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Vaspin and visfatin/Nampt are interesting interrelated adipokines playing a role in the pathogenesis of type 2 diabetes mellitus

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Abstract

Recently, vaspin and visfatin/Nampt have been identified as interesting novel adipokines having insulin-sensitizing and insulin-mimetic effects, respectively. However, the relationship between them has not been elucidated; and their circulating levels in type 2 diabetes mellitus (T2DM) have not been adequately studied. Therefore, this study was designed to investigate whether their levels are altered in Egyptian T2DM patients and to study the correlation of these novel adipokines with each other and with insulin resistance, interleukin-6 (IL-6), and other biochemical parameters. The levels of vaspin, visfatin/Nampt, IL-6, insulin, and other parameters were measured in nonobese and obese T2DM patients together with matched healthy nondiabetic control subjects. Vaspin, visfatin/Nampt, and IL-6 levels were measured by enzyme-linked immunosorbent assay, whereas insulin levels were measured by chemiluminescence technique. Vaspin and visfatin/Nampt levels were found to be significantly elevated in nonobese (1.62 ± 0.22 and 25.9 ± 3.44 ng/mL, respectively) and obese T2DM patients (2.76 ± 0.38 and 45.4 ± 4.60 ng/mL, respectively) compared with control subjects (0.42 ± 0.05 and 9.37 ± 1.98 ng/mL, respectively) at $P <$ .01. In addition, vaspin and visfatin/Nampt levels were found to be significantly positively correlated with each other and with other biochemical parameters. In conclusion, both vaspin and visfatin/Nampt might play an important role in the pathogenesis of T2DM. In addition, the 3 adipokines—vaspin, visfatin/Nampt, and IL-6—are significantly interrelated with each other. Other possible mechanisms of action for vaspin should be considered besides the inhibition of unknown substrate proteases.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder that affects more than 150 million people worldwide and is projected to increase to 439 million worldwide in 2030. Its prevalence is expected to increase exponentially around the world particularly in developing countries [1,2]. Insulin resistance and inflammation play a major role in the development of T2DM [3]. In addition, increased abdominal/visceral fat is associated with insulin resistance and T2DM [4]. Vigorous efforts have been made to delineate the relationship between increased adiposity and insulin resistance. However, the molecular mechanisms that lead to the development of insulin resistance and T2DM are far from complete elucidation. The realization that adipose tissue acts as an endocrine gland affecting whole-body energy homeostasis was a major breakthrough toward a better molecular understanding of T2DM [5,6], and growing evidence implicates adipocyte-derived factors (adipokines) as major regulators of insulin resistance [7].

Among these adipokines, the inflammatory regulator interleukin-6 (IL-6) has emerged as one of the potential mediators that link obesity-derived chronic inflammation with insulin resistance [8]. A growing body of evidence has established IL-6 as an important player in metabolic disease states, such as diabetes [8-10]. Interestingly, Hida and coworkers [11] isolated a unique insulin-sensitizing adipokine termed *visceral adipose tissue–derived serpin* (vaspin) from the visceral adipose tissue of an animal model of visceral obesity and T2DM, which was found to be a member of the serine protease inhibitor family (serpins). In addition, they found that administration of vaspin to obese mice improved glucose tolerance and insulin sensitivity—hence, insulin sensitizing. Furthermore, it has been postulated that the induction of vaspin messenger RNA expression

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in human adipose tissue could represent a compensatory mechanism associated with obesity and severe insulin resistance to antagonize the actions of unknown proteases that are up-regulated in states of decreased insulin sensitivity or impaired glucose metabolism [11,12].

