View metadata, citation and similar papers at core.ac.uk
brought to you by CORE

Vaspin: a novel adipokine, member of the family of serine protease inhibitors.

Ekaterini Koiou[1], Emmanouil Kalaitzakis[2], Konstantinos Tziomalos[3], Stylianos Mavridis[1], Konstantinos Dinas[1], Theoharis Tantanasis[1], Ilias Katsikis[1], Aristotelis Loufopoulos[1], Dimitrios Panidis[1]

[1]*Division of Endocrinology and Human Reproduction, Second Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, Hippokration Hospital, Thessaloniki, Greece*
[2]*Department of Clinics, School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece*
[3]*First Propedeutic Department of Internal Medicine, Aristotle University of Thessaloniki, AHEPA Hospital, Thessaloniki, Greece*

ABSTRACT: In 2000, the novel adipokine vaspin, which belongs to the superfamily of serpins, was isolated from visceral adipose tissue. Vaspin is mainly produced in the visceral adipose tissue and is related to insulin resistance, blood glucose levels, sex hormones (women have higher levels compared to men) and nutritional status. Moreover, vaspin levels are modulated by weight loss and several agents, and it possibly constitutes a connecting link between obesity and its associated metabolic disorders. Many patients with polycystic ovary syndrome have insulin resistance, obesity (mostly visceral) and glucose intolerance, conditions associated with abnormalities in the production of vaspin. The role of vaspin in the regulation of human metabolism is unclear at present, but it appears that vaspin might represent a novel marker of obesity and insulin resistance. However, the controversial findings of existing studies on vaspin stress the need for further research in women with obesity and metabolic disorders in order to elucidate the role of this adipokine in these diseases and particularly in the polycystic ovary syndrome.

Key Words: Vaspin, Serpins, Obesity, Insulin resistance, Polycystic ovary syndrome.

INTRODUCTION

Serine Proteases

Serine proteases are proteolytic enzymes. More than one third of all known proteolytic enzymes are serine proteases. Over 18,000 serine proteases are grouped into 12 superfamilies and 40 families. Serine proteases are widely distributed in nature and are found in all kinds of living organisms including viral genomes[1].

The active site of serine proteases contains three critical aminoacids: serine, histidine and aspartate. These residues are often referred to as the "catalytic triad"[2].

Serine proteases play diverse roles in human health, from non-specific digestion to highly regulated functions like embryonic development, immune response, blood coagulation and facilitation of sperms entering the egg[1,3].

Serine proteases catalyze peptide bond cleavage of attacking substrate in a two-step process. Initially, the catalytic serine performs a nucleophilic attack on the peptide bond of the substrate. This releases the new N-terminus and forms an ester-bond between the enzyme and the substrate. This covalent enzyme-substrate complex is called acylenzyme intermediate. Subsequent to this, this ester bond is hydrolysed and the new C-terminus is released.

Corresponding author: Emmanouil Kalaitzakis, 11 Mina Vista Street, 543 52 Thessaloniki, Greece, Tel. +30 2310 924601, e-mail: emmanouil111@gmail.com

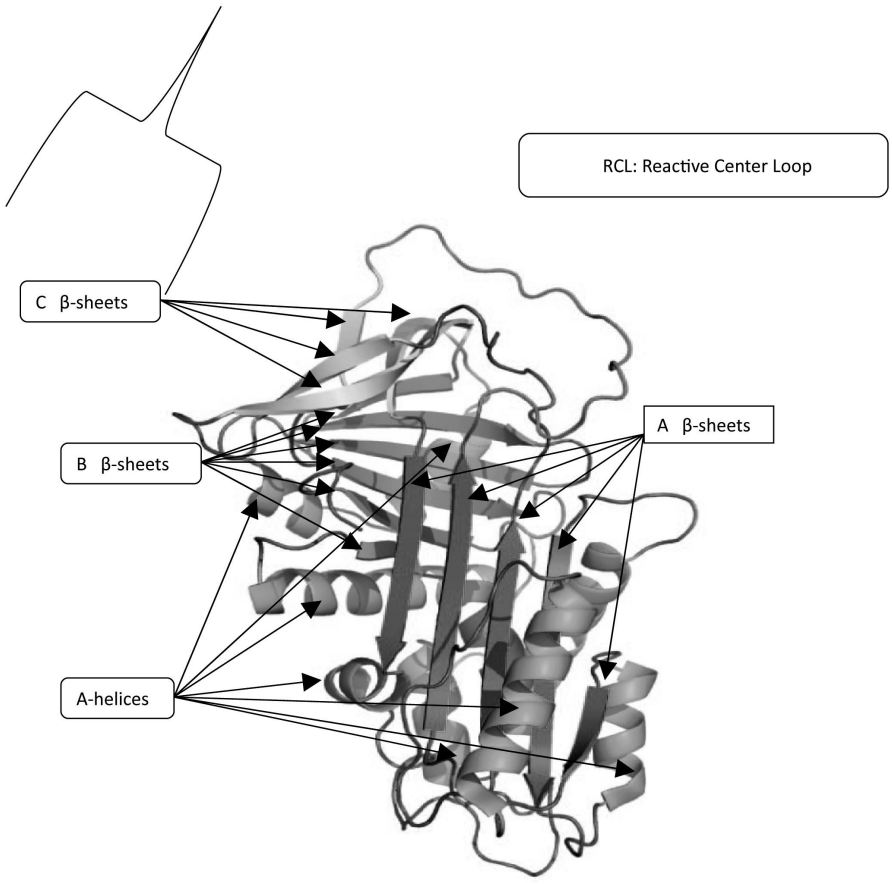

Figure 1. The structure of the archetypical serpin, α1-Antitrypsin (SERPIN A1)[6].

