Serum Visfatin Concentration and Biochemistry Parameter Relationship of Obese Adolescents in Thailand.
การศึกษาหาระดับวิสฟาตินและตัวชี้วัดทางชีวเคมีที่สัมพันธ์กับวัยรุ่นอ้วนในประเทศไทย

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บทคัดย่อ

วิสฟาติน เป็น adipokine ที่ถูกค้นพบขึ้นใหม่ ซึ่งเป็นผลผลิตจากเนื้อเยื่อไขมันภายในช่องท้อง (abdomen) และมีส่วนร่วมในการสร้างเนื้อเยื่อไขมัน นอกจากนั้นยังมีคุณสมบัติเสมือนอินซูลิน วิสฟาตินจะถูกขับออกในพลาสมาระดับสูงมักจะคู่ขนานไปกันกับความอ้วน และบทบาทที่ยังไม่ทราบอย่างชัดเจนยังมีอีกมากในวัยรุ่น การวิจัยในครั้งนี้มีวัตถุประสงค์เพื่อหาระดับความเข้มข้นของวิสฟาตินในวัยรุ่นไทย (อายุ 15 - 18 ปี) และความสัมพันธ์ของวิสฟาตินกับตัวชี้วัดทางชีวเคมีอื่นๆ ในวัยรุ่นอ้วน วิธีการวิจัยในนักเรียน 77 คน ที่ยินยอมเป็นอาสาสมัคร และได้รับความเห็นชอบจากคณะกรรมการจริยธรรมในการวิจัยจากมหาวิทยาลัยมหาสารคาม ผลการศึกษาพบว่า ค่าเฉลี่ยของซีรัมวิสฟาติน เท่ากับ 52.91 นาโนกรัมต่อมิลลิลิตร ในวัยรุ่นอ้วน และ 23.80 นาโนกรัมต่อมิลลิลิตร ในกลุ่มปกติ ในการศึกษาจะเห็นว่าได้ว่า ระดับซีรัมวิสฟาตินมีระดับสูงขึ้นในวัยรุ่นอ้วน ซึ่งสังเกตพบว่าระดับซีรัมวิสฟาตินมีความสัมพันธ์เชิงบวกกับน้ำหนักตัว ($p<0.001$) และไตรกลีเซอไรด์ ($p<0.05$) ในวัยรุ่นอ้วน ในขณะเดียวกันระดับซีรัมวิสฟาตินในกลุ่มปกติมีความสัมพันธ์อย่างมีนัยสถิติในระดับต่ำกับปริมาณเนื้อเยื่อไขมันใต้ผิวหนังบริเวณต้นแขนส่วนหน้า ($p<0.05$) และค่าระดับในน้ำตาลในเลือด ($p<0.05$) สรุปการทดลองครั้งนี้พบความสัมพันธ์ของวิสฟาตินกับน้ำหนักตัวและซีรัมไตรกลีเซอไรด์ในวัยรุ่นอ้วน

Abstract

Visfatin is a recently discovered adipokine that is produced by the intra-abdominal adipose tissue which facilitates adipogenesis and has insulin-mimetic properties. The plasma levels of visfatin increase in parallel with obesity, but its role in adolescents remains largely unknown. This research aimed to determine serum visfatin concentration levels in Thai adolescents (15-18 years) and the relationship between visfatin

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concentration and biochemical parameters in obese adolescents. The research protocol and 77 consenting students who enrolled in this study were approved ethically by Mahasarakam research ethics committee. The mean serum visfatin level was 52.91 ng/ml in the obese adolescent while it was 23.80 ng/ml in the normal group. The data demonstrated that serum visfatin level was elevated in obesity, and positive correlations were observed between serum visfatin levels with body weight ($p<0.001$) and serum triglyceride ($p<0.05$) in obese adolescents. Even though the serum visfatin levels were slightly low in the normal group, there was still a statistically significant relationship with bicep skinfold thickness ($p<0.05$) and fasting blood sugar ($p<0.05$). In conclusion, these findings showed the relationship between visfatin levels and body weight and serum triglyceride in obese adolescents.

คำสำคัญ: วิสฟาติน, โรคอ้วน และเด็กวัยรุ่น
Keywords: visfatin, obesity and adolescents

Introduction

The prevalence of childhood obesity is increasing dramatically in developed countries (Jin et al., 2008), and obese children are at greater risk of becoming abnormally obese adults in the future. Hence research about childhood obesity is of paramount importance in preventing obesity-related mortality and morbidity in adults.