Another remarkable adipokine isolated by Fukuhara et al [13] is visfatin/Nampt, which is also known as *pre–B cell colony-enhancing factor* (PBEF). Visfatin/Nampt is a pleiotropic protein that is highly conserved in evolution and unique in its structural and functional profile [14]. In fact, visfatin/Nampt was originally identified by Samal and colleagues [15] in 1994 as pre–B-cell colony-enhancing factor that acts as a growth factor for early-state B cells. However, Fukuhara et al rediscovered visfatin/Nampt as an adipokine that is mainly synthesized and secreted in visceral fat—hence, the name *visfatin* [13]. Surprisingly, visfatin/Nampt was shown to activate its target cells by binding to the insulin receptor, although at a site distinct from insulin, and to exert a variety of insulin-mimetic effects including enhancing glucose uptake and inhibiting hepatocyte glucose release and insulin-like effects in the insulin-transduction pathway [13,16]; and its insulin-mimetic effects were inhibited by an insulin receptor-specific inhibitor [16,17]. On the other hand, visfatin/Nampt was found to have important proinflammatory and immunomodulating properties [17-19] and was also found to function as nicotinamide phosphoribosyl transferase (Nampt), the rate-limiting enzyme in the NAD biosynthetic pathway from nicotinamide in mammals [20]. Actually, in mammals, visfatin/Nampt has 2 different forms: intracellular and extracellular visfatin/Nampt. Whereas the function of intracellular visfatin/Nampt as a NAD biosynthetic enzyme has been firmly supported by its biochemical and structural analyses, the significance and function of extracellular visfatin/Nampt have been a matter of debate [21].

All these data indicate that both vaspin and visfatin/Nampt are potential candidates to play a role in the pathogenesis of T2DM. However, their circulating levels in T2DM have not been adequately studied; and their correlation with insulin resistance or obesity is still controversial. Therefore, the current study was designed to determine the circulating levels of vaspin and visfatin/Nampt in Egyptian T2DM patients compared with healthy control subjects and to study the correlation of these 2 interesting adipokines with one another and between each of them with other parameters like IL-6, insulin resistance, as well as other biochemical and anthropometric parameters. According to our knowledge, the interrelation between vaspin, visfatin/Nampt, and IL-6 has not been elucidated before our current work.

2. Subjects and methods

2.1. Study population and anthropometric measurements

This study was approved by the ethical committee of National Institute of Diabetes and Endocrinology, Cairo, Egypt; and informed consent was obtained from every subject before participating in the study. A total of 75 subjects (58 men and 17 postmenopausal women) were enrolled in the study: 56 patients with T2DM and 19 age- and sex-matched nondiabetic healthy control subjects. The definition of a *nondiabetic* is a subject who has a fasting plasma glucose (FPG) level lower than 110 mg/dL and has no family history of T2DM. The 56 patients with T2DM were recruited from the outpatient clinic of the National Institute of Diabetes and Endocrinology and were further classified into nonobese group (body mass index [BMI] <30 kg/m^2) and obese group (BMI >30 kg/m^2). Both the nonobese diabetic group and the control nondiabetic group were selected to have matching BMI. The characteristics of the subjects are summarized in Table 1. The exclusion criteria were age less than 30 or more than 65 years, type 1 DM, insulin treatment, renal or hepatic disease, acute or chronic inflammatory disease, thyroid dysfunction, ischemic cardiovascular disease, retinopathy, alcohol or drug abuse, cancer, acute or chronic infections, any hematologic disorder (assessed by making complete blood count for every subject), and smoking. Subjects taking thiazolidinediones, fenofibrate, statins, spironolactone [22], or hormonal therapy were also excluded. The control subjects were not suffering any health problems and were not receiving any medications or dietary supplements. The diabetic subjects were selected not to have long duration of T2DM to help avoid the presence of diabetic complications, which was further confirmed by physical examination and laboratory investigations.

Before inclusion, all the study subjects underwent careful physical examination, detailed history, and laboratory investigations to exclude any condition that may interfere with glucose tolerance. Anthropometric parameters measured included BMI and waist-to-hip ratio (WHR). Standing height and body weight were measured in light clothing without shoes. The BMI was calculated as weight divided by squared height (in kilograms per square meter). Waist and hip circumferences were measured to the nearest 0.1 cm at the narrowest point between the lowest rib and the uppermost lateral border of the iliac crest. The hips were measured at their widest point.