Serpins

Regulation of protease activity in vivo from endogenous factors is critical for body homeostasis[4]. Such processes require timely and tightly regulated proteolytic activity. Initiation of protease activity is largely controlled by zymogen activation. Cessation of protease activity is achieved in vivo by endogenous protease inhibitors[1].

Endogenous protease inhibitors are proteins, which were initially identified in blood plasma and represent > 10% of the total plasma proteins. Among the different classes of protease inhibitors in blood plasma, the majority is serine protease inhibitors, which appear to be involved in various pathways[4].

Serine protease inhibitors consist the superfamily of serpins (SERPINS: SERine Protease InhibitorS)[4,5,6]. It is important to emphasize that even though all the serpins share the same tertiary structure,[4] they have different functions.

Serpin structure and protease inhibition mechanism

Serpins consist of three β-sheets (A, B and C), 8-9 α-helices (termed hA-hI) and the reactive center known as reactive center loop (RCL), which is responsible for the interaction with the serpin targets[6] (Figure 1).

The serpin inhibition of protease proceeds via an initial formation of a noncovalent and reversible complex, (also known as the Michaelis complex) where the sequence of the RCL is recognized by the protease as a substrate and there is no conformational change within the protease or the body of the serpin. Subsequent peptide bond hydrolysis results in an acylenzyme intermediate that progresses to either a kinetically trapped loop-inserted covalent complex (inhibitory pathway) or a cleaved serpin and free protease (non inhibitory or substrate pathway)[7].

The RCL of a serpin acts as a substrate for its cognate protease. Indeed, serpins form covalent com-

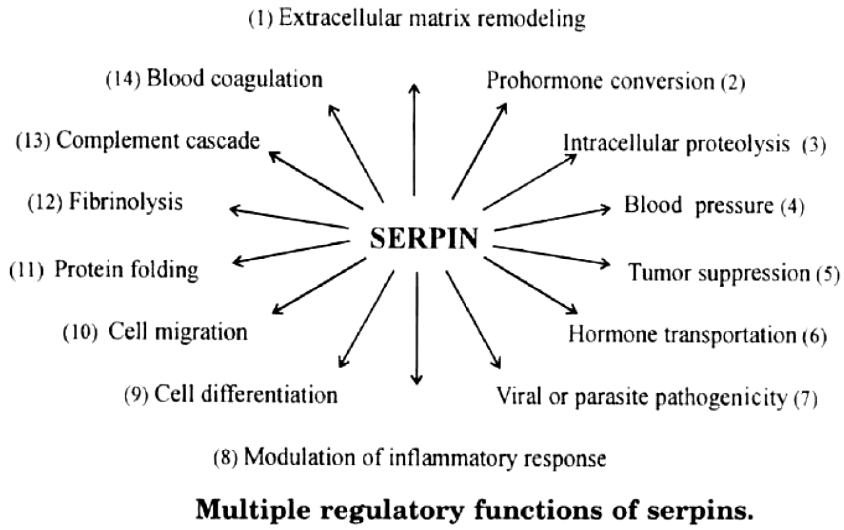

Figure 2. Regulatory functions of serpins[4].

plexes with target proteases. Prior to hydrolysis of the acyl-enzyme intermediate, after the RCL is cleaved, the serpin rapidly undergoes the S-to-R transition. Since the RCL is still covalently attached to the protease via the ester bond, the S-to-R transition causes the protease to be moved from the top to the bottom of the serpin. At the same time, the protease is distorted into a conformation, where the acylenzyme intermediate is hydrolysed extremely slowly. The protease thus remains covalently attached to the target serpin and is thereby inhibited. Further, since the serpin has to be cleaved to inhibit the target protases, inhibition consumes the serpin as well. Serpins are therefore irreversible 'suicide' enzyme inhibitors[6,7].

Certain serpins spontaneously undergo the S to R transition as part of their function, to form a conformation termed the latent state. In latent serpins the first strand of the C-sheet has to peel off to allow full RCL insertion. Latent serpins are unable to interact with proteases and are not protease inhibitors. The transition to latency represents a control mechanism for the serpin PAI-16.

Proteolytic cleavage of the serpins can result in a much more stable protein with new biological properties, including their chemo-attractant behavior. These structural transformations of serpins provide opportunities for regulation of the activity and properties of the inhibitor and are likely important in vivo in the regulation of processes like immunoregulation, cell migration, cell apoptosis control, blood coagulation and fibrinolysis, complement activation and inflammation[8,9].

The primary function of most serpins is the regulation of proteolysis by inhibition and this affects many biochemical pathways. However, many serpins have alternative, non-inhibitory functions, such as hormone transport (cortisol-binding globulin and thyroxine binding globulin)[10,11], blood pressure regulation[12] and tumor suppression[8]. The function of serpins is either intracellular or extracellular. Changes in the structure of the serpin molecule, especially of the active center, or deficient synthesis, can lead to pathologic conditions, collectively termed serpinopathies[13].

Serpins and obesity

Human serpins are involved in diverse biologic functions. For some serpins, their biologic functions appear to be directly related to protease inhibition. The large number of serpins, their ubiquitous presence in different organisms (e.g. metazoan, plantae and viruses) and the great variety of biological functions in which they are involved (Figure 2)[4], raise substantial research interest.

The multiple regulatory functions of serpins (Figure 2) and especially their participation in inflammatory processes and in fibrinolysis and coagulation

cascades, in which obesity is involved, lead to the investigation of the relationship between serpins and obesity.

Adipose tissue has long been overlooked in terms of its physiological impact and was considered only as a passive energy store in the form of fat. However, it is now clear that the adipose tissue is a major source for many factors, which link obesity with many different associated diseases[14]. Adipose tissue is currently considered an organ that not only stores energy but also acts as a multifunctional endocrine tissue, that expresses and secretes a variety of bioactive peptides, known as "adipokines", which act at both the local (autocrine-paracrine) and the systemic (endocrine) level[15]. The adipokines regulate systemic processes, including food intake and nutrient metabolism, insulin sensitivity, stress responses, reproduction, bone growth and inflammation[16]. Similarly, adipokines contribute to the pathogenesis of endothelial dysfunction, dyslipidemia and impaired coagulation. Perturbed adipokine secretion affects the function of various organs and results in many different metabolic disorders[14,15,16].

