Journal of Taibah University Medical Sciences (2015) 10(3), 327–332

Taibah University

Journal of Taibah University Medical Sciences

www.sciencedirect.com

Original Article

Serum vaspin levels are associated with decreased insulin sensitivity in newly diagnosed type 2 diabetes mellitus in Bangladesh

Fatema Tasnim, MPhil [a], M Omar Faruque, PhD [b,*], Zahid Hassan, PhD [b] and Liaquat Ali, PhD [c]

[a] *Department of Biochemistry and Cell Biology, Bangladesh Institute of Research & Rehabilitation in Diabetes, Endocrine and Metabolic Disorders, Dhaka, Bangladesh*
[b] *Department of Physiology and Molecular Biology, Bangladesh University of Health Sciences, Dhaka, Bangladesh*
[c] *Department of Biochemistry and Cell Biology, Bangladesh University of Health Sciences, Dhaka, Bangladesh*

Received 16 August 2014; revised 5 February 2015; accepted 6 February 2015; Available online 28 March 2015

الملخص

أهداف البحث: تهدف هذه الدراسة إلى استكشاف العلاقة بين مستويات الفاسبين في الدم وحساسية الأنسولين وعوامل القياسات البشرية.

طرق البحث: أجريت الدراسة على ٦٥ حالة شخصت حديثا بالنوع الثاني من السكري، (العمر: ٤٢ ± ٨ سنوات، ومؤشر كتلة الجسم (٢٥.٣٣ ± ٣.٩ كجم/م^{٢}) و ٦٥ شخصا من الأصحاء بدون تاريخ عائلي لداء السكري (العمر: ٤٠ ± ٨ سنوات، ٢٣.٨ ± ٣.٩ كجم/ م^{٢}). وقد تم قياس مستوى السكر والدهون بالدم، كما تم قياس مستوى الأنسولين والفاسبين بالدم في حالة الصيام، ومن هذه العينة تم تقييم حساسية الأنسولين والقدرة على إفراز الأنسولين.

النتائج: لقد كانت المستويات المطلقة للأنسولين في الدم أعلى لدى مرضى السكري بالمقارنة مع الأشخاص الأصحاء (١٦.٠ ± ٧.٩ مقابل ١٠.٩ ± ٣.٣ على التوالي، عامل إحصائي = ٠.٠٠٠١). وكان متوسط حساسية الأنسولين لدى مرضى السكري أقل بكثير بالمقارنة بالأشخاص الأصحاء (٤٨.٠ ± ٣١ مقابل ٧٦ ± ٥٥، على التوالي، عامل إحصائي = ٠.٠٠١). كما كان متوسط القدرة على إفراز الأنسولين لدى مرضى السكري أقل تقريبا بالنصف من الأشخاص الأصحاء (٧١ ± ٤٠ مقابل ١٣١ ± ٤٦، على التوالي، عامل إحصائي = ٠.٠٠١). وكان مستوى الفاسبين لدى مرضى السكري أقل مقارنة بالأشخاص الأصحاء (٠.٦٢ ± ٠.٢٦ مقابل ٠.٨٣ ± ٠.٢٨ على التوالي، عامل إحصائي <٠.٠٠١). كما أظهر الفاسبين علاقة سلبية مع محيط الخصر (عامل إحصائي = ٠.٠٤٣) وعلاقة إيجابية مع حساسية الأنسولين (عامل إحصائي = ٠.٠٠٧) بين جميع المشاركين. تبين من الدراسات والحسابات الإحصائية أن علاقة الفاسبين بمرضى السكري هامة للغاية (عامل إحصائي = ٠.٠٠٨).

الاستنتاجات: إن مستوى الفاسبين بالدم يرتبط بشكل إيجابي مع حساسية الأنسولين ويرتبط سلبا مع السكر في الدم، ومؤشر كتلة الجسم ونسبة الخصر إلى الطول.

الكلمات المفتاحية: داء السكري من النوع الثاني؛ الفاسبين؛ الحساسية للأنسولين؛ القدرة على إفراز الأنسولين

Abstract

Objectives: The present study aimed to explore the relationship of circulating vaspin levels with insulin sensitivity and anthropometric factors.