2.2. Blood sampling

All of the blood samples were drawn after overnight fasting, and the samples were divided into 4 aliquots. The first aliquot of blood was collected on Vacutainer tubes (BD, Franklin Lakes, NJ) containing sodium fluoride for plasma preparation used for the assay of FPG. The second aliquot of blood was collected on Vacutainer tubes containing sodium EDTA for complete blood count and for the assay of glycosylated hemoglobin (HbA_{1c} %). The third aliquot of blood was collected on Vacutainer tubes containing sodium EDTA for plasma preparation used for the assay of visfatin/Nampt. The fourth aliquot of blood was collected on plain Vacutainer tubes for serum preparation used for the assay of lipids profile, routine parameters (creatinine, urea, and

Table 1
Clinical and laboratory characteristics of the studied groups

Factor	Controls	Nonobese T2DM	Obese T2DM	*P*1	*P*2	*P*3
n	19	37	19	–	–	–
Age (y)	47.16 ± 1.80	52.4 ± 1.32	49.84 ± 1.73	NS	NS	NS
Sex (M/F)	16 M/3 F	29 M/8 F	13 M/6 F	–	–	–
Diabetes duration (y)	–	6.44 ± 0.75	5.79 ± 1.11	–	–	–
BMI (kg/m^2)	26.06 ± 0.67	27.15 ± 0.37	35.16 ± 0.59	NS	<.0001	<.0001
Waist (cm)	91.32 ± 1.43	92.46 ± 0.98	111.63 ± 1.73	NS	<.0001	<.0001
WHR	0.88 ± 0.01	0.90 ± 0.004	0.97 ± 0.01	NS	<.0001	<.0001
Creatinine (mg/dL)	0.92 ± 0.036	0.85 ± 0.02	0.87 ± 0.03	NS	NS	NS
Urea (mg/dL)	15.53 ± 2.03	12.05 ± 0.60	11.42 ± 0.55	NS	NS	NS
ALT (U/L)	25.00 ± 2.73	36.03 ± 2.14	31.89 ± 2.84	.008	NS	NS
FPG (mg/dL)	87.10 ± 2.73	227.27 ± 13.36	239.26 ± 11.44	<.0001	<.0001	NS
HbA_{1c} (%)	5.75 ± 0.13	10.52 ± 0.42	9.59 ± 0.56	<.0001	<.0001	NS
Insulin (μU/mL)	7.74 ± 0.71	10.00 ± 1.00	16.98 ± 2.39	NS	<.0001	.002
HOMA-IR	1.68 ± 0.16	5.29 ± 0.56	9.97 ± 1.52	.008	<.0001	<.0001
TG (mg/dL)	102.74 ± 6.80	185.76 ± 11.78	202.16 ± 17.90	<.0001	<.0001	NS
TC (mg/dL)	181.53 ± 6.4	221.86 ± 6.48	228.84 ± 7.02	.001	<.0001	NS
HDL-C (mg/dL)	43.95 ± 2.27	43.14 ± 1.17	40.16 ± 1.87	NS	NS	NS
LDL-C (mg/dL)	117.18 ± 6.42	141.65 ± 5.14	148.26 ± 8.01	.029	.012	NS
IL-6 (pg/mL)	6.43 ± 0.35	9.31 ± 0.45	11.84 ± 1.15	.007	<.0001	.022
Vaspin (ng/mL)	0.42 ± 0.053	1.62 ± 0.22	2.76 ± 0.38	.004	<.0001	.006
Vaspin (adjusted [ng/mL])[a]	0.29 ± 0.35	1.52 ± 0.24	3.09 ± 0.48	.006	<.0001	.034
Visfatin/Nampt (ng/mL)	9.37 ± 1.98	25.9 ± 3.44	45.4 ± 4.60	.006	<.0001	.001
Visfatin/Nampt (adjusted [ng/mL])[a]	6.37 ± 4.91	26.28 ± 3.38	47.71 ± 6.76	.001	<.0001	.043
Type of OHA (SU/MET/SU + MET/none)	–	10/2/15/10	8/0/6/5	–	–	–
Antihypertensive treatment (β-blocker/Ca channel blocker/none)	–	1/1/35	2/0/17	–	–	–

Results are expressed as mean ± SEM. *P*1, for controls and nonobese T2DM; *P*2, for controls and obese T2DM; *P*3, for nonobese T2DM and obese T2DM. OHA indicates oral hypoglycemic agent; none, does not take oral hypoglycemic agent (recently diagnosed); SU, sulfonylurea; MET, metformin; NS, not significant.

