Abdominal subcutaneous and visceral adipose tissues were collected (4–5 pieces of approximately 0.5 cm$^3$ in size) and immediately snap-frozen in liquid nitrogen and stored at −70 °C until the analysis was performed.

### *Analysis of adiponectin, visfatin, and omentin gene expression in adipose tissues*

RNA isolation was performed using TRIzol® reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Approximately 1 μg of RNA was reverse transcribed to complementary DNA (cDNA) using iScript cDNA Synthesis Kit (Bio-RAD, Hercules, CA, USA). Low density lipoprotein receptor-related protein 10 (*LRP10*) was used as a reference gene because it is the most stably expressed gene in human adipose tissue [19]. The primer sequences of adiponectin (*ADIPOQ*), visfatin (*NAMPT*), omentin (*ITLN1*), and *LRP10* for the real-time polymerase chain reaction