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REVIEW

The contribution of different adipose tissue depots to plasma plasminogen activator inhibitor-1 (PAI-1) levels

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ABSTRACT

Increased plasma plasminogen activator inhibitor-1 (PAI-1) level is considered a mechanistic pathway through which obesity contributes to increased cardiovascular disease risk. Abdominal adipose tissue specifically, is a major PAI-1 source with visceral adipose tissue (VAT), an ectopic fat depot, generally considered to produce more PAI-1 than subcutaneous adipose tissue. However, this does not necessarily lead to increased plasma PAI-1 levels. This review provides an overview of studies investigating the association between body fat distribution and plasma PAI-1 levels. It discusses factors that influence this relationship and also considers the contribution of other tissue to plasma PAI-1 levels, placing the relative contribution of adipose tissue into perspective. In conclusion, the relationship between VAT and plasma PAI-1 levels is not fixed but can be modulated by a number of factors such as the size of the subcutaneous adipose tissue depot, ethnicity, possibly genetics and other obesity-related metabolic abnormalities.

1. Introduction

Plasminogen activator inhibitor-1 (PAI-1), the main inhibitor of fibrinolysis, contributes to increased cardiovascular risk in overweight and obese individuals [1]. Elevated plasma PAI-1 levels are considered to be a biochemical marker of obesity [2] and also as a component of the metabolic syndrome (MetS), which is characterised by dyslipidaemia, hypertension, glucose intolerance and increased abdominal fat distribution [3,4]. The association between PAI-1 and obesity, especially central obesity, has been well established in both animal and human studies [5–11] and is largely considered to be the result of PAI-1 production by adipose tissue [5,6,12,13]. PAI-1 is produced by a variety of cells contained in adipose tissue; these include pre-adipocytes, mature adipocytes, stromal cells, endothelial cells, smooth muscle cells and monocytes/macrophages [14].

Abdominal fat deposition takes place in two main fat depots [subcutaneous adipose tissue (SCAT) and intra-abdominal or visceral fat tissue (VAT)], which is considered to be an ectopic fat depot. Ectopic fat refers to the storage of fat in non-adipose tissue like the liver, skeletal muscle, viscera, pancreas and the heart [15,16]. Although, it is not yet certain how ectopic fat accumulation takes place, it is postulated to be related to an overflow of triglycerides into other organs as SCAT loses the ability to expand and to store excess energy [17]. According to this theory, the expandability of SCAT protects other organs against ectopic fat deposition [18]. In support thereof, SCAT does not seem to be associated with a linear increase in cardiovascular risk factors in obesity [19]. Furthermore, when compared to VAT, abdominal and thigh SCAT were observed to be protective against obesity-associated metabolic complications such as insulin resistance [20,21].

It is generally accepted that ectopic fat, and for the purpose of this review, VAT specifically, produces more PAI-1 than SCAT [5,22]. Consistently, computed tomography (CT) data from obese individuals have shown higher PAI-1 levels in VAT than SCAT [8,23]. Also, *PAI-1* gene expression, determined by means of adipose biopsies, has been shown to be higher in the omental adipose tissue compared to SCAT during acute systemic inflammation which was also accompanied by increased plasma PAI-1 levels [9]. However, contradicting evidence also exists, as some studies have shown comparable PAI-1 antigen ($PAI\text{-}1_{ag}$) secretion from VAT and SCAT [24], or even higher *PAI-1* messenger ribonucleic acid (mRNA) expression and increased rate of $PAI\text{-}1_{ag}$ synthesis in SCAT than in VAT [25]. Although adipose tissue PAI-1 concentration and production have important local effects, it is the concentration of PAI-1 in the blood that contributes to the development of CVD

Abbreviations: BMI, body mass index; CT, computed tomography; IL-1, interleukin-1; IL-6, interleukin-6; NRF, National Research Foundation; MetS, metabolic syndrome; mRNA, messenger ribonucleic acid; PAI-1, plasminogen activator inhibitor-1; $PAI\text{-}1_{act}$, PAI-1 activity; $PAI\text{-}1_{ag}$, PAI-1 antigen; PCR, polymerase chain reaction; SCAT, subcutaneous adipose tissue; TGF-β1, transforming growth factor B; TNF-α, tumour necrosis factor-α; VAT, visceral adipose tissue; VLDL, very low density lipoproteins; WC, waist circumference; WHtR, waist-to-height ratio.

