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The Role of Plasminogen Activator Inhibitor-1 in the Metabolic Syndrome and Its Regulation

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Abstract

Obesity is a major risk factor for cardiovascular disease (CVD). Lipid abnormalities, hypertension, impaired glucose tolerance or diabetes, are cardiovascular risk factors that are frequently present in patients with obesity. Haemostatic and fibrinolytic disturbances are also considered to be important risk factors for CVD hence, a potential link between CVD, obesity and the metabolic syndrome arises. Regulation of the fibrinolytic system can occur at the level of plasminogen activators and plasminogen activator inhibitor-1 (PAI-1). PAI-1, a glycoprotein, is one of the most important inhibitors of fibrinolysis. Regulation of this serine protease inhibitor may have a beneficial effect on other conditions associated with the metabolic syndrome. Human adipose tissue is a source of PAI-1. PAI-1 production may in turn be controlled by a number of hormones and cytokines which are secreted by adipose tissue in addition to dietary factors. In this review we summarise the current knowledge regarding the role of altered fibrinolytic function in obesity, CVD and hence the metabolic syndrome. Regulatory factors including different dietary components, weight loss and dietary intervention will also be discussed.

Keywords: metabolic syndrome, CVD, PAI-1, dietary factors, weight loss

1. Introduction

The metabolic syndrome is a group of physical and metabolic abnormalities that result in an increased cardiovascular risk. These abnormalities include obesity, hyperglycemia, hypertension, high plasma triglycerides and low concentrations of high density lipoprotein (NCEP Report, 2002). Increased plasmatic coagulation, reduced fibrinolysis, decreased endothelial thrombo resistance and platelet hyper-reactivity (Bonetti, Lerman, & Lerman, 2003; Sobel & Schneider, 2004; Nieuwdorp, Stroes, Meijers, & Büller, 2005; Palomo, Alarcon, Moore-Carrasco, & Argiles, 2006) are also seen when the coagulation system is switched towards a pro-thrombotic state.

Cardiovascular diseases (CVDs) are a group of disorders of the heart and blood vessels. The World Health Organisation (WHO) estimates that by 2020, heart disease and stroke will become the leading cause of death and disability worldwide, surpassing infectious diseases (Murray & Lopez, 1997). Obesity is a major risk factor for cardiovascular disease (Alberti, Zimmet, Shaw, & Grundy, 2006). Lipid abnormalities, hypertension, impaired glucose tolerance and diabetes are classic cardiovascular risk factors associated with obesity, while haemostatic and fibrinolytic disturbances are also considered important factors.

Disturbances in the haemostatic and fibrinolytic systems include platelet hyperaggregability, hypercoagulability and hypofibrinolysis. These are all a part of the atherosclerotic process. Platelet hyperactivity is associated with an increase in circulating von Willebrand factor (vWF). Hypercoagulability with increases in fibrinogen, factor VII (FVII) and factor VIII (FVIII), and hypofibrinolysis is associated with increased PAI-1 levels (Juhan & Vague, 1990). A hypercoagulable and hypofibrinolytic state has been shown to predispose individuals to cardiovascular disease (Juhan, 1996). The fibrinolytic pathway provides an important endogenous antithrombotic pathway and is involved in the dissolution of blood clots. PAI-1 plays an important role in this system by limiting fibrinolysis. Elevated plasma levels of PAI-1 is a biochemical marker of obesity and may contribute to the increased risk of atherothrombotic events in patients with obesity and the metabolic syndrome. Hence the link between CVD, obesity and the metabolic syndrome arises. Recently, Cesari et al. (2013), reviewed PAI-1

linking it to fibrinolysis and age related subclinial and clinical conditions, where it was found to be an extremely promising marker, that may be used in a number of areas including prognostic evaluation, disease monitoring, and as a treatment target of age-related conditions in the future. The aim of this review is to summarise the current knowledge regarding the role of altered fibrinolytic function in obesity, CVD and hence the metabolic syndrome, and to focus on other factors including the role of PAI-1 in obesity and CVD, and its regulation through weight loss, diet and bariatric surgery.