Nowadays, adipose tissue is no longer considered as an inert tissue mainly devoted to energy storage but is emerging as an active endocrine organ. It releases a variety of factors, so-called adipocytokines, i.e. leptin, resistin, adiponectin, acylation stimulating protein, visfatin, and others (Koerner et al., 2005, Maslowska et al., 2005, Fukuhara et al., 2005). The roles of adipocytokines have been investigated in obesity (Juge et al., 2005, Zahorska et al., 2000, Olszanecka et al., 2004). Excess adipose tissue is the most important risk in the development of insulin resistance and type 2 diabetes mellitus (T2DM) (Bloomgarden, 2002). The expansion of adipose tissue mass results from increased number and size of adipocytes (Bakker et al., 2004). Adipocyte number is determined by recruitment of preadipocytes to adipocytes as well as cell death. The differentiation process of preadipocytes into mature adipocytes presumably occurs throughout the human life span. In addition, subcutaneous abdominal preadipocyte differentiation in vitro is inversely correlated with central obesity (Permana et al., 2004). Both blunted differentiation potential of preadipocytes and hypertrophic adipocytes are associated with insulin resistance.

Visfatin, also known as pre-B cell colony-enhancing factor (PBEF), is an adipocytokine that is highly expressed in visceral fat and was originally isolated as a secreted factor that synergizes with interleukin (IL)-7 and stem cell factors to promote the growth of B cell precursors (Samal et al., 1994). The accumulation of adipose tissue is the result of, at least visfatin involved in the pathogenesis of overweight and obesity (Rafael, 2005). Visfatin directly binds to and stimulates the insulin receptor, exerting insulin-mimetic affects in vitro and in vivo. Moreover, visfatin is mainly secreted by visceral adipose tissues (Fukuhara et al., 2005). High level of plasma visfatin concentrations in morbidly obese subjects are reduced after weight loss (Haider et al., 2006). However, it has not been elucidated how visfatin modulates glucose and lipid metabolism.

The controlling mechanisms of visfatin secretion have not yet been characterized. Fukuhara et al. (2005) found that visfatin expression in visceral fat is increased in obese subjects, and the plasma concentrations of visfatin correlates much more strongly with the amount of visceral fat than that of subcutaneous adipose tissue (Fukuhara et al., 2005).

In the KKAy mouse, a model of obesity with T2DM, visfatin expression in visceral adipose tissue and plasma visfatin co ncentrations increases as obesity develops, while visfatin expression in subcutaneous fat and fat liver showed little change. In mice fed with a high-fat diet, visfatin expression in visceral mesenteric fat and plasma visfatin concentrations were higher than those in control animals.

A study between visfatin and metabolic parameters, such as obesity and insulin resistance in adolescents, has recently been undertaken. It was found that plasma visfatin was markedly elevated in obesity (Haider et al., 2006). A study of obesity found that fat tissue induces insulin resistance (Samal et al., 1994). The physiological range of serum visfatin levels has so far not been identified making interpretation of clinical studies difficult in which the serum visfatin level and the relationship between visfatin concentration and related biochemical parameters in obese and normal adolescents have not been investigated. Because adolescents are relatively free from co-morbidity compared with adults, we examined the role of serum visfatin levels as a marker of adolescent obesity or insulin resistance. This has not been studied in Thai adolescents to date.

The aims of the present study were to determine the serum concentration of visfatin and the possible relationship between biochemical parameters and visfatin in obese and normal adolescents.

Methodology

Subject

Forty-two obese and thirty-five normal Thai adolescents (age 15-18 years) living in Mahasarakham Thailand, participated in the study. All subjects in the study were diagnosed as simply slightly obesity, without diabetes mellitus, hypertension and hypothyroidism. The normal group consisted of healthy normal-weight controls recruited among adolescents referred by regular health check-up, with no history of obesity in childhood. All subjects and their parents gave written informed consent approved by the ethics committee of Mahasarakham University. The specimens and data collecting were done from November 1, 2008 to March 31, 2009. All subjects had no history of illness, and were interviewed as healthy 1 week before blood collection. This research protocol was approved by the ethics committee of Mahasarakham University.

Collection of serum

Blood samples were taken after 12 h fasting for analysis of fasting blood sugar, triglyceride, and serum visfatin. Blood samples were immediately analyzed for fasting blood sugar and triglyceride. Serum for visfatin analysis was divided into aliquots and stored at -80 ^{o}C until serum visfatin was assayed.

Anthropometry measurements

Anthropometric parameters including height, weight, left mid-arm circumferences, bicep, and tricep skin fold thickness were measured. The body mass index (BMI) was calculated as the body weight divided by height squared (kilograms per-square meter) and was used as an indicator of obesity.

