

## Visfatin levels in non-obese, obese, and insulin resistant adolescents

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### Abstract

**Background** Preterm infants are vulnerable to iron deficiency (ID) Background Adipose tissue is not merely a site for energy storage, but is also the largest endocrine organ, secreting various adipocytokines. Plasma visfatin, an adipocytokine predominantly secreted from visceral adipose tissue, has insulin-mimetic effects, and has been closely linked to insulin resistance.

**Objective** To compare plasma visfatin levels between obese and non-obese adolescents, as well as between obese adolescents with and without insulin resistance.

**Methods** This cross-sectional study was conducted in students who attended three senior high schools in Padang. Subjects comprised 28 obese and 28 non-obese adolescents. The age of the subjects ranged from 14-18 years. Obesity criteria were based on body mass index (BMI) measurements. Fasting serum glucose level was measured by glucose hexokinase photometry and serum insulin was measured by chemiluminescence immunoassay. Plasma visfatin was measured by enzyme-linked immunosorbent assay (ELISA). The insulin resistance index was estimated from fasting serum insulin and glucose levels using the homeostatic model assessment for insulin resistance (HOMA-IR). Differences in the variables were tested using independent T-test and Mann-Whitney test, depending on the distribution of the variables.

**Results** The mean plasma visfatin level was significantly higher in the obese than in the control group [2.55 (SD 1.54) vs. 1.61 (SD 0.64) ng/mL, respectively; (P=0.005)]. The insulin resistant group had significantly higher mean plasma visfatin level than the non-resistant group [3.61 (SD 1.59) vs. 1.96 (SD 1.18) ng/mL, respectively; (P=0.004)].

**Conclusion** Obese adolescents with insulin resistance have significantly higher plasma visfatin levels compared to those without insulin resistance. [Paediatr Indones. 2016;56:291-6. doi: 10.14238/pi56.5.2016.291-6].

**Keywords:** obese adolescents; insulin resistance; plasma visfatin

Visfatin was identified as an adipocytokine by Fukuhara *et al.* in 2005. Visfatin is predominantly expressed in visceral adipose tissue (VAT), compared to subcutaneous adipose tissue (SAT). This 55-kDa protein, consisting of 491 amino acids, was previously called pre- $\beta$  cell colony enhancing factor 1 (PBEF 1). Visfatin is secreted by macrophages (CD14+) in VAT induced by hypoxia.<sup>1,2</sup> Under hypoxic conditions, hydroxylase processes are rendered inactive. Consequently, hypoxia-inducible factor-1a (HIF-1a) is stabilized and moves into the nucleus where it binds to hypoxia response elements (HREs) within target genes and initiates the transcription of visfatin.<sup>3,4</sup> Visfatin binds directly to insulin receptors and exerts insulin-mimetic effects.<sup>5</sup>

Past studies have reported that serum visfatin levels are elevated in obesity.<sup>6-11</sup> However, some studies reported no differences in plasma visfatin levels between obese and non-obese people.<sup>12,13</sup> Visfatin levels were positively correlated to BMI, the ratio of waist and hip circumference, the incidence

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of dyslipidemia, insulin concentration, HOMA-IR scores, and metabolic syndrome.<sup>8-11,14</sup> Plasma visfatin levels decrease as insulin resistance is ameliorated and body weight is decreased.<sup>15</sup> In insulin resistant patients, increased plasma visfatin acts to compensate for their insulin resistance and hyperglycemia. This theory was supported by significantly elevated plasma visfatin in patients with type 2 diabetes. Furthermore, HOMA-IR and plasma visfatin levels were positively correlated, as observed with significantly decreased plasma visfatin after 3 months of controlled blood glucose.<sup>16,17</sup>

Indonesian studies on plasma visfatin have been limited. Dinisari *et al.* (2009) reported significantly elevated plasma visfatin in adults with central obesity compared with non-obese adults.<sup>18</sup> However, more visfatin studies in children and adolescents are needed. The aim of this study was to investigate the differences in plasma visfatin levels between obese and non-obese adolescents, and between obese adolescents with and without insulin resistance.