Many adipokines have been identified, including leptin, adiponectin, visfatin, tumor necrosis factor-a (TNF-α), interleukin-6 (IL-6), plasminogen activator inhibitor-1 (PAI-1)[15], C-reactive protein (CRP)[17,18].

It is important to stress that there are serpins that belong to the fibrinolytic system. The best known serpin of this system is PAI-1 (SERPIN E1). Other examples of serpins that inhibit proteases involved in the generation and control of plasmin function are PAI-2 (SERPIN B2) and α2-antiplasmin (SERPIN F2)[19]. PAI-1 is a major regulator of the fibrinolytic system, i.e. the natural defense mechanism against thrombosis. The major sources of PAI-1 synthesis are hepatocytes and endothelial cells, but platelets, smooth muscle cells, and adipocytes also produce PAI-1[20]. The increased gene expression and secretion of PAI-1 by adipose tissue contribute to the elevated plasma PAI-1 levels in obesity, which strongly correlate with features of the insulin resistance syndrome, namely elevated fasting plasma insulin and triglyceride levels, high BMI, and visceral fat accumulation. Both the levels and the functional impact of PAI-1 are affected by adipose tissue mass. PAI-1 levels are also influenced from various metabolic disturbances including the metabolic syndrome and type 2 diabetes mellitus, conditions that are associated with increased adipose tissue mass[21,22]. This increase is associated with up-regulated PAI-1 gene expression, which results in increased plasma PAI-1 levels. Moreover, in patients with visceral obesity, PAI-1 is implicated in the pathogenesis of cardiovascular disease[23,24]. The cardinal feature in all the afore-mentioned diseases is insulin resistance, which is induced by the increase in visceral adipose tissue mass[25].

Adipose tissue-induced insulin resistance

Insulin resistance and the compensatory hyperinsulinaemia, which are induced by the increase in adipose tissue mass, are considered important contributors to the biological and metabolic disturbances observed in obesity. A variety of molecules, including free fatty acids, TNF-α[26], leptin[27], adiponectin and resistin play a role in the pathogenesis of obesity and its associated diseases, e.g. type 2 diabetes mellitus. In type 2 diabetes mellitus, adipokines modify insulin sensitivity in peripheral tissues. The activation of adipokine excretion triggers multiple reactions, following the insulin receptor phosphorylation, while in the same time adipokines activate other molecules[28] which exert endocrine actions in various target tissues, including muscles, liver and hypothalamus. Some adipokines (e.g. TNF-α, IL-6 and resistin) induce insulin resistance and inflammation whereas others (e.g. leptin and adiponectin) are considered necessary for glucose homeostasis and energy control[29]. TNF-α, IL-6, resistin and PAI-1 contribute to the pathogenesis of obesity-related metabolic disorders and finally to atherothrombosis[29].

Vaspin: a new adipokine that belongs to the serpin superfamily

To assess the relationship between obesity and insulin resistance, various experimental models are being used, particularly the Otsuka Long-Evans Tokushima fatty rats (OLETF rats), which comprise a genetic model of type 2 diabetes mellitus. These rats are characterized by visceral obesity, insulin resistance, hyperinsulinemia, hyperglycemia, hypertension and dyslipidemia[30]. The OLETF rats are being compared with their diabetes-resistant counterparts, the Long-Evans Tokushima Otsuka rats (LETO rats), which are

characterized by a minimal volume of omental fat, absence of insulin resistance and no development of type 2 diabetes mellitus. In these experiments, various tissue samples (from brain, heart, lung, spleen, kidney, small intestine, muscle, brown adipose tissue and subdermal adipose tissue) were harvested from both rat strains[31]. In the harvested tissue, gene isolation and identification was performed. Among the genes upregulated in the visceral adipose tissue of OLETF rats, ten known and three novel genes were isolated. Some of these genes showed visceral adipose tissue-specific expression and were not detected in neither subcutaneous nor brown adipose tissue[31].

The isolated genes were classified into three groups: the first group comprised of known genes with identified functions. Some of these had already been reported to be upregulated in situations of increased visceral adipose tissue [enzymes related to glucose and lipid metabolism including lipoprotein lipase (LPL), phosphoenol-pyruvate carboxykinase (PEPCK), cholesterol esterase (CE) and c-Cbl-associated protein (CAP)]. The second group comprised of known genes with unknown function in the adipose tissue (nucleolar phosphoprotein of 140 kDa (Nopp140), trans-Golgi network 38 (TGN 38), selenoprotein P, thrombospondin 1 and contrapsin-like protease inhibitor). The third group comprised of three new, uncharacterized genes, which were exclusively expressed in the visceral adipose tissue of OLETF rats.

Among these novel genes, OL-64 was exclusively expressed in the visceral adipose tissue of OLETF rats and was not identified in brown adipose tissue or in subcutaneous adipose tissue, neither in any other organ[32]. The study of the aminoacid sequence of the OL-64 gene revealed a 40% homology with antitrypsin. The gene product of OL-64 is a novel member of the serine protease inhibitor (serpin) gene family and was named vaspin (Visceral Adipose tissue-derived SerPIN). The gene that encodes for vaspin has a molecular size of 1.8 kbs[31] and maps on the chromosome 14 (14q32.13). The structure of vaspin molecule follows the characteristic serpin structure and consists of three β-sheets (A, B, C), nine α-helices, and one active site loop (Figure 3).