2. Metabolic Syndrome

It is estimated that 20-25% of the worlds' adult population may be classified as having the metabolic syndrome (Alberti et al., 2006). The metabolic syndrome can be defined as any individual having type 2 diabetes, or impaired glucose tolerance, or impaired fasting glycemia, or insulinresistanceplus any two (or more) of the following four risk abnormalities: obesity, dyslipidaemia, hypertension, or microalbuminuria (Alberti & Zimmet, 1998). Individuals with the metabolic syndrome as defined by WHO, have an 2.87-3.30 fold increased risk of death from coronary heart disease (CHD), 2.63-2.96 times risk of death from CVD, and an 1.87-2.11 times increased risk of death from any cause with the presence of the metabolic syndrome (Lakka et al., 2002).

A hypercoagulable state accompanies the metabolic syndrome. Basal markers associated with thrombosis, fibrinolytic activity and function and endothelial thrombo-resistance and platelet hyper-reactivity (Anand et al., 2003) are interlinked and hence the strong link between CVD, obesity and fibrinolysis becomes more complex.

3. Haemostasis and Platelet clot Formation

Haemostasis is the process of 'arresting the escape of blood by either natural or artificial means' (Miller & Keane, 1978), in which the blood vessels, the platelets, the coagulation cascade, and the fibrinolytic system have important roles. Coagulation is a cascade of events involving two pathways the intrinsic and the extrinsic pathways, which converge to form a common pathway leading to insoluble fibrin clot formation. Numerous clotting factors and at least twelve distinct plasma glycoproteins are involved in both processes, however both pathways are distinguishable since the intrinsic pathway is activated by surface-mediated reactions, whereas the extrinsic pathway is activated by tissue-derived factors (Thompson & Harker, 1988).

3.1 Antithrombotic Mechanisms and Fibrinolysis

Under normal physiological conditions several antithrombotic mechanisms act to prevent fibrin clot formation and preserve blood flow to specific sites of vascular injury. Endothelial cells (Hirsh, Salzman, Marder, & Colman, 1994), and numerous proteins and pathways including antithrombin II, Protein C (Dahlback & Villoutreix, 2005), tissue factor pathway inhibitor (TFPI) (Price, Thompson, & Kam, 2004), and the fibrinolysis pathway display antithrombotic effects. Antithrombin is the major plasma protease inhibitor of both thrombin and the other clotting factors in coagulation. It forms a complex between the active site of the enzyme and the reactive centre of antithrombin, hence, neutralizing thrombin and other activated coagulation factors. Protein C is a plasma glycoprotein activated by thrombin on thrombomodulin, a transmembrane proteoglycan binding site for thrombin on endothelial cell surfaces. When activated Protein C becomes an anticoagulant (Dahlback & Villoutreix, 2005), and cleaves and inactivates factors V and VIII. This reaction is accelerated by protein S, a glycoprotein that undergoes vitamin K-dependent posttranslational modification. TFPI is a plasma protease inhibitor that regulates the TF-induced extrinsic pathway of coagulation (Steffel, Luscher, & Tanner, 2006). It inhibits the TF/FVIIa/FXa complex by turning off the TF/FVIIa initiation of coagulation, which then becomes dependent on the "amplification loop" via FXI and FVIII activation by thrombin. Finally, the fibrinolysis pathway is a process by which a fibrin clot is digested by plasmin a major protease enzyme, to fibrin degradation products. If any thrombin escapes the inhibitory effects of the above mentioned physiologic anticoagulant systems the endogenous fibrinolytic system is activated (Cesarman-Maus, & Hajjar, 2005).