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[reviewed by 26,27] and therefore the effect of body fat distribution on plasma PAI-1 levels has important pathophysiological consequences for the development of obesity-related CVD. Evidence regarding the contribution of the different fat depots to plasma PAI-1 levels seems conflicting. While it has been shown that adipose tissue from different body fat depots contributes to plasma PAI-1 levels [6,8,23,28,29], others found PAI-1 expression in different adipose tissue depots not to be directly related to plasma PAI-1 levels [30,31]. What is also often not considered is the relative contribution of the different fat depots to plasma PAI-1 levels, in relation to other PAI-1 producing pathways associated with obesity such as insulin resistance, increased triglycerides, inflammation and endothelial dysfunction and their influence on the different PAI-1 producing tissues (adipose tissue, hepatocytes, smooth muscle cells, platelets and endothelial cells) in the body. This review aims to provide an overview of *ex vivo* and *in vivo* studies investigating the association between fat distribution and plasma PAI-1 levels. It also discusses the contribution of other PAI-1 producing pathways found in obesity in order to draw conclusions regarding the contribution of different adipose tissue depots to plasma PAI-1 levels.

2. *Ex vivo* studies

Several human *ex vivo* studies have been carried out that show adipose tissue to be an important source of PAI-1 (Table 1). These studies can be divided into studies investigating adipocyte PAI-1 content and or gene expression and those investigating plasma PAI-1 levels. Studies investigating adipocyte PAI-1 content and or gene expression strongly support a greater production and expression of *PAI-1* mRNA in VAT compared with SCAT [12,13,22]. When using human adipose tissue in culture from obese individuals, Alessi et al. [5] found PAI-1_{ag} concentration to be greater in VAT than SCAT. The association between VAT and PAI-1 seems to be related to structural and functional differences between VAT and SCAT. Visceral adipose tissue, present in the abdominal viscera in the mesentry and omentum, produces more pro-inflammatory cytokines, such as tumour necrosis factor-α (TNF-α), transforming growth factor β (TGF-β1), interleukin-6 (IL-6) and IL-1 [28,32,33], and also contains more stromal cells, one of the main cellular components of adipose tissue that produces PAI-1 [12] as well as macrophages which also produce PAI-1 [34,35].

However, there are some contradicting findings in human *ex vivo* studies. Eriksson et al. [25] for example reported that *PAI-1* expression from abdominal SCAT was approximately two times higher than abdominal VAT in severely obese white individuals. Additionally, females were found to have a significant depot-specific PAI-1 secretion, with SCAT producing higher levels of PAI-1 than VAT [25], while these differences were not observed in men. Differences in findings could possibly be explained by the cell size of the different adipose tissue depots or even differences in study populations (as will be discussed below). In contrast with other studies [5,25,28], Eriksson et al. [25] expressed their results as nanograms of PAI-1 expressed/10^7 cells, and not ng/g adipose tissue. Since the cell size of the SCAT was found to be larger than that of VAT, it could have influenced the results. In a review, Skurk and Hauner [14] even conclude that large fat cells produce more PAI-1 than small fat cells regardless of fat depot. Furthermore, Morange et al. [28] reported a high correlation between PAI-1 production by VAT and SCAT and concluded that there could be a similar regulatory pathway of PAI-1 in these two adipose depots, despite their different anatomic and metabolic characteristics. Differences can also be seen between different SCAT depots. When investigating *PAI-1* expression from two subcutaneous territories (abdominal and femoral) in obese and lean individuals, the results indicated a higher *PAI-1* mRNA content in the abdominal SCAT of the obese participants, which was also correlated with plasma PAI-1_{ag} levels, compared with the lean participants, while no difference in the *PAI-1* mRNA content of the femoral SCAT was observed [29].