After isolating and cloning vaspin molecule, it was revealed that vaspin cDNA consists of 1236, 1242 and 1245 nucleotides in rats, mice and humans, respectively. The corresponding proteins consist of 412, 414, and 415 aminoacids, respectively[32].

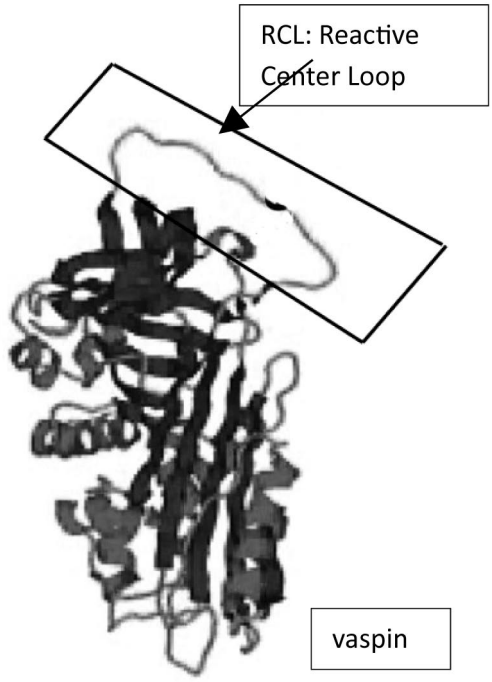

Figure 3. The molecular structure of vaspin[32].

Discovery, isolation, cloning and identification of vaspin was followed by intensive research on the biological actions of this adipokine[32].

Vaspin levels and biological actions in experimental models

Vaspin was identified in the serum of both OLETF and LETO rats. The vaspin serum levels are higher in OLETF rats than LETO rats during the period of maximal insulin resistance. In OLETF rats, vaspin serum levels significantly decline when these rats developed severe hyperglycemia. In turn, treatment with insulin and thiazolidinediones (TZD) increase serum vaspin levels in OLETF rats.

Experimental studies in the Imprinting Control Region (ICR) mice suggest that vaspin sensitizes peripheral tissues to insulin action. ICR mice do not develop obesity when fed a normal diet but when they are fed a lipid- and sucrose-enriched diet develop obesity, hyperinsulinemia and hyperglycemia, which resembles metabolic syndrome.

Administration of recombinant vaspin (rh-vaspin) in these obese, hyperinsulinemic and hyperglycemic ICR mice reduced significantly their glucose levels, 120 minutes after the intraperitoneal injection of glucose, while insulin levels were not altered. This result suggests sensitization of peripheral tissues to insulin action by vaspin, which attenuates insulin resistance in these diet-induced obese mice.

Recombinant vaspin administration also suppressed the expression of leptin, resistin and TNF-α whereas it increased the expression of adiponectin and glucose transporter-4, suggesting that vaspin administration down-regulates the expression of insulin resistance-promoting genes.[32]. Therefore, it appears that vaspin improves the insulin sensitivity of peripheral tissues by modulating gene expression, mainly in visceral and subcutaneous white adipose tissue.

In conclusion, vaspin is a novel adipokine that belongs to serpins and is expressed mainly in the visceral white adipose tissue but is also present in serum. Obesity and insulin resistance increase both vaspin expression in the visceral adipose tissue and serum vaspin levels, whereas the latter decline as diabetes worsens in rats. Administration of insulin and TZD up-regulate vaspin expression in the adipose tissue and serum vaspin levels. This up-regulation of vaspin synthesis might represent a compensatory response to antagonize the action of yet-unknown proteases derived from fat or other tissues, which antagonize insulin action. Therefore, the up-regulation of vaspin expression might constitute a defense mechanism against insulin resistance. Administration of rh-vaspin in rats suppressed gene expression of the adipokines leptin, resistin, and TNF-α, which increase the resistance of peripheral tissues to insulin, and increased the expression of glucose transporter-4 and adiponectin, which ameliorate insulin resistance. These findings support the hypothesis that vaspin down-modulates the expression of genes related to insulin resistance, normalizing their expression mainly in adipose tissue[32].

Vaspin levels and biological actions in humans

The expression of vaspin mRNA in obese humans correlates with adipose tissue mass, while vaspin mRNA is not detectable in lean humans with normal glucose tolerance. These findings indicate that the expression of vaspin mRNA in the adipose tissue is regulated in a fat depot-specific manner and could represent a compensatory response associated with obesity, insulin resistance and type 2 diabetes mellitus[33]. It therefore appears that vaspin is a new marker of obesity and impaired insulin sensitivity. At present, there is no clear proof of a causal link between vaspin levels and visceral fat accumulation or insulin resistance[34]. However, it has been shown that vaspin levels are also gender-dependent, since women have significantly higher vaspin levels compared to men[35]. Women with normal glucose tolerance have vaspin levels up to 2.5 times higher than men[36]. A recent study showed that serum vaspin levels present a meal-related diurnal variation[37]. Serum vaspin levels show a preprandial rise, 1-2 hours before the beginning of the meal. The higher levels were observed before lunch and the lowest before breakfast. Postprandially, vaspin levels decline gradually to preprandial levels within 2 hours after meal. Vaspin concentration also showed a nocturnal rise, with a peak at nighttime, when vaspin levels were approximately 250% higher than lowest daytime levels. This diurnal variation of serum vaspin concentration was exactly reciprocal to that of insulin and glucose variation. Insulin levels increase 4.7-8.3 times within thirty minutes after the beginning of the meal and gradually decline postprandially to reach preprandial levels before the next meal. In addition, insulin levels remain low during sleep[37].

It has been observed that serum vaspin concentration is exactly reciprocal to insulin levels[37]. In contrast, in other studies; insulin administration in OLETF rats resulted in an increase in serum vaspin levels but did not affect vaspin excretion in adipose cell culture, suggesting that insulin does not regulate vaspin gene expression through a direct action on adipose cells[32].