3.2 CVD and Obesity and Fibrinolysis

The WHO definition of overweight and obesity is a body mass index (BMI) > 25 kg m^{-2} and BMI > 30 kg m^{-2}, respectively. A strong relationship exists between obesity, coagulation and fibrinolysis (Darvall, Sam, Silverman, Bradbury, & Adam, 2007). This relationship relates to fibrinogen, TF, factor VII and VIII and PAI-1. Hyperfibrinogenaemia is associated with an increased incidence of CHD (McDermott et al., 2003) and obesity (Woodward et al., 1997). Fibrinogen promotes atherosclerosis through vascular smooth muscle and endothelial cell proliferation and promotes arterial and venous thrombosis through increased fibrin formation, platelet aggregation and plasma viscosity (Reiner et al., 2001). TF initiates the coagulation cascade when it is exposed to blood and binds with factor VIIa. Obese patients exhibit increased TF-mediated coagulation, mainly due to raised adipocyte and monocyte TF expression, in addition, to elevated levels of C-reactive protein (CRP),

transforming growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α), angiotensin II and insulin (Loskutoff & Samad, 1998; Visser, Bouter, McQuillan, Wener, & Harris, 1999). Increased levels of factor VII and factor VIII correlate with measures of obesity (Cushman et al., 1996), while individuals with high triglycerides and low levels of high density lipoprotein cholesterol are also found with high factor VII and VIII levels (Cushman et al., 1996; Woodward et al., 1997). PAI-1 inhibits t-PA, which in turn reduces the cleavage of plasmin from plasminogen, and is, therefore, the primary physiological inhibitor of fibrinolysis *in vivo* (Conlan et al., 1993). However, the increased levels of PAI-1 found in obesity may predispose to micro- and macro-vascular, arterial and venous, thrombosis (Folsom, Wu, Rosamond, Sharrett, & Chambless, 1997). Obesity is one of the most common clinical conditions associated with increased PAI-1 production, where a strong correlation exists between BMI and plasma PAI-1 concentration, and elevations in plasma levels of PAI-1 are likely to contribute to the increased risk of atherothrombotic events in patients with obesity and metabolic syndrome.

4. PAI-1

Acting in the plasma and tissue, plasminogen activator inhibitor-1 has multiple functions in the cardiovascular and renal systems where it can either promote thrombosis or fibrosis (Price et al., 2004). Plasminogen activator inhibitor-1 is one of the most important inhibitors of fibrinolysis. Two plasminogen activators have been identified including t-PA and urokinase-type plasminogen activator (u-PA), while, inhibition occurs at the level of PAI-1, or at the level of plasmin, mainly by α-2 antiplasmin.

Plasminogen activator inhibitor-1 is a glycoprotein, composed of 379 amino acids and has an apparent molecular weight of 48 kDa. PAI-1 is a member of the superfamily of serine-protease inhibitors (serpins) and serves as a pseudosubstrate for plasminogen activators. In healthy individuals the ratio of PAI-1 to t-PA is 4:1. The majority of PAI-1 circulates briefly in plasma mean (mean PAI-1 antigen levels in healthy adults vary between 15 and 30 ng ml^{-1}) and is removed via a hepatic clearance mechanism. The circulating half-life of PAI-1 is approximately five minutes, hence, only a fraction of the secreted active PAI-1 has the opportunity to react with plasma t-PA. There appears to be no endogenous mechanism for recycling PA-PAI-1 complexes. The main source of plasma PAI-1 is unknown but the liver, endothelial cells and thrombocytes are known to produce PAI-1 (Bastelica et al., 2002b), and several cell types including endothelial, vascular smooth muscle cells (VSMCs), platelets, hepatocytes, fibroblast and adipocytes and mesangial express PAI-1 (Booth, Simpson, Croll, Bennett, & MacGregor, 1988; Chomiki, Henry, Alessi, Anfosso, & Juhan-Vague, 1994; Lijnen, & Collen, 1997; Brogren, Wallmark, Deinum, Karlsson, & Jern, 2011). After release into the blood stream, PAI-1 is present either in an active form or complexed with either t-PA or vitronectin. Vitronectin is a multi-functional glycoprotein found in blood and extracellular matrices, it converts PAI-1 into an inactive latent form (Loskutoff, & Samad, 1998). It is likely that vitronectin-bound PAI-1 represents the physiologically relevant form of the inhibitor in tissue. Plasminogen activators' main role is in fibrinolysis where they convert inactive zymogen plasminogen into the active fibrinolytic protease plasmin (Cushman et al., 1996).Within the fibrinolytic pathway PAI-1 specifically inhibits the tissue-type (t-PA) and urokinase-type (u-PA) plasminogen activators (Cushman et al., 1996). This highlights the association between PAI-1 and CVD progression and prevention.

