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REVIEW

Vaspin in obesity and diabetes: pathophysiological and clinical significance

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Abstract Vaspin (visceral adipose tissue-derived serpin; serpinA12) was originally identified as an adipokine, which is predominantly secreted from visceral adipose tissue in Otsuka Long-Evans Tokushima fatty (OLETF), an animal model of obesity and type 2 diabetes. Consistent with that higher vaspin serum concentrations and increased vaspin mRNA expression in human adipose tissue were found to be associated with obesity, insulin resistance, and type 2 diabetes in humans. However, the mechanisms how vaspin secretion may be linked to deterioration of glucose metabolism and insulin sensitivity are not entirely understood. Vaspin serum concentrations show a food intake-related diurnal variation. Vaspin is also expressed in the skin, hypothalamus, pancreatic islets, and stomach. Administration of vaspin to obese mice improves glucose tolerance, insulin sensitivity, and reduces food intake. Until now molecular target(s) of vaspin and its mode of action are unknown. Thus, identification of the proteases, which are inhibited by vaspin may lead to the development of novel strategies in the treatment of obesity, diabetes and insulin resistance. This review discusses the clinical relevance of vaspin in the pathophysiology of obesity and type 2 diabetes.

Keywords Vaspin · Adipokines · Obesity · Type 2 diabetes · Visceral obesity

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Introduction

Adipose tissue is a highly active endocrine organ secreting a number of bioactive molecules called adipokines [1–3]. Adipokines participate in various metabolic processes including the regulation of appetite control, insulin sensitivity and insulin secretion, energy expenditure, cardiovascular function, and inflammation [2–6]. Adipocyte and adipose tissue dysfunction belong to the primary defects in obesity and may link obesity to several health problems including increased risk of insulin resistance, type 2 diabetes, fatty liver disease, hypertension, dyslipidemia, atherosclerosis, dementia, airway disease, and some cancers [3, 4]. With the development of adipose tissue inflammation, dysfunction signals from adipose tissue are shifted towards a pro-inflammatory, atherogenic, and diabetogenic adipokine pattern [3, 4, 7]. Although it is difficult to determine the quantitative contribution of adipose tissue to the low grade inflammatory state in obesity, increased production of pro-inflammatory adipokines significantly contribute to maintain the inflammatory process in obesity [3, 7] and may cause insulin resistance in the liver, muscle, and other organs [8, 9].

After the discovery of leptin as an adipose tissue-derived hormone [10], several other adipokines have been discovered and found to contribute to insulin resistance, metabolic disturbances, and cardiovascular risk [1, 3, 7]. Adipokines play an important role in the cross-talk between adipose tissue and other tissues and organs including the brain, liver, vascular system, skeletal muscle, and others [3, 4]. For example, leptin controls food intake and energy expenditure and has atherogenic and growth properties [3, 6, 11]. Leptin decreases orexigenic and increases anorexigenic peptide synthesis in the hypothalamus thereby decreasing appetite [11]. Originally, leptin was cloned in 1994 as the protein