Vaspin in the polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is one of the commonest endocrine disorders in women of reproductive age and the commonest cause of anovulatory infertility in western countries. The commonest clinical manifestations of PCOS are menstrual irregularity (because of anovulation) and signs of hyperandrogenemia, including hirsutism, seborrhoea, acne and androgenic alopecia[38].

Women with PCOS frequently exhibit insulin re-

Table 1. The four phenotypes of polycystic ovary syndrome (PCOS) according to the revised diagnostic criteria proposed by the Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group[48,49].

Phenotypes		Oligo- or anovulation	Biochemical or clinical hyperandrogenemia	Polycystic ovaries
PCOS	1	+	+	+
	2	+	+	-
	3	-	+	+
	4	+	-	+

sistance[34] and obesity, with 38-88% of women with PCOS being overweight or obese[39,40]. The majority of women with PCOS, irrespective of their body weight, develop a particular type of insulin resistance, which is characteristic of the syndrome and of incompletely understood pathogenesis[41,42,43].

Given that 1) women with PCOS have hyperinsulinemia independently of obesity, 2) hyperinsulinemia is not a feature of hyperandrogenic states in general, 3) only obese PCOS women are at risk for impaired glucose tolerance, and 4) PCOS is associated with a unique type of insulin resistance, it appears that the adverse effects of PCOS and obesity on insulin action are additive or synergistic[42].

All PCOS women are at risk for insulin resistance and the associated metabolic abnormalities of the insulin resistance syndrome (dyslipidemia, impaired fibrinolysis, cardiovascular disease, hypertension, gestational and type 2 diabetes mellitus (GDM)[44]. Indeed, in the United States, the prevalence of type 2 diabetes mellitus is 10 times higher in young women with PCOS than in age-matched controls[43]. Moreover, 30-50% of obese women with PCOS after the age of 30 years develop impaired glucose tolerance or type 2 diabetes mellitus[45,46,47].

The diagnostic criteria that have been proposed for PCOS have been revised by the Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group[48,49]. According to this expert group, PCOS is diagnosed - after the exclusion of related disorders - when two of the following three features are present: 1) oligo- or anovulation, 2) clinical signs and/or biochemical hyperandrogenism, or 3) polycystic ovaries on ultrasound. After adding this third criterion, i.e. polycystic ovarian morphology in ultrasound, there are four phenotypes of PCOS (Table 1).

According to the National Institutes of Health (NIH) criteria proposed in April of 1990, PCOS was diagnosed when both hyperandrogenism and/ or hyperandrogenemia and oligoovulation or anovulation were present[50].

These two major definitions of PCOS are the subject of intense controversy[51,52] and this impacts both clinical diagnosis and research[52].

Lately, the Androgen Excess Society, in a position statement, defined PCOS as a predominantly hyperandrogenic syndrome and concluded that PCOS should first be considered as a disorder of androgen excess or hyperandrogenism (disorder in biosynthesis, use or metabolism of androgens)[53]. Thus, ovulatory hyperandrogenic women with polycystic ovaries on ultrasound examination (phenotype 3, Table 1) exhibit a mild form of the syndrome. Chronic oligo- or anovulatory women with polycystic ovaries on ultrasound examination also constitute a mild form of PCOS[54] (phenotype 4, Table 1), and their metabolic characteristics are considered too mild or without risk of developing any metabolic disorder, which are characteristic of women with PCOS[55,56].

Many patients with polycystic ovary syndrome (PCOS) have insulin resistance, obesity (mostly visceral), glucose intolerance and abnormalities in the

secretion of steroid hormones from the ovaries and the adrenal gland, conditions associated with abnormalities in the production of vaspin. Accordingly, recent studies evaluated vaspin levels in women with PCOS.

Currently, there are only four studies that assessed serum vaspin levels in women with PCOS with controversial results. The first study included a small number of subjects (12 women with PCOS and 12 controls)[57]. Serum vaspin levels were evaluated and vaspin gene (mRNA) expression was determined in both subcutaneous and omental adipose tissue in vitro. Additionally, the effects of glucose, insulin and steroid hormone administration on vaspin gene expression and on vaspin levels in the adipose tissue were examined. Moreover, the effects of metformin treatment on serum vaspin levels and on clinical, hormonal and metabolic features of PCOS were evaluated. This study reported: a) higher serum vaspin levels in women with PCOS than in controls ($p < 0.05$), b) increased vaspin mRNA and vaspin levels in the omental adipose tissue of PCOS women ($p < 0.05$) and increase ($p < 0.001$) in vaspin protein levels and secretion into conditioned media after adding glucose to omental adipose tissue explants and c) a significant decrease in serum vaspin levels in PCOS women after six months of metformin treatment ($p < 0.001$).

Similar results, i.e. decreased vaspin levels, were reported in female diabetic patients, who were receiving metformin compared with matched controls[58]. In addition, female patients with glycosylated hemoglobin (HbA_{1c}) levels > 7% had higher serum vaspin levels than patients with HbA_{1c} levels < 7%. Thus, the impaired glucose regulation in diabetic patients is associated with increased serum vaspin levels. Additionally, decreased serum vaspin levels were reported in female patients with microangiopathy (diabetic retinopathy, nephropathy and neuropathy). In contrast, in non diabetic subjects, serum vaspin levels did not correlate with indices of insulin sensitivity[59].