4.1 PAI-1 as a Cardiovascular Risk Factor

PAI-1 is an important CVD risk factor not only in terms of increased CHD events, but also in terms of increased risk of bleeding post cardiac surgery due to high and low plasma PAI-1 levels, respectively (Ozolina et al., 2012). Hamsten et al. (1987) carried out a prospective study and showed a causal relationship between impaired fibrinolytic function and myocardial infarction. High plasma PAI-1 activity was independently related to reinfarction within three years of a primary event in men who had a first myocardial infarction before the age of forty five. An independent relationship between low fibrinolytic activity and increased risk of future episodes of CHD, was also seen in the The Northwick Park Heart Study (Meade, Ruddock, Stirling, Chakrabarti, & Miller, 1993).However, Hamsten et al. (1987) showed that PAI-1 activity or t-PA antigen or activity were not predictive for recurrent myocardial infarction. In addition in a cohort of patients with angina pectoris, no correlation was observed with PAI-1 activity, while, high basal t-PA antigen levels were found to be associated with an increased risk of myocardial infarction (Jansson, Olofsson, & Nilsoon, 1993). Johansson et al. (2000) showed t-PA/PAI-1 complex to be independently associated with the development of a first-ever stroke, especially haemorrhagic stroke, but no such association for PAI-1 could be demonstrated. This same predictive effect of high levels of t-PA antigen for stroke (Hamsten, & Eriksson, 1995) and myocardial infarction (Thompson, Kienast, Pyke, Haverkate, & van de Loo, 1995; Wiman et al., 2000) was demonstrated in various other studies. It is probable that the association between a high t-PA antigen concentration and coronary artery disease is explained by high plasma PAI-1 activity and a reduced, rather than enhanced, fibrinolytic activity (Hamsten & Eriksson, 1995). Additionally, the prospective Caerphilly study followed nearly 2000 middle aged men and found t-PA antigen,

but not PAI-1 activity, to be associated with the development of major CHD (Wiman et al., 2000), while the relationship with t-PA largely disappeared after adjusting for other risk factors. Clearly the fibrinolysis system has a role in CVD with t-PA being a predictive agent.

4.2 Adipose Tissue Expression of PAI-1 and Its Regulation

Plasma PAI-1 levels have been shown to exhibit a circadian variation (Kluft, Jie, Rijken, & Verheijen, 1988),to be affected by age (Yarnell, Sweetnam, Rumley, & Lowe, 2000), gender, the menopause (Margaglione et al., 1998), diet (Johansen, Seljeflo, Høstmark, & Arnesen, 1999), and lifestyle factors (Yarnell et al., 2000). Other important determinants of plasma PAI-1 in a given individual include hormonal, inflammatory, and metabolic factors in addition to the molecular clock, BMI, genetic determinants (Naran, Chetty, & Crowther, 2008), and nitric oxide and cyclic nucleotides (Bouchie et al., 2000).

Much research has been carried out on the participation of the adipose tissue itself in the association of obesity, insulin resistance and hypofibrinolysis. Adipose tissue from mice and rats has been shown to expresses relatively high levels of PAI-1 (Sawdey & Loskutoff, 1991; Visser et al., 1999). Human adipocytes have also been shown to be a source of PAI-1, in fact a higher expression and secretion of PAI-1 per fat cell were found in adipocytes from obese than from lean subjects (Cigolini et al., 1996; Morange et al., 1999; Gottschling-Zeller, Aprath, Skurk, & Hauner, 2000).This indicates that an enlarged adipose tissue may directly contribute to circulating PAI-1 levels in humans (Cigolini et al., 1996), and hence be closely associated with an impairment of fibrinolysis (McGill, Schneider, Arfken, Lucore, & Sobel, 1994).