[a] Mean ± SEM by general linear model with adjustment of age, sex, and BMI.

alanine aminotransferase [ALT]), insulin, vaspin, and IL-6 levels. Plasma and serum samples were divided into aliquots and kept at −80°C for subsequent assay.

2.3. Laboratory analyses

Fasting plasma glucose and serum biochemical parameters including triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), creatinine, urea, and ALT were measured using Dimension RxL analyzer (Dade Behring, Newark, DE) automated biochemistry analyzer. Low-density lipoprotein cholesterol (LDL-C) level was calculated by the formula of Friedewald et al [23]. The HbA_{1c} % was measured in whole blood with ion-exchange high-performance liquid chromatography using the Bio-Rad D-10 system (Bio-Rad Laboratories, Hercules, CA).

The concentration of serum insulin was determined by chemiluminescence using Automated Chemiluminescence Siemens ACS: 180 System Analyzer (Siemens Medical Solutions Diagnostics, Tarrytown, NY). The homeostasis model assessment of insulin resistance index (HOMA-IR) was calculated from fasting insulin and glucose by the following equation: HOMA-IR = fasting insulin (in micro-units per milliliter) × fasting glucose (in milligrams per deciliter)/405 [24].

Serum IL-6 and vaspin levels were determined by enzyme-linked immunosorbent assay (ELISA) using commercially available kits: Human IL-6 Immunoassay (Quantikine, R&D Systems, Minneapolis, MN) and Human vaspin ELISA kit (Adipogen, Seoul, South Korea), respectively. Plasma visfatin/Nampt levels were determined by ELISA (Human Visfatin ELISA kit, Phoenix Pharmaceuticals, Belmont, CA). All ELISA procedures were done by Hyprep Automated ELISA system (Hyperion Inc, Miami, FL) according to the manufacturer's instructions.

2.4. Statistical analysis

Results are expressed as mean ± SEM. Kolmogorov-Smirnov test was done to evaluate the distribution of variables. Different groups were compared by analysis of variance, and post hoc Bonferroni was applied to compare individual groups. Any skewed data were further analyzed by Kruskal-Wallis and Mann-Whitney *U* test. The general linear modeling was used to control for potential confounders (eg, age, sex, BMI). Any skewed data were logarithmically transformed before performing simple and multiple

linear stepwise regression analyses to study the association between each of IL-6, vaspin, and visfatin/Nampt with other biochemical parameters and to study the association between vaspin and visfatin/Nampt adjusted for the effects of other covariates; generally, in the multiple linear stepwise regression analysis, the independent variables included demographic factors (age, sex, and duration), various metabolic parameters (BMI, FPG, HbA_{1c} %, insulin, HOMA-IR, TG, TC, HDL-C, LDL-C), and variables found to be significantly associated with the examined dependent variable (IL-6, vaspin, or visfatin/Nampt) in univariate analysis. All statistical analyses were performed using Windows-based SPSS statistical package (SPSS version 17.0; SPSS, Chicago, IL). P values < .05 were considered to be significant. All statistical analyses were done under supervision of the Institute of Statistical Studies and Research, Cairo University, Egypt.