The second study[60] of vaspin levels in PCOS included a larger number of PCOS women and controls (42 PCOS women and 42 obese nonhyperandrogenic women; 26 subjects were severely obese, 15 with PCOS). Serum vaspin levels were determined in all women. In addition, serum vaspin levels were evaluated in the 26 severely obese patients after bariatric surgery and in 34 PCOS patients after treatment with either metformin (n = 19) or an antiandrogenic oral contraceptive pill (n = 15). In this study, serum vaspin concentration was not significantly influenced by PCOS or obesity. Circulating vaspin levels were also similar in women with normal or impaired glucose tolerance. After bariatric surgery, which resulted in significant weight loss and reduction in waist circumference, serum vaspin levels decreased significantly, irrespective of the presence or absence of PCOS. Moreover, serum vaspin levels decreased slightly after treatment with metformin for 24 months and increased significantly in women treated with oral contraceptives. The authors concluded that the change in vaspin levels might represent a compensatory mechanism against insulin resistance and glucose intolerance.

In the third study[61] serum vaspin levels were determined in 24 patients with PCOS, in 23 women with polycystic ovaries (PCO) and in 24 controls. Patients with PCOS or PCO had higher serum vaspin levels than controls.

We recently reported our findings in 79 patients with PCOS and 50 healthy female volunteers[62]. Normal weight patients with PCOS (n = 25) were treated with metformin 850 mg bid for 6 months. Overweight/ obese patients with PCOS (n = 54) were prescribed a normal-protein, energy-restricted diet for 6 months; half of them were also given orlistat 120 mg tid and the rest were given sibutramine 10 mg qd. At baseline and after 6 months, serum vaspin levels and anthropometric, metabolic and hormonal features of PCOS were determined. Overall, patients with PCOS had higher vaspin levels than controls ($p = 0.021$). Normal weight patients with PCOS had higher vaspin levels than normal weight controls ($p = 0.043$). Vaspin levels were non-significantly higher in overweight/ obese patients with PCOS than in overweight/obese controls. In normal weight patients with PCOS, metformin reduced vaspin levels non-significantly. In overweight/ obese patients with PCOS, diet plus orlistat or sibutramine did not affect vaspin levels. Vaspin levels were independently correlated with body mass index in women with PCOS ($p = 0.001$) and with waist circumference in controls ($p = 0.015$). In conclusion, our results suggest that serum vaspin levels are elevated in PCOS

but neither a small weight loss, nor metformin affect vaspin levels significantly.

As a conclusion, current data on serum vaspin levels in PCOS women are controversial and there is a need for additional larger studies to elucidate the role of vaspin in PCOS.

Βασπίνη: μια νέα λιποκίνη, μέλος της οικογένειας των αναστολέων των πρωτεασών της σερίνης.

Αικατερίνη Κόιου[1], Εμμανουήλ Καλαϊτζάκης[2], Κωνσταντίνος Τζιόμαλος[3], Στυλιανός Μαυρίδης[1], Κωνσταντίνος Δίνας[1], Θεοχάρης Ταντανάσης[1], Ηλίας Κατσίκης[1], Αριστοτέλης Λουφόπουλος[1], Δημήτριος Πανίδης[1]

[1]*Μονάδα Ενδροκρινολογίας και Ανθρώπινης Αναπαραγωγής, Β΄ Μαιευτική και Γυναικολογική Κλινική, Α.Π.Θ., Ιπποκράτειο Νοσοκομείο, Θεσσαλονίκη, Ελλάδα*

[2]*Κλινική Παραγωγικών Ζώων, Τομέας Κλινικών, Κτηνιατρική Σχολή, Α.Π.Θ., Θεσσαλονίκη, Ελλά δα*

[3]*Α΄ Προπαιδευτική Παθολογική Κλινική, Α.Π.Θ., ΑΧΕΠΑ, Θεσσαλονίκη, Ελλάδα*

ΠΕΡΙΛΗΨΗ: Το 2000 απομονώθηκε από το σπλαγχνικό λιπώδη ιστό μία νέα λιποκίνη, που ανήκει στην υπεροικογένεια των σερπινών, η βασπίνη. Η βασπίνη παράγεται, ενδεχομένως, από το σπλαγχνικό λιπώδη ιστό και σχετίζεται με την αντίσταση στην ινσουλίνη, τα επίπεδα του σακχάρου, τις ορμόνες, το φύλο (υψηλότερες τιμές στις γυναίκες από τους άνδρες) και τη διατροφή. Επιπλέον, επηρεάζεται από την απώλεια βάρους και τη λήψη φαρμάκων. Έτσι, η βασπίνη φαίνεται να συνδέει την παχυσαρκία με τις μεταβολικές της επιπτώσεις στον άνθρωπο. Έχει, μάλιστα, αναφερθεί ότι η λιποκίνη αυτή θα μπορούσε να θεωρηθεί ένας καινούργιος βιο-δείκτης για την παχυσαρκία και την αντίσταση στην ινσουλίνη. Πρέπει, πάντως, να σημειωθεί ότι στις διάφορες μελέτες τα ευρήματα είναι αντικρουόμενα, γεγονός που καθιστά ελκυστικό και αναγκαίο τον περαιτέρω έλεγχο της βασπίνης σε διάφορες παθολογικές καταστάσεις, όπως το σύνδρομο των πολυκυστικών ωοθηκών.