There are contradictory findings however in relation to body fat distribution and depot specific differences, which are contributing factors in the secretion of PAI-1 from adipose tissue. In obese rats mesenteric fat cells produce significantly more PAI-1 than subcutaneous adipocytes, and PAI-1 mRNA increased only in visceral fat during the development of obesity (Shimomura et al., 1996). Mixed findings have been obtained in human studies, where more PAI-1 has been shown to be produced in omental fat (Gottschling-Zeller et al., 2000), visceral fat (Morange et al., 1999), and in isolated human adipocytes (Gottschling-Zeller et al., 2000) as compared to subcutaneous human fat. In addition, the stromal cell fraction of adipose tissue has also been shown to synthesize substantial amounts of the inhibitor (Bastelica et al., 2002).

Consequently, a strong correlation exists between BMI and plasma PAI-1 levels. De Taeye et al. (2005) hypothesized that PAI-1 influences adipocyte biology and the development of obesity. De Taeye et al. (2005) stated that the increase in adipose PAI-1 production associated with increased fat mass is of benefit, in that it interferes in processes which contribute to further adipose accumulation. PAI-1 deficiency in turn helps prevent the development of obesity by protecting against insulin resistance and by altering adipocyte differentiation.

Adipose tissue secretes a variety of substances that help regulate metabolic homeostasis. These substances include leptin, TNF-α, interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), PAI-1, angiotensinogen, visfatin, retinol-binding protein-4, serum amyloid A (SAA), resistin, as well as anti-inflammatory factors e.g. adiponectin (Fried, Muga, Misore, & Duffy, 1998; Yarnell et al., 2000; Steppan et al., 2001; Weisberg et al., 2003; Wellen & Hotamisligil, 2003; Xu et al., 2003). The PAI-1 promoter is very responsive to a variety of metabolic and hormonal factors that are associated with obesity, especially cytokines.

One potential mechanism by which adipose tissue derived PAI-1 is increased is via inflammation. Individuals with obesity are thought to be in a chronic low-grade proinflammatory state. This is due to the presence of leukocytosis, increased plasma levels of the proinflammatory cytokines interleukin-1, IL-6 and TNF-α, elevated acute phase proteins, for example CRP (Lemieux et al., 2001), and increased levels of markers of endothelial cell dysfunction and activation (Lyon & Hsueh, 2003). These markers can act to regulate PAI-1 production in adipose tissue either locally in an autocrine manner, or distally as an endocrine hormone (Bastelica et al., 2002b).

TNF-α is a pro inflammatory cytokine and has been shown to be a common link between obesity, insulin resistance, hyperinsulinemia, systolic blood pressure (Porter, Cutchins, Fine, Bai, & DiGirolamo, 2002) and plasma triglyceride and glucose homeostasis (Ventre et al., 1997). TNF-α and TGF-β cytokines have been clearly shown to be involved in the regulation of PAI-1 (Sawdey & Loskutoff, 1991), where the expression of both cytokines has been shown to be elevated in adipose tissue in both obese rodents and humans (Huben & Hauner, 1999; Alessi et al.,2000; Birgel, Gottschling-Zeller, Röhrig, & Hauner, 2000). While IL-6 and IL-1 are two additional cytokines that are overproduced in the setting of obesity, interleukin-1β (IL-1β) has been found to significantly increase the expression of PAI-1 in human adipocytes (Bastelica et al., 2002a). While Kralisch et al. (2006) showed that PAI-1 expression and secretion is upregulated by IL-6 in rodent fat cells (Kralischa et al., 2006) and in human adipocytes (Rega et al., 2005). Adiponectin has important metabolic and inflammation regulating effects (Shimabukuro et al., 2003), where it has important anti-diabetic, anti-atherosclerotic and

anti-inflammatory activities (Havel, 2002; Matsuda et al., 2002; Stefan, & Stumvoll, 2002; Kumada et al., 2003; Ryo et al., 2004) and a role in glucose and lipid homeostasis (Berg, Combs, & Scherer, 2002). Adiponectin levels are negatively correlated with CRP, IL-6, and PAI-1 levels (Engeli et al., 2003; Shetty, Economides, Horton, Mantzoros, & Veves, 2004), and adiponectin gene expression and secretion are inhibited by TNFα and IL-6 (Havel, 2002; Meier & Gressner, 2004).