Biochemical measurements and tests

Blood was collected after overnight fasting for analysis of blood sugar and triglyceride (Using routine laboratory proceeded by Mahasarakham hospital professional staff).

Serum visfatin

Radioimmunometric assay was used to determine serum visfatin levels. Visfatin was analyzed using a commercially available kit (Phoenix Peptides, Karlsruhe, Germany).

Statistical methods

The data were analyzed as normal distribution data and were expressed as mean ± standard deviation.

The relations between biochemical parameters, anthropometric indices and visfatin were analyzed by Pearson correlation coefficient (95% CI).

Results

Seventy-seven adolescents participated in this study, included 42 obese and 35 normal adolescents. The mean age of obese (16.97 ± 0.92 years) and normal adolescents (16.90 ± 0.76 years) was similar (Table 1).

As shown in Table 1, anthropometric indices and biochemical parameters included weight, height, BMI, bicep, tricep skinfold thickness, fasting blood sugar, triglyceride and visfatin. Serum visfatin levels were higher in obese (52.91 ± 19.74 ng/ml) than in the normal group (23.80 ± 15.10 ng/ml) ($p<0.001$). Fasting blood sugar levels were normal in both groups, as all subjects did not have T2DM (*as Thai medical DM council*; fasting blood sugar cut off point < 126 mg/dL).

Significantly positive correlations were found between serum visfatin and anthropometric indices and biochemical parameters values, highly significant with weight ($p<0.001$) and triglyceride ($p= 0.017$) in obese when compared to the normal group (Table 2), while serum visfatin in the normal group showed moderate statistical significance with bicep skinfold thickness ($p=0.046$) and fasting blood sugar ($p=0.034$). Obese adolescents had no statistically significant correlation ($p > 0.05$) and, consequently, a relatively low power for an association was found between serum visfatin levels and bicep, tricep skinfold thickness. However, there was a potential association between visfatin and obesity.

Discussion and Conclusion

The prevalence and magnitude of childhood obesity are increasing dramatically in the developing countries. Obesity is becoming an important public health problem in childhood and presents numerous problems. Similarly to the risks of obesity in adulthood, childhood obesity is also a leading cause of T2DM, and increases the risk of cardiovascular diseases (Wajchenberg, 2000, Jian et al., 2006). Excess adiposity is the most important, because adipose tissue produces visfatin. Studies on visfatin alterations in adolescents may be useful in understanding some complications of obesity. Hua et al. (2008) reported the correlation of visfatin serum levels with a variety of metabolic and clinical parameters related to the insulin resistance syndrome in obese adolescents.

In the present study, it has been demonstrated that serum visfatin level was elevated in obesity. There was moderate statistical significance between serum visfatin with bicep skinfold thickness and fasting blood sugar in the normal group. Visfatin expression in visceral fat is increased in obese subjects, therefore plasma concentrations of visfatin correlated much more strongly with the amount of visceral fat than that of subcutaneous adipose tissue (Fukuhara et al., 2005). Visfatin is predominantly secreted from visceral adipose tissue, and increased visceral fat is closely linked to insulin resistance in adults (Despres et al., 1989). In the KKAy mouse, a model of obesity with T2DM, visfatin expression in visceral adipose tissue and plasma visfatin concentrations increased as obesity developed, while visfatin expression in subcutaneous fat and liver fat showed little change. In mice fed with a high-fat diet, visfatin expression in visceral mesenteric fat and plasma visfatin concentrations were higher than those in control animals. Our results are in accordance with the report of Berndt et al. (2005) which showed that serum concentration of visfatin is increased in obesity. However, in contrast to the results obtained by Berndt et al. (2005) they did not show correlation between serum concentration of visfatin and BMI and percentage of body fat in both obese and lean groups. Barbara et al, (2007) studied serum concentration of visfatin in obese women, and they found that serum concentration of visfatin was significantly higher in obese women when compared to controls. Positive

correlations between serum concentrations of visfatin and insulin in the obese group were found. In the control group, they observed positive correlations between serum concentrations of visfatin and glucose. They suggested that the observed increase of visfatin in obesity may be a counter regulation preventing further glucose increase. Corresponding with Fukuhara et al. (2005) who reported that visfatin exerts insulin-mimetic effects that are dose-dependent and quantitatively similar to those of insulin in stimulating muscle and adipocyte glucose transport, and in inhibiting hepatocyte glucose production. Intravenous injection of recombinant visfatin in mice decreased plasma glucose in a dose-dependent fashion. In keeping with its insulin-mimetic effects, visfatin was as effective as insulin in reducing hyperglycemia in insulin-deficient diabetic mice. Visfatin also bound to and activated insulin receptor, causing receptor phosphorylation and the activation of downstream signaling molecules. However, visfatin and insulin did not compete for binding to the insulin receptor, indicating that the two proteins were recognized by different regions of the receptor.