Methods: This study was conducted with 65 newly diagnosed type 2 diabetes mellitus (T2DM) patients with age-matched 65 healthy controls. Serum glucose was measured using glucose-oxidase method, lipid profiles by enzymatic end-point methods, and fasting insulin and vaspin levels were assessed with ELISA techniques. Homeostasis model assessment for insulin sensitivity (HOMA%S) and insulin secretory capacity (HOMA%B) were estimated from the fasting glucose and insulin levels using HOMA-CIGMA software.

Results: Fasting serum insulin (μU/ml) was higher in the diabetic group than controls (16.0 ± 7.9 vs. 10.9 ±3.3, respectively, p =0.0001). The mean (±SD) HOMA%S of the diabetics was significantly lower than that of the

* Corresponding address: Department of Physiology and Molecular Biology, Bangladesh University of Health Sciences, 125/1, Darus Salam, Mirpur, Dhaka 1216, Bangladesh.
E-mail: faruqueomar@yahoo.com (M.O. Faruque)

Peer review under responsibility of Taibah University.

ELSEVIER Production and hosting by Elsevier

 http://dx.doi.org/10.1016/j.jtumed.2015.02.010

controls (48 ±31 vs. 76±55, respectively, p = 0.001). The HOMA%B of the T2DM group was nearly 50% of that of the controls (71± 40 vs. 131 ±46, respectively, p = 0.001). The T2DM group exhibited significantly lower serum vaspin (ng/ml) levels than the controls (0.62 ±0.26 vs. 0.83± 0.28, respectively, p =0.001). Vaspin levels were negatively correlated with waist circumference (r =0.17, p =0.043) and positively correlated with HOMA%S (r =0.243, p =0.007) among all of the participants. The association of serum vaspin with diabetes remained highly significant (p =0.008) in binary logistic regression analysis performed after adjusting for the effects of confounders.

Conclusions: Serum vaspin level is positively associated with insulin sensitivity and negatively correlated with serum glucose, BMI and waist-height ratio.

Keywords: HOMA%B; HOMA%S; Type 2 diabetes mellitus; Vaspin

Introduction

Adipose tissue is regarded as an endocrine organ and secretes a number of bioactive molecules, known as adipokines.[1–3] Adipokines have been reported to participate in various metabolic processes that include the regulation of appetite, insulin sensitivity, insulin secretion, energy expenditure, cardiovascular function, and inflammation.[2–6] Adipocyte and adipose tissue dysfunction contribute to the primary defects in obesity and might be linked to several health problems, including increases in the risks for insulin resistance, type 2 diabetes, fatty liver disease, hypertension, dyslipidaemia, atherosclerosis, dementia, and cancers.[3,4]

Vaspin is a member of the serine protease inhibitor family[7–9] and has been found to be expressed in the visceral adipose tissue of Otsuka Long-Evans Tokushima Fatty (OLETF) rats at the age at which obesity and insulin concentrations in the plasma reaches their peaks.[10] Serum vaspin levels have been found to be markedly reduced in parallel with age and the development of severe hyperglycaemia, and this process can be reversed by insulin or pioglitazone treatment.[10] In humans, vaspin expression has been observed in the adipose tissue,[11] stomach,[12] liver, and pancreas,[13] and vaspin expression has also been observed in the hypothalamic of db/db and C57BL/6 mice.[12] The administration of recombinant vaspin to obese mice improves glucose tolerance and increases insulin sensitivity, affects the expressions of candidate genes for insulin resistance[10] and acutely reduces food intake.[12] The exact mechanisms by which vaspin is linked to the deterioration of glucose homeostasis and insulin sensitivity are not yet clearly understood. Based on existing data about the actions of vaspin, it can be postulated that vaspin inhibits a protease that plays a role in the degradation of a hormone or molecule with direct or indirect glucose-lowering effects.[10,12]