## Methods

This cross-sectional study was conducted on 28 obese and 28 non-obese adolescents, aged 15-18 years in Padang, from January to September 2015. Subjects were selected by consecutive sampling from obesity screening programs in three senior high schools in Padang. The minimum required sample size was estimated to be 25 for each group. Obesity was defined as BMI > 95<sup>th</sup> percentile, while non-obese was defined as BMI < 85<sup>th</sup> percentile, using the *World Health Organization-Centers for Disease Control* (WHO-CDC) 2000 growth reference standard.<sup>19</sup> The BMI was calculated by dividing weight in kilograms (kg) by height squared in meters (m<sup>2</sup>). Weight was measured by a Detecto scale with an accuracy of 0.1 kg. Height was measured by a microtoise with 0.1 cm accuracy. The control subject were non-obese senior high students who got permission from parents.

This study was approved by the Ethics Committee of Andalas University Medical Faculty, Padang. Subjects' parents provided informed consent. Blood specimens were collected (up to 6 cc) from the cubital vein after fasting for a minimum of 8 hours. Blood specimens were centrifuged (3000 rpm) at

room temperature for 15-20 minutes. The separated plasma was then stored at -20°C until the assays for fasting plasma glucose, insulin, and visfatin levels were performed. Plasma glucose was determined by glucose reagent kit using glucose hexokinase FS method (*ProLiNE*, Indonesia). Serum insulin was measured by chemiluminescence immunoassay (*IMMULITE 2000*, *Siemens Medical Solutions*, USA). Insulin resistance index was estimated using the following formula from the HOMA-IR: (HOMA) = fasting serum insulin x fasting serum glucose /22.5. Insulin resistance was defined as HOMA-IR level greater than 3.16.<sup>20</sup> Plasma visfatin was measured by sandwich enzyme-linked immunosorbent assay (ELISA) method, using a visfatin kit (*Adipogen*, *CH-1410 Liestal*, Switzerland). The standard error on calibration was 0.125-8 ng/mL, with a minimum limit of detection (sensitivity) of 30 pg/mL. The concentration was expressed in ng/mL.

Statistical analyses were performed with *SPSS 15* software. Demographic and clinical data are presented as means with SD, and medians with 25<sup>th</sup> percentile and 75<sup>th</sup> percentile. We used T-test and Mann-Whitney test to analyze the differences in plasma visfatin levels between obese and non-obese adolescents, and between the insulin resistant and non-insulin resistant groups. Results with P values < 0.05 were considered to be statistically significant.

## Results

An obesity screening of 1,200 students from three high schools led to identification of 135 obese students (11.25%) but only 28 obese students got parent permission and enrolled the study. The characteristics of the 28 obese and 28 non-obese adolescents are summarized in **Table 1**. The obese and non-obese groups had similar median ages. Mean insulin levels and HOMA-IR scores were significantly higher in the obese group than in the non-obese group (P<0.05). However, median blood glucose concentrations were not significantly different between the obese and non-obese groups (P>0.05). The mean plasma visfatin level was significantly higher in the obese than the non-obese group [2.55 (SD 1.54) ng/mL vs. 1.61 (SD 0.64) ng/mL, respectively; (P<0.05)].

The prevalence of insulin resistance in the obese subjects was 10/28 (35.71%). The mean BMI of the insulin resistant group was higher than that of the non-insulin resistant group, but the difference was not statistically significant [34.36 (SD 4.49) kg/m<sup>2</sup> vs. 31.31 (SD 3.39) kg/m<sup>2</sup>, respectively; (P>0.05)]. However, the mean plasma visfatin concentration was significantly higher in the insulin resistant group than the non-insulin resistant group [3.61 (SD 1.59) ng/mL vs. 1.96 (SD 1.18) ng/mL, respectively; (P<0.05)] (Table 2).