product of the ob gene mutation, which leads to extreme obesity in the *ob/ob* mouse model [10]. The importance of altered leptin signalling for the development of obesity and diabetes is further supported by the discovery that a mutation in the leptin receptor gene causes obesity and diabetes in *db/db* mice [12]. The most abundant protein secreted by white adipose tissue is adiponectin [13, 14]. Adiponectin has important anti-diabetic, anti-atherogenic, and anti-inflammatory properties and accumulating evidence suggests that adiponectin may also have anti-cancer properties and be cardioprotective [13]. In contrast to many other adipokines, adiponectin serum concentration is decreased in obesity, type 2 diabetes and other insulin-resistant states [13, 14]. Furthermore, adiponectin expression and secretion increase upon improved insulin sensitivity and weight loss [13, 14]. Another example for the complexity of adipokine effects is apelin, which is in addition to adipose tissue expressed in the central nervous system, particularly in the hypothalamus and in many peripheral tissues [15]. Apelin has been shown to be involved in the regulation of cardiovascular and fluid homeostasis, food intake, cell proliferation, and angiogenesis [15]. During the past decade it became clear, that adipose tissue secretes many more molecules including pro-inflammatory cytokines and adipokines including TNFα, transforming growth factor β (TGFβ) and interferon-γ, C-reactive protein (CRP), interleukins (IL)-1, -6, -8, -10, plasminogen activator inhibitor-1 (PAI-1), fibrinogen, haptoglobin, angiopoietin-related proteins, metallothionein, complement factor 3, serum amyloid A (SAA) protein, anandamide, and 2-AG as well as chemoattractant cytokines, such as monocyte chemotactic protein-1 (MCP-1), progranulin and macrophage inflammatory protein-1α [1, 3, 4, 6–8]. The search for novel adipokines linking obesity to related co-morbidities has become a major topic in obesity research. In this context, visceral adipose tissue-derived serpin (vaspin) has gained interest in obesity research after the observation that its expression in adipose tissue is related to worsening of metabolic parameters in a rat model of obesity [16]. Administration of vaspin to obese mice improves glucose tolerance, insulin sensitivity, and altered gene expression of candidate genes for insulin resistance [16], suggesting vaspin as an attractive candidate for drug development [17]. In addition, we could demonstrate that central vaspin administration leads to reduced food intake and has sustained blood glucose-lowering effects [18].

Sources of vaspin and mechanism of action

Vaspin was identified as member serpin A12 of the serine protease inhibitor family [19–21], which has been found to be expressed in visceral adipose tissue of Otsuka Long-Evans Tokushima Fatty (OLETF) rats at the age when obesity and insulin plasma concentrations reach a peak [16]. In OLETF rats, vaspin serum levels were markedly reduced in parallel with ageing and the development of severe hyperglycemia, a process which could be reversed by insulin or pioglitazone treatment [16]. Human vaspin protein consists of 415 amino acids and homology analyses indicated that vaspin has ~40% identity with α_1-anti-trypsin [16]. Serpins inhibit serine proteases by a unique suicide mechanism. They contain an exposed reactive centre loop (RCL) that is presented to the target protease as a pseudosubstrate. The amino acid sequence of the RCL determines which serine protease will be inhibited by the serpin. Binding of the protease to the RCL induces conformation changes of the serpin which thus deforms the reactive centre of the protease and inactivates it. Noteworthy, the target protease of vaspin has not been identified yet. It has been shown that recombinant human vaspin failed to inhibit protease activity of trypsin and other known common proteases [16]. Recently, a significant association of vaspin SNP rs2236242 with type 2 diabetes has been reported in 2,759 participants of the KORA F3 study [22]. Kempf et al. [22] found that the AA genotype is independently of obesity associated with an increased diabetes risk and suggests vaspin as candidate gene for impaired glucose metabolism.

Vaspin expression has been found in human adipose tissue [23], stomach [18], liver, pancreas [24] as well as in hypothalamus of *db/db* and C57BL/6 mice [18]. Lean human individuals had undetectable vaspin mRNA in visceral and subcutaneous (SC) fat, whereas the frequency of subjects with detectable vaspin mRNA expression in visceral adipose tissue increased from overweight to obese individuals [23]. In accordance with the original data from OLETF rats, we found significantly higher vaspin gene expression in visceral compared to SC adipose tissue [23]. Further analysis of vaspin mRNA expression in adipocytes and cells of the stromal vascular fraction (SVF) revealed vaspin expression in the SVF in addition to preadipocytes and mature adipocytes of human omental adipose tissue [25]. In addition, skin has been shown to express relatively high vaspin mRNA levels, both in mice and humans [26]. There are no data on the effects of vaspin loss or gain of function animal models available yet. However, administration of recombinant vaspin to obese mice improves glucose tolerance, insulin sensitivity, affected gene expression of candidate genes for insulin resistance [16] and acutely reduces food intake [18]. The exact mechanisms how vaspin secretion may be linked to deterioration of glucose metabolism and insulin sensitivity are not understood. From the previous data on vaspin action, it can be postulated that vaspin inhibits a protease which plays a role in the degradation of a hormone or molecule with direct or indirect glucose lowering effects (Fig. 1).