Elevated serum vaspin levels have been found to be associated with obesity, impaired insulin sensitivity, and fitness level.[2,14,15] Circulating vaspin is significantly correlated with serum leptin concentration, which supports the notion that vaspin closely reflects body fat mass.[14] In overweight women with polycystic ovary syndrome and insulin resistance, metformin has been shown to decrease circulating vaspin in parallel with improvements in insulin sensitivity.[15] The effects of metformin on circulating vaspin have been confirmed in drug-naive patients with diabetes[2,16] and extended by the finding that improved glucose metabolism and insulin sensitivity are the strongest predictors of changes in serum vaspin concentrations. In contrast to these data, several studies have failed to identify any associations between circulating vaspin and insulin sensitivity[17–20] or the parameters of obesity and fat distribution.[19,20] In one study, serum vaspin concentration was found to be significantly correlated with fasting insulin, HOMA-IR, and the ratio of visceral to subcutaneous vaspin expression.[21] However, in a systematic comparison of BMI-, age-, and gender-matched insulin-sensitive and insulin-resistant healthy obese individuals found that the vaspin levels were indistinguishable between these groups,[22] which prompted the suggestion that the associations between circulating vaspin, fat distribution, and insulin sensitivity are more complex. Therefore, the findings regarding the relationships between circulating vaspin levels, insulin resistance and glucose metabolism are conflicting and warrant further elucidation. Based on this background, the present study aimed to investigate the circulating levels of the novel adipocytokine vaspin in newly diagnosed type 2 diabetic subjects with the goal of exploring the mechanisms that underlie insulin resistance and insulin secretory defects in diabetes.

Materials and Methods

This was an observational analytical study. Sixty-five newly diagnosed type 2 diabetic subjects (male 35, female 30) aged between 30 and 60 years were recruited along with an additional 65 healthy subjects (male 33, female 32) without family histories of diabetes. The subjects were considered to have T2DM based on the WHO guidelines.[23] Patients with severe illnesses and pregnant women were excluded from the study.

On a prescheduled morning, we requested that the subjects come to the laboratory after an overnight fast (8−10 h) so that we could acquire fasting blood samples. Informed written consent was obtained, and the subjects were then given 75 g glucose dissolved in 250 ml water. The blood was taken by venepuncture in the fasting condition and 2 h after the glucose load. Ten to fifteen minutes after collection, the blood samples were centrifuged for 10−15 min at 3000 rpm to obtain the sera and were maintained frozen at −30 °C until analysis.

Serum glucose was measured by the glucose-oxidase (GOD-PAP) method, serum lipid profiles were assessed with the enzymatic colorimetric method, and serum insulin

and vaspin were assessed with the enzyme-linked immunosorbent assay (ELISA) method (Linco Research Inc., 6 Research Park Drive, St. Charles, Missouri 63304, USA and Aviscera Bioscience, Inc., 2348 Walsh Ave., Suite C Santa Clara, CA 95051, USA, respectively). Fasting serum glucose levels <6 mmol/l and post-prandial (2 h after the 75 g glucose load) serum glucose levels <7.8 mmol/l were considered to be within the normal ranges. Regarding the serum cholesterol and TG, levels <200 mg/dl and <150 mg/dl, respectively, were considered to be within the normal ranges. Insulin secretory capacity (HOMA%B) and insulin sensitivity (HOMA%S) were estimated from the fasting serum glucose and fasting insulin concentrations using the HOMA-CIGMA software.[24]

Statistical analyses

The statistical analyses were performed using the Statistical Package for Social Science (SPSS) software version 11.5 (SPSS Inc., Chicago, Illinois, USA) for Windows. The data are expressed as the mean ±SD (standard deviation). The differences between the groups were calculated using Student's t-tests, and $p < 0.05$ was considered statistically significant.

Results

Clinical and biochemical characteristics of the study subjects

The mean (±SD) ages of the control (40 ± 6) and T2DM (42 ± 8) subjects were not significant different. The mean (±SD) BMIs of the control (23.8 ± 3.9) and T2DM (25.3 ± 3.9) subjects were significantly different. The mean (±SD) waist circumferences of the control (91.5 ± 6.9) and T2DM (95.0 ± 9.8) subjects were also significantly different. The mean (±SD) waist−hip ratios (WHR) of the control and diabetic subjects were 0.92 ± 0.04 and 0.93 ± 0.03, respectively, and this difference was not significant. The mean (±SD) waist-to-height ratio (WHtR) of the control subjects (0.60 ± 0.08) was significantly ($p = 0.0001$) greater than that of the T2DM subjects (0.55 ± 0.05). The mean (±SD) systolic and diastolic blood pressures of the control (115 ± 15 and 79 ± 8, respectively) and T2DM (116 ± 17 and 77 ± 9) subjects were not different (Table 1).