**Table 1.** Clinical and laboratory characteristics of the study population

Characteristics	Obese (n=28)	Non-obese (n=28)	P value
Gender, n			
Male	4	13	0.02*
Female	24		
Median age (range), years	16 (15-18)	16 (15-18)	0.392**
Mean BMI (SD), kg/m <sup>2</sup>	32.36 (4.00)	21.85 (2.49)	
Median plasma blood glucose (range), mg/dL	78 (69-99)	77.5 (68-99)	0.465**
Mean insulin level (SD), µIU/mL	12.73 (7.13)	5.65 (3.59)	0.001***
Mean HOMA-IR (SD)	2.63 (1.58)	1.06 (0.62)	0.001***
Mean plasma visfatin (SD), ng/mL	2.55 (1.54)	1.61 (0.64)	0.005***

\*Chi-square test; \*\* Mann-Whitney test; \*\*\* T-test

## Discussion

The prevalence of obesity in this study (11.25%) was higher than the reported 2013 national prevalence of 10.8% in 13-15-year-old adolescents (*Riset Kesehatan Dasar, Riskesdas*).<sup>21</sup> In addition, the obesity prevalence in our study was higher than that reported by Adhianto et al.<sup>21</sup> (11% in 552 children aged 11-17 years in Denpasar and Bandung), and Andriani<sup>23</sup> (10.35% in 13-15-year-old adolescents in Padang).

In our study, 10/28 obese adolescents (35.7%) had insulin resistance, based on HOMA-IR scores >3.16. Similarly, Pulungan et al.<sup>24</sup> in Jakarta found that the prevalence of insulin resistance in obese

adolescents aged 12-15 years was 38%, while Lestari<sup>25</sup> in Padang reported 31.6%. Similar prevalences were reported in other countries, including Bolivia (40%) in 2008 and Pakistan (35%) in 2011.<sup>26,27</sup> Ghergherechi et al. found that the prevalence of glucose tolerance disorders and insulin resistance in obese children and adolescent were 14.7% and 31.8%, respectively.<sup>28</sup> Insulin resistance is one component of metabolic syndrome.<sup>29</sup> Obese adolescents with insulin resistance have 4.1 times the risk of suffering from metabolic syndrome.<sup>30</sup> Furthermore, insulin resistance in

**Table 2.** Comparison of clinical and laboratory characteristics of the obese adolescent group

Variables	Insulin resistance (n=10)	Non-insulin resistant (n=18)	P value
Gender			
Male	2	2	0.452*
Female	8	16	
Median age (range), years	16.5 (15-18)	16 (15-18)	0.710**
Mean BMI (SD), kg/m <sup>2</sup>	34.36 (4.49)	31 (3.39)	0.061***
Median plasma blood glucose (range), mg/dL	85 (71-94)	77 (69-99)	0.064**
Mean insulin level (SD), µIU/mL	20.38 (5.6)	8.48 (3.27)	0.001***
Mean HOMA-IR (SD)	4.06 (3.35-7.10)	1.39 (0.74-2.83)	
Median plasma visfatin (SD), ng/mL	3.61 (1.5)	1.96 (1.18)	0.004***

\*Chi-square test; \*\* Mann-Whitney test; \*\*\* T-test

obese adolescents is a risk factor for hypertension, dyslipidemia, glucose intolerance, type 2 diabetes, and fatty liver. Insulin resistance has also been connected with systemic inflammation, endothelial dysfunction, and early formation of atherosclerotic plaque, all of which can increase the risk of cardiovascular disorders in the future.<sup>31,32</sup>

We found that the plasma visfatin level in the obese group was significantly higher than in the non-obese group. This result was in agreement with a Turkish study that reported plasma visfatin levels of 4.0 (SD 3.0) ng/mL in the obese group and 2.2 (SD 1.5) ng/mL in the non-obese group (P=0.014).<sup>9</sup> Similar results were also found by Araki et al. in Japan,