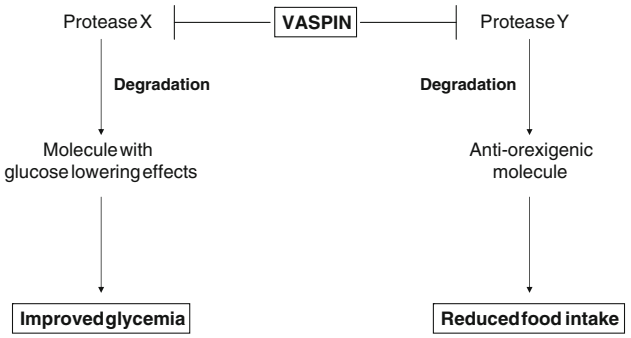

Fig. 1 Proposed mechanism of vaspin action. Vaspin has been shown to improve glycemia and reduce food intake in rodent models. These observations suggest that vaspin inhibits proteases which degrade molecules with glucose lowering effects as well as anti-orexigenic factors. Noteworthy, "protease X" and "protease Y" may represent the same molecule

Serum vaspin concentration in obesity and metabolic diseases

We and others recently found a sexual dimorphism in circulating vaspin with higher serum concentrations in lean healthy women compared to men [27, 28]. In addition, vaspin serum concentration correlates with age in lean individuals with normal glucose tolerance [27]. Körner et al. [24] recently showed that gender differences in circulating vaspin levels develop during pubertal progression in girls. In children, vaspin serum concentration has been shown to increase with worsening insulin resistance and was acutely down-regulated following glucose provocation in insulin-resistant adolescents independent of obesity [24]. Interestingly associations of circulating vaspin with age, gender, and BMI are abrogated in obese patients with chronic metabolic and cardiovascular diseases [29, 30]. Elevated vaspin serum concentrations have been associated with obesity, impaired insulin sensitivity, and fitness level [27, 31, 32]. Circulating vaspin significantly correlates with leptin serum concentrations supporting the notion that vaspin closely reflects body fat mass [27]. The association between circulating vaspin and parameters of obesity and insulin sensitivity seems to be abolished in patients undergoing chronic hemodialysis [33]. In overweight women with polycystic ovary syndrome and insulin resistance, metformin decreases circulating vaspin in parallel to improvements of insulin sensitivity [31]. The effects of metformin on circulating vaspin have been confirmed in drug-naïve patients with type diabetes [32, 34] and extended by the finding that improved glucose metabolism and insulin sensitivity are the strongest predictors of changes in vaspin serum concentrations.

In contrast to these data, several studies did not find an association between circulating vaspin and insulin sensitivity [28, 33, 35, 36] or parameters of obesity and fat distribution [35, 36]. The observation that vaspin is predominantly expressed in visceral adipose tissue [16, 19] has been recently challenged by data in Korean women, who had significantly higher vaspin expression in SC compared to visceral fat [37]. Interestingly, in this study, serum vaspin concentration significantly correlated with fasting insulin, HOMA-IR, and the ratio of visceral to SC vaspin expression [37]. In contrast to these and our own previous data [19, 37] in a systematic comparison between BMI-, age-, and gender-matched insulin sensitive versus insulin resistant healthy obese individuals, we found indistinguishable vaspin levels between these groups [38] suggesting that the association between circulating vaspin, fat distribution, and insulin sensitivity is more complex and may be regulated by so far unrecognized factors.