The serum lipid profiles of the control and diabetic subjects were similar, although the total cholesterol was significantly different between the groups. The mean fasting serum creatinine and GPT values of the control and diabetic subjects were within the normal ranges, but the diabetic subjects exhibited shown significantly higher values (Table 1).

The mean fasting serum insulin levels of the control (10.9 ± 3.3) and T2DM (16.0 ± 7.9) subjects were significantly ($p = 0.0001$) different. The insulin secretory capacity (HOMA%B) and insulin sensitivity (HOMA%S) were calculated using the fasting glucose and fasting insulin, and both of these values were found to be significantly decreased in the T2DM subjects compared to the Controls. The mean (±SD) fasting vaspin level of the control (0.83 ± 0.28) subjects was significantly higher than that of the T2DM subjects (0.62 ± 0.26) (Table 1).

Table 1: Clinical and biochemical measurements from the study subjects.

Variables	Control (n = 65)	T2 DM (n = 65)	*p*-Values
Age (yrs)	40 ± 6	42 ± 8	*0.23*
BMI (kg/m^2)	23.8 ± 3.9	25.2 ± 4.0	*0.04*
WC(cm)	91.5 ± 6.9	95.1 ± 9.8	*0.01*
WHR	0.92 ± 0.04	0.93 ± 0.03	*0.06*
WHtR	0.55 ± 0.05	0.60 ± 0.08	*0.0001*
SBP (mmHg)	115 ± 15	116 ± 17	*0.8*
DBP (mmHg)	79 ± 8	77 ± 9	*0.17*
Fasting glucose	5.1 ± 0.4	8.9 ± 3.2	*0.001*
Glucose2hAG	5.6 ± 0.7	16.4 ± 5.3	*0.001*
Fasting Insulin (μU/ml)	10.9 ± 3.3	16.0 ± 7.9	*0.0001*
TG (mg/dl)	167 ± 102	188 ± 84	1.29/0.19
T Cholesterol (mg/dl)	192 ± 25	203 ± 27	2.35/0.02
HDL-c (mg/dl)	36 ± 9.8	38 ± 10.3	0.09/0.36
LDL-c (mg/dl)	122 ± 27	124 ± 26	0.40/0.68
HOMA%B	131 ± 46	71 ± 40	*0.0001*
HOMA%S	76 ± 55	48 ± 31	*0.001*
Serum vaspin (ng/ml)	0.83 ± 0.28	0.62 ± 0.26	*0.001*

BMI, body mass index; WC, waist circumference; WHR, waist−hip ratio; WHtR, waist−height ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure. The *p*-values were calculated with Student's t-tests, and $p < 0.05$ was considered statistically significant.

When the serum vaspin levels were analyzed according to BMI, the subjects with BMIs >27 exhibited significantly lower vaspin levels compared to their counterparts with BMIs ≤27 (Figure 1). Correlation analyses revealed that serum vaspin level was negatively correlated with waist−height ratios and serum glucose and positively correlated with diastolic blood pressure and insulin sensitivity (Figure 2).

Discussion

For the first time, this article reports on the measurements of fasting serum vaspin in a group of Bangladeshi subjects consisting of healthy controls and newly diagnosed T2DM subjects. The mean (±SD) fasting vaspin level in the control subjects was 0.83 ± 0.28 ng/ml (no difference between the males and females was observed) which was approximately half of that which has been observed in healthy subjects of Caucasoid origin.[25] However, the vaspin level of Bangladeshi

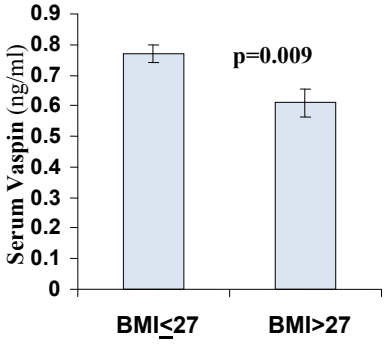

Figure 1: Serum vaspin statuses of the study subjects according to BMI.