With regards to *ex vivo* studies, it should be kept in mind that the results can be influenced by the data collection and analytical method used. For example, data employing cultured human adipose tissue explants suggest a direct contribution of visceral adipose tissue to plasma PAI-1 levels [8,28,36], while studies using native human adipose tissue found similar or even lower *PAI-1* mRNA expression in VAT compared to SCAT [5,36,37]. Lindeman et al. [30] suggest that the increased PAI-1 release from adipose tissue explants is likely related to an incubation artefact rather than being a true reflection of the *in vivo* situation.

Furthermore, an increased adipocyte PAI-1 content does not necessarily relate to increased plasma PAI-1 levels. As an example, in SCAT, elevated PAI-1 gene expression was found to be present during very low-calorie diets in obese participants, while plasma PAI-1 levels were decreased [38]. Furthermore, when comparing differences between VAT and SCAT depots, no correlation was found between *PAI-1* mRNA and plasma PAI-1 (antigen and activity) in either one of the two fat depots [39]. On the other hand, Morange et al. [28] investigated the correlation between plasma PAI-1 and PAI-1 measured in adipose tissue explants by means of real-time polymerase chain reaction (PCR). They found a correlation between plasma PAI-1 (antigen and activity) and the PAI-1_{ag} level measured in cultured SCAT explants. This relationship was not investigated in VAT explants.

Due to the fact that *ex vivo* investigations are performed under controlled conditions outside the human body, it is also difficult to assess the total contribution adipose tissue might make to plasma PAI-1 levels when compared with other PAI-1 producing cells such as hepatocytes, platelets and endothelial and vascular smooth muscle cells. It is also possible that VAT and PAI-1 levels are concurrently related to abnormal fat metabolism, rather than the one bringing about the other [30]. Notwithstanding the fact that PAI-1 has a strong association with body fat distribution, these results suggest that assumptions regarding plasma PAI-1 levels based on adipose tissue *PAI-1* content and gene expression should not be made.

3. In vivo studies

In vivo human studies support the strong association between PAI-1, obesity and body fat distribution and provide more evidence for the influence of adipose tissue depots on plasma PAI-1 levels [9,23,40–43]. *In vivo* studies often use anthropometric indicators [such as body mass index (BMI), waist-hip ratio (WHR), waist circumference (WC), weight loss programs etc.] and CT scan to assess the association of body fat distribution or differences in fat depots (SCAT and VAT) with plasma PAI-1 levels. However, *ex vivo* studies use methods such as mRNA quantification or freshly collected or cultured adipose tissue samples, with or without stimulating factors. When considering the methodological differences between *in vivo* and *ex vivo* studies, it is not surprising that some studies have indicated that *ex vivo* results do not always lead to *in vivo* changes in PAI-1 plasma levels [38,39].

Several studies have used anthropometrical indicators like WC, BMI and WHR to investigate possible associations between plasma PAI-1 levels and body fat distribution [43–45]. BMI, as a general marker of body fat, was found to be strongly associated with PAI-1 [6,29,46] confirming the association of PAI-1 with total obesity. BMI does, however, not reflect body fat distribution and waist circumference, as a measure of central obesity, was demonstrated to be a significant contributor to plasma PAI-1 levels [4,41,43,44] independent of BMI [41], supporting the strong association between plasma PAI-1, VAT and central obesity. Consistently, when compared with CT scan and ultrasound, WC has been regarded as a useful surrogate for the measurement of visceral fat [47], whereas WHR and BMI seem to be associated with VAT or plasma PAI-1 to a lesser extent [47,48].

On the other hand, a stronger correlation between BMI and plasma PAI-1, as compared with WC has also been demonstrated [42]. However, in this study WC and WHR correlated with plasma PAI-1 to a similar degree. Furthermore, after adjusting for WC, an inverse association

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