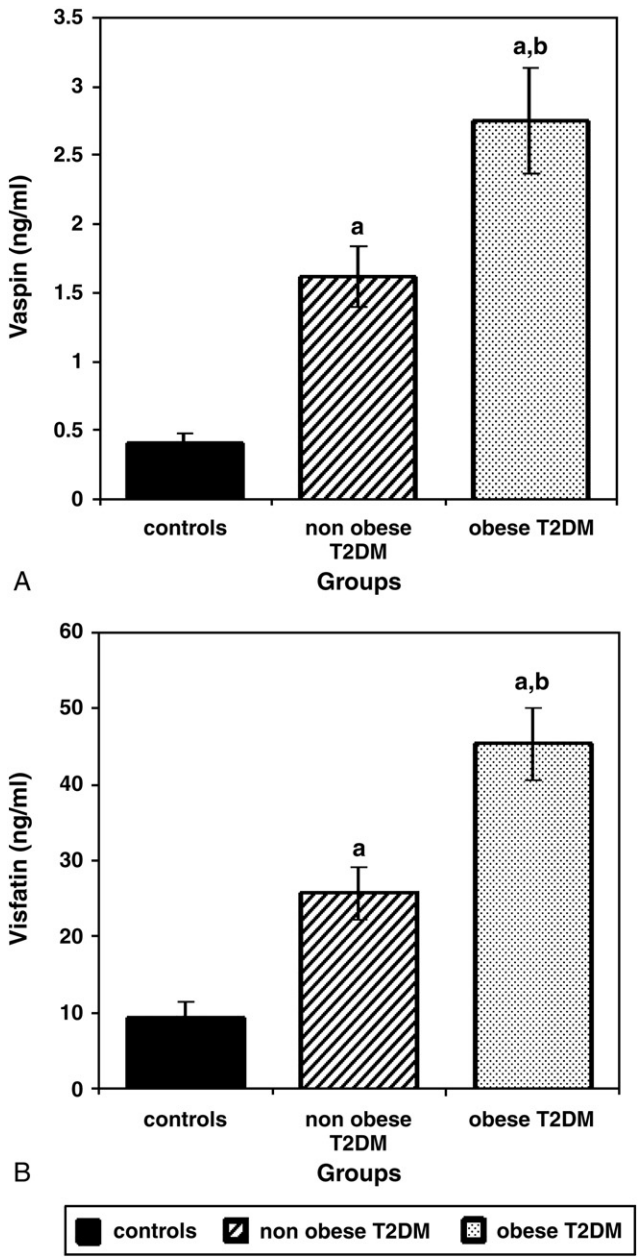

Fig. 1. A, Serum vaspin levels in the studied groups. B, Plasma visfatin/Nampt levels in the studied groups. [a]Significantly different from control group at $P < .05$. [b]Significantly different from non-obese T2DM group at $P < .01$.

3. Results

The clinical characteristics as well as the levels of circulating insulin, HOMA-IR, IL-6, vaspin, and visfatin/Nampt of the studied subjects are shown in Table (1). Concerning serum insulin levels, no significant difference was found between the serum insulin levels of nonobese diabetic patients compared with controls; however, they were significantly elevated in obese diabetic subjects as compared with both controls and nonobese diabetic subjects at $P < .01$. Additionally, the HOMA-IR index was significantly elevated in both nonobese and obese T2DM patients compared with control subjects at $P < .01$. Concerning the serum levels of IL-6, they were found to be significantly elevated in both nonobese and obese T2DM patients compared with control subjects at $P < .01$ and significantly elevated in the obese as compared with the nonobese T2DM group at $P < .05$.

As for serum vaspin as well as plasma visfatin/Nampt levels, as shown in Fig. 1 and Table 1, they were found to be significantly elevated in nonobese and obese T2DM patients compared with healthy control subjects at $P < .01$. Furthermore, as shown in Table 1, even after adjustment for the effect of covariates as age, sex, or BMI, vaspin and

Table 2
Simple linear regression analysis using vaspin, visfatin, or IL-6 as dependent variable