The increases in PAI-1 associated with obesity may reflect factors that regulate PAI-1 gene expression. For example, leptin, is a hormone that plays a key role in regulating energy intake controls satiety, energy expenditure and neuroendocrine functions (White & Tartagliam, 1996), and modulates the fatty acid oxidation in the muscle. Leptin secretion is increased by TNF-α (Kershaw & Jeffreym, 2004). Leptin also increases the synthesis of CRP by acting on IL-6 receptors in the liver, thus possibly contributing to the chronic inflammatory state of obesity (Monzillo et al., 2003). Ekström et al. (2012) recently showed that an acute systemic inflammation in humans increases gene expression and protein synthesis of PAI-1 in adipose tissue, with the increase being most prominent in omental adipose tissue. Hence, PAI-1 synthesis in adipose tissue due to acute systemic inflammation may be a link between inflammation and impaired endogenous fibrinolysis.

Genetic factors may play a role in PAI-1 regulation. Over 180 single nucleotide polymorphisms have been described in the PAI-1 gene. The PAI-1-675 4G/5G polymorphism, is a single guanosine insertion/deletion, which contains an additional binding site for a DNA binding protein (Eriksson, Kallin, van't Hooft, Båvenholm, & Hamsten, 1995). This may play a role as a repressor during transcription and in turn influence plasma PAI-1 concentrations (Wiklund et al., 2005; Naran, Chetty, & Crowther, 2008). Associations between increased levels of PAI-1 and obesity, and in turn between elevated levels of PAI-1 and the presence of PAI-1 promoter 4G allele have been described in adults. Hence, this polymorphism may be a marker of genetic susceptibility for these diseases (Ozel, Aktas, & Akar, 2008). Estelles et al. (2001) evaluated the effect of weight loss and the influence of the PAI-1 promoter 4G/5G genotype on the fibrinolytic system and lipid parameters in obese children. A decrease in BMI had a favourable effect on the fibrinolytic system due to a decrease in PAI-1 levels, whereas, the 4G/5G genotype had no influence of on PAI-1 levels. De la Cruz-Mosso et al. (2013) studied the 4G/5G polymorphism in the PAI-1 gene in Mexican children, and found it to be associated with an increase of body adiposity, as shown by increased waist-hip ratio, waist circumference, and subscapular skinfold thickness. Furthermore, De Lange et al. (2013) found that polymorphisms in the promoter area of the PAI-1 gene had less of an effect on PAI-1 activity levels than obesity. Significant gene-environment interactions were also identified where urbanisation was shown to affect the phenotypic expression of the 4G/5G polymorphism, with larger differences being seen across the 4G/5G genotypes in the urban community than in the rural community. Naran et al. (2008) found that metabolic syndrome-related factors had little influence on PAI-1 levels in white subjects, whereas in African and Indians subjects, these variables had a major influence on PAI-1 levels, but, only in those with the 5G/5G genotype. Ethnic differences in PAI-1 levels are largely due to differences in the frequency of the 4G and 5G alleles at the −675 locus. Hence, in Indian and African, but not white populations, the potential of metabolic syndrome-related factors to influence PAI-1 levels is modulated by the −675 genotype. Subjects from a white European population who are homozygous for the 4G allele (4G/4G genotype) have plasma concentrations of PAI-1 approximately 25% higher than subjects who are homozygous for the 5G allele (genotype 5G/5G) (Eriksson, Kallin, van't Hooft, Båvenholm, & Hamsten, 1995). Many factors including gene-environemental interactions, in addition to the PAI-1 polymorphism, have an effect on PAI-1 levels. These findings may be taken into consideration when developing treatments addressing CVD risk factors.

5. Regulation of PAI-1 Production by Dietary Factors

There is currently only limited information on the role of dietary factors on PAI-1 gene expression, however, effects are seen nontheless. Several studies have reported regulatory effects of vitamin D on PAI-1 production in a number of cell types (Chen et al., 2011). The hormonal metabolite of vitamin D, 1,25(OH)2D3, was shown to enhance plasminogen activator activity and decrease PAI-1 production in ROB cells and osteogenic sarcoma cells (Fukumoto, Allan, & Martin, 1994). In addition, activated vitamin D analogs were shown to suppress PAI-1 in human coronary artery smooth muscle cells (Wu-Wong, Nakane, & Ma, 2006; Wu-Wong, Nakane, & Ma, 2007). Hence, *in vitro* models show that vitamin D is a physiological inhibitor of PAI-1 production.