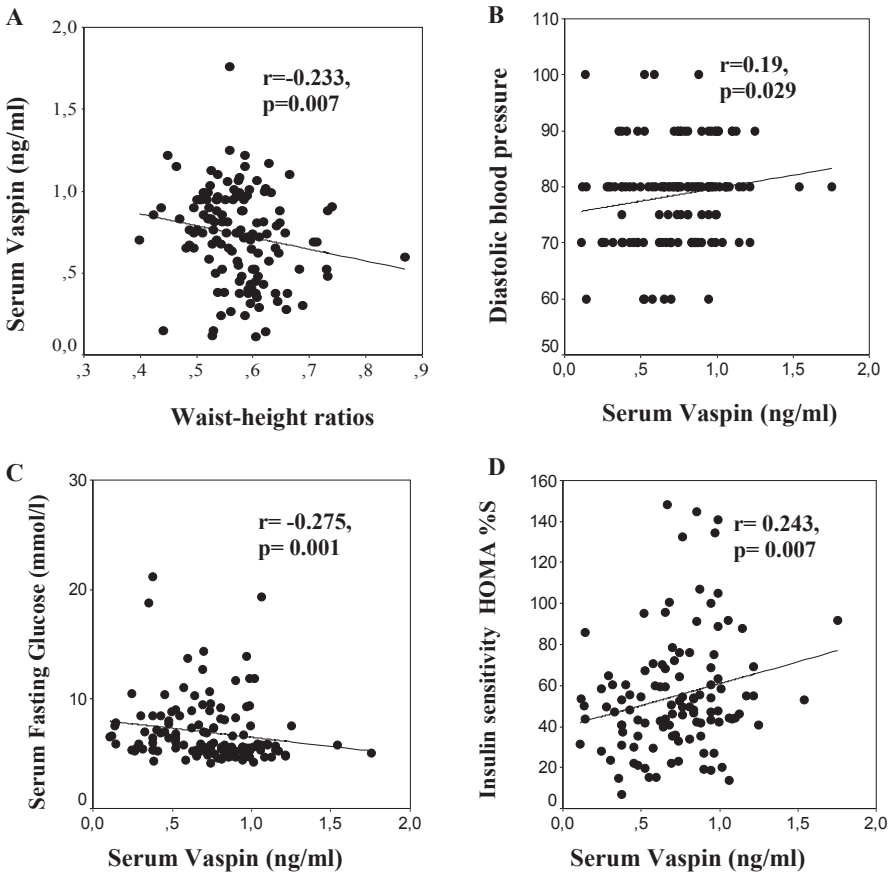

Figure 2: Correlations of serum vaspin with waist-to-height ratio (A), diastolic blood pressure (B), fasting serum glucose (C) and insulin sensitivity (D).

subjects is much higher than that of healthy Turkish women[2] (0.18 ± 0.10), and this value was found to be 0.69 ± 0.31 ng/ml in a group of healthy Chinese[26] subjects, which is higher than that of the Turkish subjects but lower than that of the subjects of the present study. These findings are consistent with the existing notion that vaspin level varies considerably across different races.

The present study demonstrated that the serum vaspin level of the newly diagnosed T2DM subjects (0.62 ± 0.26 ng/ml) was significantly (p = 0.00l) lower than that of the Controls (0.83 ± 0.28 ng/ml). In a previous study, the serum vaspin levels of newly diagnosed T2DM subjects were found to be similar to those of the present study and were not significantly different from those of the controls.[26] This same study demonstrated that previously diagnosed diabetic subjects exhibit significantly lower vaspin levels compared to controls and newly diagnosed T2DM counterparts.[26] These findings are consistent with data that have been obtained in an Egyptian population.[27]

One study found that diabetic subjects with microvascular complications exhibit serum vaspin levels that are significantly lower than those of their counterparts without complications.[28] Microvascular complications are understood to result from prolonged uncontrolled hyperglycaemia; therefore, this study indicated that the worsening of diabetes is attributable to the lack of insulin and reflected by decreased serum vaspin levels.[28] In the present study, although the diabetic subjects were newly diagnosed and presented with higher mean (±SD) blood glucose levels, they exhibited significantly higher serum insulin values. The significantly lower vaspin levels in these subjects might support the hypothesis that this molecule increases insulin sensitivity by as yet unidentified mechanism(s). This view is supported by the observations that Long-Evans Tokushima fatty rats exhibit decreased vaspin expression with worsening glucose control and that the administration of recombinant human vaspin improves glucose tolerance and insulin sensitivity.[10] In the present study, the serum vaspin levels of those with BMIs ≥27 kg/m^2 were found to be higher than those of their counterparts with BMIs <27 kg/m^2, which further strengthens the view that obesity and/or insulin resistance in T2DM subjects might possibly be due to lower serum vaspin levels.