Variable	IL-6[a]		Vaspin[a]		Visfatin/Nampt[a]	
	β (r)	P	β (r)	P	β (r)	P
Age	0.136	.246	0.203	.08	−0.013	.915
Sex	0.109	.350	0.127	.279	.09	.441
Duration	0.086	.602	−0.256	.116	−0.083	.614
BMI[a]	0.283	.014*	0.423	<.0001†	0.434	<.0001†
WHR	0.296	.01*	0.376	.001†	0.280	.015*
FPG	0.562	<.0001†	0.494	<.0001†	0.283	.014*
HbA_{1c} (%)	0.444	<.0001†	0.368	.001†	0.17	.145
Insulin[a]	0.133	.254	0.279	.015*	0.188	.105
HOMA-IR[a]	0.455	<.0001†	0.552	<.0001†	0.357	.002†
TG	0.374	.001†	0.374	.001†	0.282	.014*
TC	0.185	.112	0.341	.003†	0.278	.016*
HDL-C	−0.154	.188	−0.061	.604	−0.111	.343
LDL-C	0.084	.475	0.246	.034*	0.224	.053
IL-6[a]	–	–	0.397	<.0001†	0.431	<.0001†
Vaspin[a]	.0.397	<.0001†	–	–	0.503	<.0001†
Visfatin/ Nampt[a]	0.431	<.0001†	0.503	<.0001†	–	–

[a] Logarithmically transformed values were used.
* Significant at $P < .05$ level.
† Significant at $P < .01$ level.

visfatin/Nampt levels remained to be significantly different among the studied groups.

We next analyzed the correlation between each of IL-6, vaspin, and visfatin/Nampt levels with each other and with other parameters. Concerning serum IL-6 levels, as shown in Table 2, simple linear regression analysis revealed that they were found to be significantly positively correlated with BMI, WHR, FPG, HbA_{1c} %, HOMA-IR, TG, vaspin, and visfatin/Nampt levels, whereas in multiple linear stepwise regression analysis using IL-6 as dependant variable and age, sex, duration, BMI, FPG, HbA_{1c} %, insulin, HOMA-IR, TG, TC, HDL-C, LDL-C, vaspin, and visfatin/Nampt) as independent variables, only FPG ($\beta = 0.305$, $P = .04$) and TG ($\beta = 0.374$, $P = .014$) remained to be significantly associated with IL-6.

Furthermore, as shown in Table 2, simple linear regression analysis revealed that serum vaspin was significantly positively correlated with BMI, WHR, FPG, HbA_{1c} %, insulin, HOMA-IR, TG, TC, LDL-C, IL-6, and visfatin/Nampt levels, whereas in multiple linear stepwise regression analysis using vaspin as dependant variable and age, sex, duration, BMI, FPG, HbA_{1c} %, insulin, HOMA-IR, TG, TC, HDL-C, LDL-C, IL-6, and visfatin/Nampt as independent variables, only visfatin/Nampt remained significantly associated with vaspin ($\beta = 0.402$, $P = .011$).

With respect to visfatin/Nampt levels, simple linear regression analysis revealed that they were significantly positively correlated with BMI, WHR, FPG, HOMA-IR, TG, TC, IL-6, and vaspin levels, while in multiple linear stepwise regression analysis using visfatin/Nampt as dependant variable and age, sex, duration, BMI, FPG, HbA_{1c} %, insulin, HOMA-IR, TG, TC, HDL-C, LDL-C, IL-6, and vaspin as independent variables, only vaspin remained significantly associated with visfatin/Nampt ($\beta = 0.402$, $P = .011$).

4. Discussion

The number and diversity of identified adipokines are growing rapidly [25]; and understanding of the diverse effects of distinct adipokines as well as the interplay between these bioactive mediators is still incomplete [26] and, if fully elucidated, would provide much better understanding for the molecular basis of T2DM. Vaspin and visfatin/Nampt were identified as interesting insulin-sensitizing and insulin-mimetic adipokines, respectively [11,13]. Our findings confirm that T2DM is associated with increased circulating levels of vaspin and visfatin/Nampt. Furthermore, the results of the current study show that vaspin and visfatin/Nampt levels are affected by both factors: T2DM and obesity.