Studies have shown that glucose is able to induce PAI-1 production in various cell types including vascular smooth muscle cells (Chen et al., 1998), human mesangial cells (Tada, Tsukamoto, Ishii, & Isogai, 1994), and human adipose tissue (He, Bruun, Lihn, Pedersen, & Richelsen, 2003). Hyperglycemia can induce PAI-1 production (Gabriely et al., 2002), in addition, it has been shown that normal subjects infused with glucose and intralipids to induce hyperinsulinaemia combined with hyperglycaemia and hypertriglyceridaemia, which are characteristic of type 2 diabetes, have significantly increased PAI-1 concentrations (Gabriely et al., 2002).

In terms of other carbohydrates, MacKay et al. (2012) investigated the effect of six weeks of consumption of whole grain wheat sourdough bread versus refined white bread on PAI-1 in adults with normal or impaired carbohydrate metabolism, and found no affect on PAI-1 levels. Conversely, it was found that adults who adopted a low glycemic index diet for three weeks significantly attenuated circulating PAI-1 (Jarvi et al., 1999; Rizkalla et al., 2004) along with an associated reduction in postprandial glucose and insulin (Jarvi et al., 1999). Djoussé et al. (1998) examined the relation of dietary fiber intake to PAI-1 and fibrinogen concentrations and found higher fiber intake to be inversely associated with PAI- 1, but not with fibrinogen concentration.

In relation to proteins, studies have shown that both human and bovine caseins are potent accelerators of the rate of plasminogen activation by u-PA and/or t-PA (Markus, Hitt, Harvey, & Tritsch, 1993; Heegaard, Rasmussen, & Andreasen, 1994; Politis, White, Zavizioin, Goldberg, Guo, & Kindstedt, 1995). This may be due to the fact that the clotting of milk and the process of blood clotting are two coagulation processes which on the molecular levels share numerous common features (Christy & Macleod, 1989; Fiat & Jollès, 1989).

Marckmann et al. (1992) showed a reduction in fat intake did not affect t-PA and PAI-1 activity or antigen levels. In addition, a low-fat, high-fibre diet increased fibrinolytic activity, without affecting PAI-1 antigen (Marckmann, Sandström, & Jespersen, 1993). Mixed findings have been obtained in relation to fatty acids. Fatty acids have been shown to stimulate PAI-1 release in HepG2 cells (Banfi, Risé, Musssoni, Galli, & Tremoli, 1997), and to increase PAI-1 mRNA expression (Kariko, Rosenbaum, Kuo, Zurier, & Barnathan, 1995), secretion (Banfi et al., 1997) and transcription (Nilsson et al., 1998) *in vitro*. Barcelli et al. (1985) suggested that n-3 fatty acids may enhance plasma fibrinolysis, where as numerous studies reported no or even worsening of fibrinolysis (Prisco et al., 1994; Eritsland, Arnesen, Seljeflot, & Kierulf, 1995). Hansen et al. (2000) conducted a double blind, placebo-controlled trial and found no relationship between changes in serum triglycerides or phospholipid n-3 fatty acids and changes in PAI-1 activity. A review of other similar trials, found an overall 17.7% increase in PAI-1 activity following n-3 supplementation, while, only two studies were able to demonstrate a significant increase in PAI-1 attributable to n-3 fatty acid supplementation. Meta-analysis of observationalstudies on dietary and non-dietary intake of n-3polyunsaturated fatty acids and CHD, showed a reduction in overall mortality, mortality due to myocardial infarction, and sudden death in patients with CHD (Bucher, Hengstler, Schindler, & Meier, 2002). Doenyas-Barak et al. (2012) investigated the effects of statins and n-3 fatty acid consumption (1.9 g day^{-1}) in hypercholesyerolemic patients and found a reduction in platelet aggregation, IL-6, and an improvement in daytime blood pressure. These effects were not associated with lipid profile improvement or oxidative stress amelioration. Hence the effect of n-3 fatty acids on PAI-1 activity in plasma is favourable.