Mahaweerawar *et al.* in Thailand, and Tascular *et al.* in Turkey.<sup>8,10,11</sup> In addition, Korpicka *et al.* reported significantly different plasma visfatin levels in normal BMI, overweight, and obese adolescents ( $P=0.034$ ).<sup>33</sup> Plasma visfatin levels in obese children were found to be increased two-fold compared to children with normal BMI.<sup>12</sup>

Visfatin is produced by macrophages in fat tissue, especially visceral fat tissue. Visfatin gene expression was higher in visceral fat tissue than in subcutaneous fat, and positively correlated with plasma visfatin levels.<sup>1,34</sup> Plasma visfatin is a superior marker to both CT scan and MRI for predicting the extent of visceral fat tissue in obese children, as it is much less costly and does not expose children to radiation. Plasma visfatin concentration has a strong correlation with the area of visceral fat tissue ( $r=0.604$ ;  $P<0.01$ ), as a concentration of 20 ng/mL Visfatin is equivalent to visceral fat  $>100$  cm<sup>2</sup>, with sensitivity 88.9% and specificity 91.5%.<sup>8</sup>

In our obese subjects, mean plasma visfatin rate was significantly higher in those with insulin resistance than those without insulin resistance [3.61 (SD 1.59) ng/mL *vs.* 1.96 (SD 1.18) ng/mL, respectively; ( $P=0.004$ )]. Tascular *et al.* reported similar findings in Turkey, as well as a positive correlation between plasma visfatin concentration and HOMA-IR values.<sup>11,35</sup> Visfatin has insulin-mimetic effects. In normal conditions, visfatin binds the insulin receptor at a different site from insulin (non-competitive), inducing phosphorylation of insulin receptor substrates 1 and 2, (IRS1 and IRS2) and binding to phosphatidylinositol 3-kinase (PI3K). The IRS1 and IRS2 also induce the phosphorylation of protein kinase B (Akt) and mitogen-activated protein kinase (MAPK), resulting in activating the signal to increase glucose uptake. Normally, hyperglycemia stimulates visfatin secretion. Insulin resistance creates a state of chronic hyperglycemia due to decreased insulin receptor sensitivity. Hence, the secretion of visfatin is increased, however, the receptor was the same, so that visfatin increasing in plasma caused by decreased of insulin receptor sensitivity.<sup>1</sup>

A study in Taiwan found strong correlations between plasma visfatin concentration and the expression of macrophage CD68 and TNF-alpha genes, in fat tissue biopsies of obese adults. In visceral fat tissue, plasma visfatin is predominantly secreted

by macrophages. Increased plasma visfatin levels increase cytokine pro-inflammatory molecules (TNF-alpha, IL-6, and IL-1 $\beta$ ). As such, plasma visfatin secretion by macrophages in visceral fat tissue can be used as a marker for chronic inflammation in obesity. Insulin resistance in obesity is related to mild chronic inflammation of the fat tissue, with increased macrophage infiltration in adipocyte tissues. Positive correlations between plasma visfatin level and insulin resistance is mediated through increased adipocyte tissue inflammation.<sup>36</sup>

Plasma visfatin levels in adolescents with normal BMI vary according to the reagent kits used.<sup>37</sup> For our purposes, plasma visfatin in those with normal BMI was 0.1-1.5 ng/mL.

A limitation of this study was that obesity was measured by BMI, not by CT scan or MRI, which are more accurate for assessing body fat. Also, we did not assess lipid profiles in order to diagnose metabolic syndrome in our subjects.

We conclude that obese adolescents with insulin resistance have significantly higher plasma visfatin levels compared to those without insulin resistance. In obese adolescents, we suggest examining plasma visfatin levels to identify insulin resistance. Further clinical research is needed to identify an appropriate plasma visfatin cut-off point for identifying insulin resistance in obese children and adolescents.

## Conflict of Interest

None declared.

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