As for vaspin, this finding could be explained by its induction, being insulin sensitizing, as a compensatory mechanism in states of impaired glucose tolerance [11]. It is noteworthy to point that vaspin messenger RNA expression was previously found to be absent in lean normal glucose–tolerant individuals and was more frequently detected in T2DM patients [12]. Moreover, we found serum vaspin levels to be significantly positively correlated with markers of insulin resistance and glycemic control such as FPG, HOMA-IR, insulin, and HbA_{1c} %. Previously, glucose was found to cause a significant dose-dependent increase in vaspin net protein production and secretion from human omental adipose tissue explants [27], which indicates that vaspin is induced by hyperglycemia and explains the significant correlation between vaspin and glucose levels in the current study. In addition, insulin treatment was found to affect the expression and serum levels of vaspin in OLETF rats [11], which would also explain the correlation between vaspin and insulin levels in the present study. However, the correlation of vaspin with glucose and HOMA-IR levels was much stronger than that with insulin levels. Our results corroborate the finding of Kempf et al [28] who demonstrated a significant association of vaspin single nucleotide polymorphism with T2DM. The controversy in the association of vaspin levels with insulin resistance reported in previous studies may be due to different patient populations in these studies or other currently undefined factors that may affect vaspin or its substrate protease [29]. In addition, vaspin levels were found to be significantly correlated with some markers of lipid metabolism such as TG, TC, and to a lesser extent LDL-C, which indicates that vaspin may play a role in lipid metabolism or might be induced by diabetic dyslipidemia as a compensatory mechanism, especially because vaspin is an adipokine secreted by adipocytes.

Surprisingly, serum vaspin was found to be significantly positively correlated with serum IL-6 levels. This observation sheds light on the possible interplay between this unique insulin-sensitizing adipokine and IL-6 that was proven to affect insulin signaling and to modulate insulin resistance by various mechanisms, and to be a marker of systemic inflammation at the same time [8]. Interestingly, Hida and coworkers [11] have found previously that administration of vaspin to obese mice improved glucose tolerance and insulin sensitivity, and altered gene expression of candidate genes for insulin resistance such as tumor necrosis factor–α. However, the interrelation between vaspin and inflammation could be further clarified through investigating the association between vaspin and other proinflammatory markers such as nuclear factor–κB and high-sensitivity C-reactive protein. Our observation sheds light on the possibility that the inhibition of unknown proteases might not be the sole mechanism of action for vaspin, especially because the superfamily of serpins is involved in a number of fundamental biological processes, sometimes not related to protease inhibition [30]. In addition, the significant positive correlation of serum vaspin levels with IL-6 despite being insulin sensitizing raises the suggestion of possible pleiotropic tissue-specific actions of this interesting adipokine that need further research to be elucidated.

Visfatin/Nampt is another remarkable adipokine that has recently drawn much attention in several fields, including NAD biology, metabolism, and inflammation [21]. In the

current study, elevated visfatin/Nampt levels in patients with T2DM could have more than one possible explanation: Firstly; elevated visfatin/Nampt levels in diabetic patients may suggest the impairment of visfatin/Nampt signaling in target tissues (ie, visfatin/Nampt resistance resulting in eventual hypervisfatinemia-like insulin resistance that results eventually in hyperinsulinemia to overcome this resistance) [31]. Secondly; being insulin mimetic, the increased plasma visfatin/Nampt concentrations could be a compensatory mechanism in response to hyperglycemia aiming to ameliorate the functional consequences of insulin deficiency or resistance [32]. Thirdly, the recent discovery of visfatin/Nampt-mediated NAD biosynthesis regulating glucose-stimulated insulin secretion in pancreatic β-cells [33] could explain the elevated levels of visfatin/Nampt in T2DM patients as a compensatory mechanism for β-cell functioning. Finally, being an adipokine with proinflammatory properties [18], these elevated levels could be attributed to the chronic state of low-level inflammation in T2DM in which adipose tissue plays a pivotal role [25].