Insulin sensitivity as assessed based on HOMA%S and secretory capacity as assessed based on HOMA%B were lower in the T2DM subjects in the present study, and these findings are consistent with those of our previous reports that insulin sensitivity and secretory capacity are predominantly impaired in T2DM patients of Bangladeshi origin.[29,30] HOMA-IR has also been reported to be associated with serum vaspin in diabetics with relatively longer disease durations and the obese, including obese children.[2,15,31] In the present study, serum vaspin exhibited significant negative correlations with waist–height ratio and fasting glucose and a positive correlation with insulin sensitivity (HOMA%S) when the T2DM and control subjects were considered together (Figure 2). Among

the diabetic subjects, the circulating vaspin level exhibited significant negative associations with BMI and hip circumference, and these findings support the notions that vaspin, *via* a yet to be identified mechanism(s), increases insulin sensitivity, and low vaspin levels are implicated in the development of insulin resistance and obesity leading to diabetes. The above findings are supported by a number of studies.[2,14] Glucelic et al, 2009 demonstrated a correlation between circulating vaspin level and BMI[2] that was corroborated in the present study. Sportsmen have been found to have significantly lower serum vaspin levels (0.28 ± 0.07 ng/ml) than lean normal glucose-tolerant control subjects (0.77 ± 0.42 ng/ml), and the authors who reported this finding attributed this difference to lower BMIs.[14] The authors postulated that the sportsmen were much leaner than the normal glucose-tolerant subjects. It is understood that the tissues, particular the skeletal muscles, of subjects who are accustomed to heavy exercise take up glucose independently of insulin, which might explain the lower level of vaspin in these subjects. However, controversies still remain regarding the relationships between serum vaspin level and markers of obesity. It has been observed that morbidly obese women exhibit no relationship between BMI and serum vaspin.[28] This lack of a relationship between vaspin and BMI might be attributable to racial and gender differences or some other unexplored mechanism(s) that differentially modulates the obesity–adipokine correlation in morbidly obese women.

Thus, the findings of the present study are supported by studies of different population and indicate that diabetes patients with longer durations of disease exhibit lower levels of vaspin. Poor glucose control and insulin sensitivity/resistance might influence vaspin levels independently of the worsening of diabetes. Vaspin can act as a compensatory molecule in metabolic disorders; the administration of recombinant human vaspin improves insulin sensitivity and glucose tolerance and reverses the expression of genes in diet-induced obese mice. Vaspin has been shown to remarkably decrease with the worsening of diabetes in diabetic rats, and pioglitazone and insulin treatments were found to normalized vaspin gene expression in this animal study.[10] Moreover, vaspin levels are decreased in metformin-treated patients with polycystic ovary syndrome and in female T2DM female patients.[2,15] Therefore, the roles and significance of vaspin in diabetes and other metabolic disorders have become subjects of great interest.

In conclusion, the serum vaspin levels of newly diagnosed type 2 diabetes subjects are significantly reduced, positively associated with insulin sensitivity and negatively associated with serum glucose, BMI and waist-to-height ratio. Further studies are required to explore the mechanism(s) by which vaspin potentially modulates insulin action, and the understanding of this mechanism might explain the pathophysiological basis of reduced vaspin levels in states of obesity, insulin-resistance and long-duration diabetes state and potentially identify promising novel treatments for metabolic conditions.

Conflict of interest

The authors have no conflict of interest to declare for the publication of this manuscript.

Acknowledgments

Authors are grateful to the authority of Bangladesh University of Health Sciences (BUHS), Dhaka for the financial support and laboratory facility needed for the study and also acknowledged BIRDEM general Hospital for the space to recruit the subjects.