Λέξεις Κλειδιά: *Βασπίνη, Σερπίνη, Παχυσαρκία, Αντίσταση στην ινσουλίνη, Σύνδρομο πολυκυστικών ωοθηκών.*

REFERENCES

1. Di Cera E. Serine Proteases. Washington University School of Medicine, St. Louis, MO. Available at: http://www.scitopics.com/Serine_Proteases.html. Last updated on 18 August 2008.
2. Ekici ÖD, Paetzel M, Dalbey RE. Unconventional serine proteases: Variations on the catalytic Ser/His/Asp triad configuration. Protein Science 2008;17:2023-3.
3. Neitzel JJ. Enzyme Catalysis: The Serine Proteases. Nature Education 2010;3:21.
4. Potempa J, Korzus E, Travis J. The Serpin Superfamily of Proteinase Inhibitors: Structure, Function, and Regulation. J Biol Chem 1994;269:15957-60.
5. Irving JA, Pike RN, Lesk AM, Whisstock JC. Phylogeny of the Serpin Superfamily: Implications of Patterns of Amino Acid Conservation for Structure and Function. Genome Res 2000;10:1845-64.
6. Law RHP, Zhang Q, McGowan S, Buckle AM, Silverman GA, Wong W, et al. An overview of the serpin superfamily. *Genome Biol* 2006;7:216-27.
7. Silverman GA, Bird PI, Carrell RW, Church FC, Coughlin PB, Gettins PG et al.: 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;276:33293-6.
8. Irving JA, Pike RN, Dai W, Bromme D, Worrall DM, Silverman GA, et al. Evidence that serpin architecture intrinsically supports papain-like cysteine protease

inhibition: engineering alpha (1)-antitrypsin to inhibit cathepsin proteases. Biochemistry 2002;41:4998-5004.
9. Gettins P, Patston PA, Schapira M. The role of conformational change in serpin structure and function. Bioessays 1993;15:461-7.
10. Torpy DJ, Bachmann AW, Grice JE, et al. Familial corticosteroid-binding globulin deficiency due to a novel null mutation: association with fatigue and relative hypotension. J Clin Endocrinol Metab 2001;86:3692-700.
11. Refetoff S, Murata Y, Mori Y, Janssen OE, Takeda K, Hayashi Y. Thyroxine-binding globulin: organization of the gene and variants. Horm Res 1996;45:128-38.
12. Kim HS, Krege JH, Kluckman KD, et al. Genetic control of blood pressure and the angiotensinogen locus. Proc Natl Acad Sci 1995;92:2735-9.
13. Irving JA, Cabrita LD, Kaiserman D, Worrall MM, Whisstock JC. Evolution and classification of the serpins superfamily. In: Silverman GA and Lomas DA (eds) Molecular and cellular aspects of the serpinopathies and disorders in serpin activity. Singapore: World Scientific Publishing Co, 2007;1-34.
14. Trujillo ME, Scherer PE. Adipose tissue-derived factors: impact on health and disease. Endocr Rev 2000;627:762-78.
15. Kershaw EE, Flier JF. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 2004;89:2548-56.
16. MacDougald OA, Burant CF. The rapidly expanding family of adipokines. Cell Metabolism 2007;6:159-61.
17. Ouchi N, Kihara S, Funahashi T, Nakamura T, Nishida M, Kumada M, et al. Reciprocal association of C-Reactive protein with adiponectin in blood stream and adipose tissue. Circulation 2003;107:671-4.
18. Devaraj S, Singh U, Jialal I. The evolving role of C-Reactive protein in atherothrombosis. Clin Chem 2009;55:229-38.
19. Hernández-Espinosa D, Ordóñez A, Vicente V, Corral J. Factors with conformational effects on haemostatic serpins: Implications in thrombosis. Thromb Haemost 2007;98:557-63.
20. Wajchenberg BL. Subcutaneous and Visceral Adipose Tissue: Their Relation to the Metabolic Syndrome. Endocrine Reviews 2000;21:697-738.
21. Grundy SM. Metabolic Syndrome: A Multiplex Cardiovascular Risk Factor. J Clin Endocrinol Metab 2007;92:399-404.
22. Nigro J, Osman N, Dart AM, Peter J. Little Insulin Resistance and Atherosclerosis. Endocrine Reviews 2006;27:242-59.
23. Shimomura I, Funahashi T, Takahashi M, et al. Enhanced expression of PAI-1 in visceral fat: possible contributor to vascular disease in obesity. Nat Med 1996;2:800-3.
24. Mauras N, Del Giorno C, Kollman C, et al. Obesity without established comorbidities of the metabolic syndrome is associated with a proinflammatory and prothrombotic state, even before the onset of puberty in children. J Clin Endocrinol Metab 2010;95:1060-8.
25. Juhan-Vague I, Alessi MC, Morange PE. Hypofibrinolysis and increased PAI-1 are linked to atherothrombosis via insulin resistance and obesity. Ann Med 2000;32(Suppl 1):78-84.
26. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-α: direct role in obesity-linked insulin resistance. Science 193;259:87-9.
27. Ebihara K, Ogawa Y, Masuzaki H, Shintani M, Miyanaga F, Aizawa-Abe M, et al. Transgenic overexpression of leptin rescues insulin resistance and diabetes in a mouse model of lipoatrophic diabetes. Diabetes 2001;50:1440-8.
28. Goldberg RB. Cytokine and cytokine-like inflammation markers, endothelial dysfunction, and imbalanced coagulationin development of diabetes and its complications. J Clin Endocrinol Metab 2009;94:3171-82.
29. Guerre-Millo M. Adipose tissue secretory function: implication in metabolic and cardiovascular complications of obesity. J Soc Biol 2006;200:37-43.
30. Kawano K, Hirashima T, Mori S, Saitoh Y, Kurosum M, Natori T. Spontaneous long-term hyperglycemic rat with diabetic complications, Otsuka Long-Evans Tokushima Fatty (OLETF) strain. Diabetes 1992;41:1422-8.
31. Hida K, Wada J, Zhang H, Hiragushi K, Tsuchiyama Y, Shikata K, et al. Identification of genes specifically expressed in the accumulated visceral adipose tissue of OLETF rats. J Lipid Res 2000;41:1615-22.
32. Hida K, Wada J, Eguchi J, Zhang H, Baba M, Seida A, et al. Visceral adipose tissue-derived serine protease inhibitor: a unique insulin-sensitizing adipocytokine in obesity. Proc Natl Acad Sci 2005;102:10610-5.
33. Kloting N, Berndt J, Kralisch S, Kovacs P, Fasshauer M, Schön MR, et al. Vaspin gene expression in human adipose tissue:association with obesity and type 2 diabetes. Biochem Biophys Res Commun 2006;339:430-6.
34. Li Q, Chen R, Moriya J, Yamakawa J, Sumino H, Kanda T, et al. A novel adipocytokine, visceral adipose tissue-derived serine protease inhibitor (vaspin) and obesity. J Int Med Res 2008;36:625-9.
35. Seeger J, Ziegelmeier M, Bachmann A, Lössner U, Kratzsch J, Blüher M, et al. Serum levels of the adipokine Vaspin in relation to metabolic and renal parameters. J Clin Endocrinol Metab 2008;93:247-51.
36. Youn BS, Klöting N, Kratzsch J, Park JW, Lee N, Song ES, et al. Serum vaspin concentrations in human