Furthermore, we found plasma visfatin/Nampt levels to be significantly positively correlated with markers of insulin resistance such as FPG and HOMA-IR. Previously, visfatin/Nampt release was found to be enhanced by glucose in cultured human adipocytes in vitro and by hyperglycemia in healthy humans, which was counterregulated by insulin; however, basal visfatin/Nampt concentrations were not altered by hyperinsulinemia alone [34]. This could partially explain the absence of correlation between visfatin/Nampt and insulin levels in the current study, despite the significant positive correlation with FPG and HOMA-IR. In addition, visfatin/Nampt levels were found to be significantly positively correlated with some markers of lipid metabolism such as TG and TC. Because visfatin/Nampt is an adipokine, a direct or indirect relationship may exist between visfatin/Nampt levels and lipid metabolism. The relationship between visfatin/Nampt and lipid profile might represent a compensatory mechanism for diabetic dyslipidemia because visfatin/Nampt was previously reported to up-regulate peroxisome proliferator–activated receptor γ activity [35].

Unexpectedly, the current study did not show correlation between visfatin/Nampt levels and HbA_{1c} %. This observation is paradoxical but could be partially explained by the complex biology of visfatin/Nampt and the fact that it is involved in various processes like inflammation and NAD biology in addition to insulin resistance and metabolism [19], and the possibility that inflammation and NAD biology rather than glycemic control might have stronger chronic effects regarding the regulation of visfatin/Nampt. However, further studies are required to fully elucidate the regulation of this novel adipokine. Actually, it is noteworthy here that the diabetic patients in the current study showed relatively high levels of FPG and HbA_{1c} % levels, indicating that they were poorly controlled; and further large-scale studies to investigate the effect of glycemic control on vaspin as well as visfatin/Nampt levels would be of good value.

Moreover, we found a significant positive correlation between visfatin/Nampt and IL-6 levels, which is in accordance with the previous finding of proinflammatory effects of visfatin/Nampt, inducing the expression of both IL-6 and tumor necrosis factor–α [18]. On the other hand, Kralisch and colleagues [36] previously reported that IL-6 inhibits visfatin/Nampt transcription in 3T3-L1 adipocytes.

Interestingly, we found vaspin and visfatin/Nampt levels to be significantly associated in both simple and multiple linear stepwise regression analyses. Accordingly, this association together with the association between each of vaspin and visfatin/Nampt with IL-6 shed light on the possible interplay between these 3 adipokines. Moreover, it is noteworthy that the 3 of them were reported to be significantly affected by exercise; both IL-6 and vaspin were found to be increased by exercise [37-39], whereas visfatin/Nampt was found to be decreased [40]. Although the molecular mechanism for the effect of exercise on these adipokines is not clear, the fact that the 3 of them are affected by exercise, in addition to the significant correlations observed between them in our study, suggests that the 3 of them might be interrelated and involved in a compensatory mechanism for impaired glucose metabolism and insulin resistance observed in T2DM.

However, it is important to point out that the current study is an epidemiologic study, capable of identifying correlations between variables and not direct cause and effect. Accordingly, further experimental studies are required to unravel the molecular mechanism of the observed associations between vaspin as well as visfatin/Nampt with various metabolic parameters. In addition, although the current study had sufficient power to detect significant associations between vaspin and visfatin/Nampt with one another and between each of them with various metabolic parameters, further large-scale studies are required to gain more insight into their role in T2DM and to investigate their role in various diabetic complications as well.

In conclusion, we found that vaspin and visfatin/Nampt are significantly elevated in T2DM patients compared with healthy control subjects. Vaspin and visfatin/Nampt were found to be significantly correlated with various metabolic parameters. Our results indicate that both vaspin and visfatin/Nampt might play a role in the pathogenesis of T2DM. Furthermore, the novel finding of our study is that there might be some sort of interplay between vaspin, visfatin/Nampt, and IL-6; and further investigations are warranted to unravel the molecular mechanism of this interplay, which would help to clarify the mechanism of the putative compensatory response to insulin resistance.

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