References

1. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. **J Clin Endocrinol Metab 2004**; 89: 2548–2556.
2. Gulcelik NE, Karakaya J, Gedik A, Usman A, Gurlek A. Serum vaspin levels in type 2 diabetic women in relation to microvascular complications. **Eur J Endocrinol 2009**; 160: 65–70.
3. Bluher M. Adipose tissue dysfunction in obesity. **Exp Clin Endocrinol Diabetes 2009**; 117: 241–250.
4. Bays HE. "Sick fat," metabolic disease, and atherosclerosis. **Am J Med 2009**; 122: S26–S37.
5. Kralisch S, Bluher M, Paschke R, Stumvoll M, Fasshauer M. Adipokines and adipocyte targets in the future management of obesity and the metabolic syndrome. **Mini Rev Med Chem 2007**; 7: 39–45.
6. Van Gaal LF, Mertens IL, DeBlock CE. Mechanisms linking obesity with cardiovascular disease. **Nature 2006**; 44: 875–880.
7. Gettins PG. Serpin structure, mechanism, and function. **Chem Rev 2002**; 102: 4751–4804.
8. Law RH, Zhang Q, MacGowan S, Buckle AM, Silverman GA, Wong W, Rosado CJ, Langendorf CG, Pike RNBird PI, Whisstock JC. An overview of the serpin superfamily. **Genome Biol 2006**; 7: 216.
9. Silverman GA, Bird PI, Carrell RW, Church FC, Coughlin PB, Gettins PG, Irving JA, Lomas DA, Luke CJ, Moyer RW, Pemberton PA, Remold-O'Donnell E, Salvesen GS, Travis J, Whisstock JC. The serpins are an expanding superfamily of structurally similar but functionally diverse proteins. Evolution, mechanism of inhibition, novel functions, and a revised nomenclature. **J Biol Chem 2001**; 276: 33293–33296.
10. Hida K, Wada J, Eguchi J, Zhang H, Baba M, Seida A, Hashimoto I, Okada T, Yasuhara A, Nakatsuka A, Shikata K, Hourai S, Futami J, Watanabe E, Matsuki Y, Hiramatsu R, Akagi S, Makino H, Kanwar YS. Visceral adipose tissue-derived serine protease inhibitor: a unique insulin-sensitizing adipocytokine in obesity. **Proc Natl Acad Sci U S A 2005**; 102: 10610–10615.
11. Kloting N, Berndt J, Kralisch S, Kovacs P, Fasshauer M, Schon MR, Stumvoll M, Bluher M. Vaspin gene expression in human adipose tissue: association with obesity and type 2 diabetes. **Biochem Biophys Res Commun 2006**; 339: 430–436.
12. Kloting N, Kovacs P, Kern M, Heiker JT, Fasshauer M, Schon MR, Stumvoll M, Beck-Sickinger AG, Bluher M. Central vaspin administration acutely reduces food intake and has sustained blood glucose-lowering effects. **Diabetologia 2011**; 54: 1819–1823 (2011).
13. Korner A, Neef M, Friebe D, Erbs S, Kratzsch J, Dittrich K, Bluher S, Kapellen TM, Kovacs P, Stumvoll M, Bluher M, Kiess W. Vaspin is related to gender, puberty and deteriorating insulin sensitivity in children. **Int J Obes (Lond) 2011**; 35: 578–586.
14. Youn BS, Kloting N, Kratzsch J, Lee N, Park JW, Song ES, Ruschke K, Oberbach A, Fasshauer M, Stumvoll M, Bluher M. Serum vaspin concentrations in human obesity and type 2 diabetes. **Diabetes 2008**; 57: 372–377.
15. Tan BL, Heutling D, Chen J, Farhatullah S, Adya R, Keay SD, Kennedy CR, Lehnert H, Randeva HS. Metformin decreases the adipokine vaspin in overweight women with polycystic