Vaspin serum concentrations in cardiovascular disease

We have recently shown that low vaspin serum concentrations correlate with recently experienced ischemic events in patients with carotid stenosis despite the lack of an association between circulating vaspin and parameters of atherosclerosis severity [29]. Therefore, vaspin could serve as a novel marker of unrecognized symptoms of carotid artery stenosis. Another finding of our study was that overweight patients with carotid stenosis showed significantly highest serum vaspin concentrations compared to both obese and lean patients suggesting an U-shaped relationship between BMI and serum vaspin. Recently, we found a similar U-shaped association in healthy females [27]. The causative factors for this U-shaped relationship between BMI and circulating vaspin need to be further investigated. Extending our findings in patients with carotid stenosis, Li et al. [39] found that low vaspin plasma concentrations as well as low vaspin mRNA expression in peripheral blood mononuclear cells predict both coronary artery disease (CAD) and unstable angina pectoris. In the context of the Kozani study of 108 patients with angiographically proven stable, asymptomatic CAD and 65 healthy controls, low vaspin concentrations correlate with CAD severity [40]. In patients with nonalcoholic fatty liver disease, vaspin serum concentration predicts coronary flow reserve suggesting a complex interplay between ectopic fat disposition, serum vaspin, and liver histology in promoting an impaired hyperemic stimulation of coronary blood flow [41]. A potential role of vaspin linking adverse fat distribution to cardiovascular disease is further supported by distinct vaspin mRNA expression profiles in periaortic, pericoronary, and apical epicardial adipose tissue which correlates with either aortic or coronary atherosclerosis suggesting that locally produced vaspin may affect the atherosclerotic process [42]. Very recently, it has been shown that vaspin

protects vascular endothelial cells against free fatty acid-induced apoptosis suggesting direct beneficial effects of vaspin on the protection against atherosclerosis [43]. Noteworthy, in patients with hypercholesterolemia and moderate estimated cardiovascular risk, atorvastatin administration has been shown to increase serum vaspin levels compared to lifestyle modification [44].

Exercise-induced oxidative stress decreases circulating vaspin

A recent study demonstrated that increased fat mass, but also low cardiorespiratory fitness are associated with increased vaspin serum concentrations [45]. In a 4-weeks physical exercise program, we found increased vaspin serum concentrations, which were significantly associated with decreased BMI, but also with BMI-independent improvement in insulin sensitivity and in fitness level [27]. We therefore hypothesized that increased vaspin serum concentrations are directly related to the insulin sensitizing effects of physical activity. However, in 126 individuals with metabolic syndrome undergoing a 10-month lifestyle modification interventional program, including dietary counseling, advice on increasing physical activity and recommendations to stop or limit smoking and alcohol drinking, Kim et al. [46] did not find any changes in circulating vaspin. To further elucidate these contradictory results on the effects of physical exercise on circulating vaspin, we investigated vaspin serum concentrations in response to two different exercise interventions [47]. We measured circulating vaspin before and after 1-h resistance circle training as well as before and after a 4-week exercise intervention in healthy young men, which had been randomly assigned to groups with or without anti-oxidants supplementation, as previously described [48] to elucidate the potential role of exercise-induced reactive oxygen species (ROS) in modulating circulating vaspin in humans. We found, that increased oxidative stress following short- and long-term physical training decreases vaspin serum concentration, whereas changes in insulin sensitivity do not seem to regulate circulating vaspin [47]. Taken together, the available data on the effects of exercise on circulating vaspin suggest that increased physical activity may indirectly—via increased oxidative stress—regulate vaspin serum concentrations.