obesity and type 2 diabetes. Diabetes 2008;57:372-7.
37. Jeong E, Youn BS, Kim DW, Kim EH, Park JW, Namkoong C, et al. Circadian rhythm of serum vaspin in healthy male volunteers: relation to meals. J Clin Endocrinol Metab 2010;95:1869-75.
38. Nestler JE. Metformin for the treatment of the polycystic ovary syndrome. N Engl J Med 2008;358:47-54.
39. Balen AH, Conway GS, Kaltsas G, Techatraisak K, Manning PJ, West C, et al. Polycystic ovary syndrome: the spectrum of the disorder in 1741 patients. Hum Reprod 1995;10:2107-11.
40. Legro RS. The genetics of obesity. Lessons for polycystic ovary syndrome. Ann NY Acad Sci 2000;900:193-202.
41. Chang RJ, Nakamura RM, Judd HL, Kaplan SA. Insulin resistance in nonobese patients with polycystic ovarian disease. J Clin Endocrinol Metab 1983;57:356-9.
42. Dunaif A, Graf M, Mandeli J, Laumas V, Dobrjansky A. Characterization of groups of hyperandrogenic women with acanthosis nigricans, impaired glucose tolerance, and/or hyperisulinemia. J Clin Endocrinol Metab 1987;65:499-507.
43. Dunaif A, Segal KR, Futterweit W, Dobrjansky A. Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. Diabetes 1989;38:1165-74.
44. Dunaif A. Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. Endocrine Reviews 1997;18:774-800.
45. Ehrmann DA, Barnes RB, Rosenfield RL, Cavaghan MK, Imperial J. Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. Diabetes Care 1999;22:141-6.
46. Legro RS, Kunselman AR, Dodson WC, Dunaif A. Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. J Clin Endocrinol Metab 1999;84:165-9.
47. Legro RS, Gnatuk CL, Kunselman AR, Dunaif A. Changes in glucose tolerance over time in women with polycystic ovary syndrome: a controlled study. J Clin Endocrinol Metab 2005;90:3236-42.
48. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 2004;81:19-25.
49. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004;19:41-7.
50. Zawadski JK, Dunaif A. Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In: Dunaif A, Givens JR, Haseltine FP, Merriam GE, eds. Hershman SM, series ed. Polycystic ovary syndrome. Current issues in endocrinology and metabolism. Boston: Blackwell Scientific Publications, 1992:377-84.
51. Azziz R. Controversy in clinical endocrinology: diagnosis of polycystic ovarian syndrome: the Rotterdam criteria are premature. J Clin Endocrinol Metab 2006;91:781-5.
52. Franks S. Controversy in clinical endocrinology: diagnosis of polycystic ovarian syndrome: in defense of the Rotterdam criteria. J Clin Endocrinol Metab 2006;91:786-9.
53. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, et al, Androgen Excess Society. Positions statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an Androgen Excess Society guideline. J Clin Endocrinol Metab 2006;91:4237-45.
54. Carmina E, Lobo RA. Do hyperandrogenic women with normal menses have polycystic ovary syndrome? Ferti Steril 1999;71:319-22.
55. Broekmans FJ, Knauff EA, Valkenburg O, Laven JS, Eijkemans MJ, Fauser BC. PCOS according to the Rotterdam consensus criteria: change in prevalence among WHO-II anovulation and association with metabolic factors. Brit J Obstet Gynecol 2006;113:1210-7.
56. Welt CK, Gudmundsson JA, Arason G, Adams J, Palsdottir H, Gudlaugsdottir G, et al. Characterizing discrete subsets of polycystic ovary syndrome as defined by the Rotterdam criteria: the impact of weight on phenotype and metabolic features. J Clin Endocrinol Metab 2006;91:4842-8.
57. Tan BK, Heutling D, Chen J, Farhatullah S, Adya R, Keay SD, et al. Metformin decreases the adipokine Vaspin in overweight women with polycystic ovary concomitant with improvement in insulin sensitivity and a decrease in insulin resistance. Diabetes 2008;57:1501-7.
58. 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.
59. Loeffelholz C, Möhlig M, Arafat AM, Isken F, Spranger J, Mai K, et al. Circulating vaspin is unrelated to insulin sensitivity in a cohort of nondiabetic humans. Eur J Endocrinol 2010;162:507-13.
60. Escobar-Morreale HF, Luque-Ramirez M, San-Millan JL. Serum visceral adipose tissue-derived serine protease inhibitor concentrations in human obesity and polycystic ovary syndrome. Diabetes Care 2009;32:e6.

61. Cakal E, Ustun Y, Engin-Ustun Y, Ozkaya M, Kilinc M. Serum vaspin and C-reactive protein levels in women with polycystic ovaries and polycystic ovary syndrome. Gynecol Endocrinol 2010 Jul 13 [Epud ahead of print].

62. Koiou E, Tziomalos K, Dinas K, Katsikis I, Kalaitzakis E, Panidis D, et al. The effect of weight loss and treatment with metformin on serum vaspin levels in women with polycystic ovary syndrome. Endocr J. 2011;58:237-46.