ovary syndrome concomitant with improvement in insulin sensitivity and a decrease in insulin resistance. **Diabetes 2008**; 57: 1501–1507.
16. Kadoglou NP, Kapelouzou A, Tsanikidis H, Vitta I, Liapis CD, Sailer N. Effects of rosiglitazone/metformin fixed-dose combination therapy and metformin monotherapy on serum vaspin, adiponectin and IL-6 levels in drug-naïve patients with type 2 diabetes. **Exp Clin Endocrinol Diabetes 2011**; 119: 63–68.
17. von Loeffelholz C, Mohlig M, Arafat AM, Isken F, Spranger J, Mai K, Randeva HS, Pfeiffer AF, Weickert MO. Circulating vaspin is unrelated to insulin sensitivity in a cohort of nondiabetic humans. **Eur J Endocrinol 2010**; 162: 507–513.
18. Seeger J, Ziegelmeier M, Bachmann A, Lössner U, Kratzsch J, Bluher M, Stumvoll M, Fasshauer M. Serum levels of the adipokine vaspin in relation to metabolic and renal parameters. **J Clin Endocrinol Metab 2008**; 93: 247–251.
19. Akbarzadeh S, Nabipour I, Jafari SM, Movahed A, Motamed N, Assadi M, Hajian N. Serum visfatin and vaspin levels in normoglycemic first-degree relatives of Iranian patients with type 2 diabetes mellitus. **Diabetes Res Clin Pract 2012**; 95: 132–138.
20. Cinar N, Gulcelik NE, Aydin K, Akın S, Usman A, Gurlek A. Serum vaspin levels in hypothyroid patients. **Eur J Endocrinol 2011**; 165: 563–569.
21. Lee JA, Park HS, Song YS, Jang YJ, Kim JH, Lee YJ, Heo YS. Relationship between vaspin gene expression and abdominal fat distribution of Korean women. **Endocr J 2011**; 8: 639–646.
22. Kloting N, Fasshauer M, Dietrich A, Kovacs P, Schon MR, Kern M, Stumvoll M, Bluher M. Insulin-sensitive obesity. **Am J Physiol Endocrinol Metab 2010**; 299: E506–E515.
23. World Health Organization Consultation. *Definition, diagnosis and classification of diabetes mellitus and its complications, part 1: diagnosis and classification of diabetes mellitus, report of a WHO consultation*. Geneva: World Health Organization; 1999.
24. Levy JC, Matthews DR, Hermans MP. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. **Diabetes Care 1998**; 21: 2191–2192.
25. Tőnjes A, Fasshauer M, Kratzsch J, Stumvoll M, Blüer M. Adipokine pattern in subjects with impaired fasting glucose and impaired glucose tolerance in comparison to normal glucose tolerance and diabetes. **PLoS One 2010**; 5(11): e13911. 1–6.
26. Feng RN, Wang C, Sun CH, Guo FC, Zhao C, Li Y. Vaspin in newly and previously diagnosed Chinese Type 2 diabetic females: a case-control study. **Asian Biomed 2011**; 5: 525–529.
27. Atya HB, Hassan ZA, Amin AI, Ali SAE. Vaspin concentration in obesity, impaired glucose tolerance and Type 2 diabetes in Egypt. **Adv Res Biol Sci 2013**; 1: 6–13.
28. Auguet T, Quintero Y, Riesco D, Morancho B, Terra X, Crescenti A, Broch M, Aguilar C, Olona M, Porras J, Hernandez M, Sabench F, Del Castillo D, Richart C. New adipokines vaspin and omentin, circulating levels and gene expression in adipose tissue from morbidly obese women. **BMC Med Genet 2011**; 12: 60.
29. Roy MN, Akter S, Jafarulla M, Mollah FH, Saha AR, Ali L. Leptin and other factors as determinants of insulin secretion and sensitivity in Bangladeshi type 2 diabetic subjects. **J Bangladesh Soc Physiol 2008**; 3: 1–7.
30. Al-Mahmood AK, Hassan Z, Zinnat R, Ali L. Insulin secretion and insulin sensitivity in Bangladeshi type 2 diabetic subjects. **Intl Med J 2007**; 14: 295–298.
31. Lee MK, Jekal Y, Im JA, Kim E, Lee SH, Park JH, Chu SH, Lee HC, Oh EG, Kim SH, Jeon JY. Reduced serum vaspin concentrations in obese children following short-term intensive lifestyle modification. **Clin Chim Acta 2010**; 411: 381–385.