Vaspin administration reduces food intake and improves glucose metabolism

With the discovery vaspin in OLETF mice, Hida et al. [16] reported that administration of recombinant vaspin to obese mice improves glucose tolerance and insulin sensitivity a finding which has been confirmed in *db/db* and C57BL6 mice [18]. In this context, it should be noted that circulating vaspin levels were not significantly different in individuals with prediabetes and increasing categories of impaired glucose metabolism [49]. Interestingly, circulating vaspin levels follow a meal-related diurnal variation in humans similar to that of ghrelin [50], suggesting a previously unrecognized role of vaspin in the regulation of food intake. Serum vaspin concentrations are increased in preprandial condition followed by a significant decline in response to meals and unscheduled food ingestion after a prolonged fast significantly reduced circulating vaspin [50]. Supporting a potential role of vaspin in the regulation of food intake, we and others found vaspin expression in the hypothalamus of rodent models [18, 51] and detected vaspin in the cerebrospinal fluid of healthy individuals [18]. We could further demonstrate that peripheral and central vaspin administration decrease food intake in obese *db/db* and lean C57BL/6 mice [18]. Decreased food intake upon central vaspin administration was recently confirmed in rats supporting the hypothesis that vaspin is an adipokine triggering anorectic pathways in the hypothalamus, where reduction of NPY and increase of POMC mRNA levels mediate feeding inhibition [51]. Although the mechanism how vaspin may regulate feeding behaviour is not clear, we postulate that vaspin inhibits a protease which degrades an anti-orexigenic factor (Fig. 1).

Vaspin in weight loss intervention studies

Long-term dietary intervention typically induces a rapid weight decline that stabilizes by 6 months. This "weight loss phase" is followed by either weight stabilization or partial to full weight regain despite continued dieting [52]. We therefore tested whether vaspin may reflect continued beneficial effects of dieting despite partial weight regain among 322 participants in the 2-year Dietary Intervention Randomized Controlled Trial (DIRECT) of low-fat, Mediterranean, or low-carbohydrate diets for weight loss [53]. Interestingly, vaspin exhibited cumulative decline despite partial weight regain throughout the study [54]. The vaspin dynamics may reflect a continuous beneficial response to the switching to healthier dietary patterns. In contrast to our data, Koiou et al. [55] did not find changes in vaspin serum concentrations in different weight loss interventions including sibutramine and orlistat treatment. In prepubertal children, diet intervention did also not affect circulating vaspin [56] whereas in obese subjects, a short-term 12-weeks weight reduction program caused significantly decreased vaspin levels [57]. In the latter study, changes in serum vaspin only correlate with body weight, BMI, waist

circumference, and hip circumference in insulin resistant subjects [57]. In accordance with our diet intervention data [54], significant weight loss following bariatric surgery caused significantly reduced vaspin serum concentrations [30]. In this intervention, changes in serum vaspin concentrations significantly correlate with the reduction of circulating leptin and insulin levels and with the amelioration of insulin sensitivity [30]. It has been recently reported that acute starvation does not affect circulating vaspin [58] suggesting that changes in vaspin in long-term weight loss interventions reflect chronic adaptations of vaspin secretion. Although decreased vaspin serum concentration in weight loss intervention studies may simply reflect reduced fat mass, the continuous decline despite partial weight in the DIRECT study [54] suggests that additional factors such as healthy diets may play a role in the regulation of circulating vaspin.

Summary and conclusions

Vaspin is an adipokine which has been related to obesity, visceral fat distribution, and metabolic and cardiovascular diseases. Variants in the vaspin gene are independently of obesity associated with an increased risk for the development of type 2 diabetes. Vaspin may become an important tool in the treatment of obesity and hyperglycemia because administration of vaspin to rodent models of obesity has been shown to significantly improve hyperglycemia and to reduce food intake. In addition, anti-apoptotic effects of vaspin have been described in endothelial cells. However, until now the mechanisms underlying these beneficial vaspin effects are not known. We hypothesize that vaspin inhibit proteases which play a role in the degradation of proteins which directly or indirectly have glucose lowering and anti-orexigenic effects. Identification of the proteases (and their target proteins) which are inhibited by vaspin will lead to a better understanding of the mode of vaspin's action and may be the basis for future pharmacologic treatment strategies.

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