Serum visfatin levels were positively correlated with weight and triglyceride in obese adolescents but were not directly correlated with the BMI, which is consistent with the findings of Haider et al. (2006). Hua et al. (2008) found that serum visfatin correlated with triglyceride. The recently discovered adipocytokine visfatin preferentially produced in visceral adipose tissue, can also be found in skeletal muscle, liver, bone marrow and lymphocytes, where it was initially identified as pre-B cell colony-enhancing factor (PBEF) (Sethi and Vidal, 2005).The accumulation of adipose tissue is the result of, at least visfatin involved in the pathogenesis of overweight and obesity (Rafael, 2005). Thus, obesity of adolescents is caused by an increased caloric intake and decreased physical activity, and the obesity is related with four factors e.g. caloric intake, cortisol, somatotropin (growth hormone) and physical activity. Bouhours et al. (2007) reported that obese children often display increased linear growth. Obesity is characterized by high serum growth hormone (GH)-binding protein and normal to high insulin-like growth factor -1 (IGF-1) levels. Obesity, as in tall stature, may suggest an increase in responsiveness to GH. Hence the increased linear growth in obese children can be partly explained by the increase in GH sensitivity. Hua et al. (2008) reported that fasting serum visfatin levels have significantly negative association with age, and are independent of gender and BMI in this obese adolescent population. Studies conducted in 3T3-L1 cells (Mouse embryonic fibroblast - adipose like cell line) showed that other hormones, such as tumor necrosis factor (TNF)-α, Interleukin-6 (IL-6), GH and dexamethasone may alter the expression of visfatin (Kralisch et al., 2005). They surmised that certain hormones which abnormally fluctuate with age in obese adolescents, such as androgen, estrogen, GH, etc., may influence the secretion of visfatin.

In conclusion, the results showed the relationship between serum visfatin and body weight and triglyceride in obese adolescents. Further studies on the physiological role of visfatin may lead into glucose metabolism since it has an insulin-mimetic effect.

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Table 1. Anthropometric indices and biochemical parameters values.

Parameter	Norma (n=35) mean + SD (range)	lobese (n=42) mean + SD (range)	p- value*
Age	16.97 + 0.97 (15.00 - 18.00)	16.90 + 0.76 (15.00 - 18.00)	0.729
Body weight (kg)	51.89 + 4.66 (44.30 - 61.60)	70.38 + 11.00 (53.90 - 104.50)	< 0.001**
Height (m)	1.58 + 0.04 (1.49 - 1.68)	1.59 + 0.06 (1.47 - 1.72)	0.224
BMI (kg/m2)	20.80 + 1.41 (17.97 - 22.96)	27.69 + 3.82 (23.02 - 36.84)	< 0.001**
Bicep skinfold thickness (mm)	13.82 + 5.08 (2.56 - 22.16)	20.10 + 7.21 (2.50 - 35.66)	< 0.001**
Tricep skinfold thickness (mm)	12.52 + 3.91 (5.93 - 24.00)	22.74 + 8.99 (6.66 - 39.33)	< 0.001**
FBS (mg/dL)	83.77 + 5.28 (73.00 - 94.00)	85.61 + 7.12 (72.00 - 101.00)	0.208
TG (mg/dL)	70.77 + 44.55 (33.00 - 289.00)	96.02 + 41.24 (24.00 - 205.00)	0.012**
Visfatin (ng/ml)	23.80 + 15.09 (0.07 - 42.17)	52.91 + 19.74 (30.56 - 112.58)	< 0.001**

FBS = Fasting blood sugar * *p-value for Independent t-test*
TG = Triglyceride ** *significant at the 0.05 level*

Table 2. Pearson's correlation coefficients between serum visfatin level and parameters.

Parameter	normal (n=35)		obese (n=42)	
	r	p-value	r	p-value
Body weight (kg)	-0.151	0.386	0.529	< 0.001**
Height (m)	0.036	0.839	0.256	0.102
Bicep skinfold thickness (mm)	0.339	0.046*	0.262	0.093
Tricep skinfold thickness (mm)	-0.166	0.340	0.258	0.099
FBS (mg/dL)	0.360	0.034*	0.018	0.912
TG (mg/dL)	0.170	0.329	0.366	0.017*

* *Pearson correlation coefficient is significant at the 0.05 level*
** *Pearson correlation coefficient is significant at the 0.01 level*