In relation to n-6 fatty acids several *in vitro* studies have shown that linoleic acid increases the secretion of PAI-1 in HepG2 cells (Banfi et al., 1997; Ye, He, Wang, & Liu, 2007). Lee et al. (2012) recently carried out a population-based cross-sectional study and found that total serum n-6 fatty acids were inversely associated with PAI-1 levels. However, in parallel with the effect of n-3 fatty acids mixed findings have been obtained with n-6 fatty acids (Fleischman, Justice, Bierenbaum, Stier, & Sullivan, 1975; O'Brien, Etherington, Jamieson, Vergroesen, & Ten Hoor, 1976; Byberg, Smedman, Vessby, & Lithell, 2001; Møller et al., 1992).

An increase in platelet aggregation time and a decrease in disaggregation time has been shown in individuals where linoleic acid was increased from 2.9% to 5% of energy intake (Fleischman et al., 1975). Similar results were obtained in a crossover study (Byberg et al., 2001) and clinical trial (O'Brien et al., 1976). The Nurses' Health Study, have suggested that n-6 fatty acids may lower the CHD risk through a variety of mechanisms, including a decrease in blood pressure (Zhao et al., 2009), a reduction of thrombosis (Knapp, 1997), and an improvement in insulin sensitivity (Laaksonen et al., 2002). These results are inconsistent with the results of a study by Byberg et al. (2001) who showed an inverse association between PAI-1 acitivity and serum linoleic acid and a significant positive association with serum arachidonic acid. Linoleic acid may reduce PAI-1 levels through its cholesterol lowering effect (Willett, Howe, & Kushi, 1997; Choo et al., 2010), and has been shown to reduce platelet aggregation (Fleischman et al., 1975; O'Brien et al., 1976). Hence, n-6 fatty acids may decrease platelet aggregation so that PAI-1 is decreased during vascular injury and these reduced cholesterol levels may improve or modulate the fibrinolytic response.

Much work has been completed on the effect of fish and fish oil consumption on PAI-1 levels. A fishdiet and fish oil, but not DHA-oil, was shown to inhibit plateletaggregation*in vitro* (Agren, Väisänen, Hänninen, Muller, & Hornstra, 1997). Garcia-Rodriguez et al. (2012) investigated increased consumption of n-3 long-chain polyunsaturated fatty acids (n-3 LCPUFA) from farmed Atlantic salmon during pregnancy and found plasma inflammatory-, and vascular homeostasis-biomarkers, including PAI-1, to increase as pregnancy progressed. These biomarkers exhibited unique time-dependent profiles over the second half of gestation, however they were not affected by the consumption of two portions of salmon per week providing 3.45 g eicosapentaenoic acid

(EPA) plus docosahexaenoic acid (DHA). Further to this, EPA, but not DHA, has been shown to reduce platelet activation (Park & Harris, 2002). The addition of 6 g day^{-1} of dietary DHA for 90 day to a typical Western diet containing less than 50 mg day^{-1} of DHA, produced no observable physiological changes in blood coagulation, platelet function, or thrombotic tendencies in healthy, adult males over a three month period (Nelson et al, 1997). Furthermore, a meta-analysis of observationalstudies on fishintake and CHD showed fish consumption to be associated with a significantly lower risk of fatal and total CHD. Hence, fish consumption may be an important component of lifestyle modification for the prevention of CHD (Whelton, et al, 2004).Research studying the effect of fish oils on PAI-1 show conflicting results however. Some studies have reported impaired fibrinolytic capacity (Fumeron et al., 1991), whereas others find improvements (Barcelli et al., 1985; Calles-Escandon et al., 1996; Marckmann, Toubro, & Astrup, 1998; Morange et al., 1999), or no effect (de Maat et al., 1994; Eritsland et al., 1996; Marckmann et al., 1997). Montegaard et al. (2010) investigated the acute effect of high or low n-3 LCPUFA consumption from a saturated fat beverage on plasma PAI-1, t-PA, and platelet aggregation, in men with metabolic syndrome. Both fat loads resulted in a significant increase in whole blood triacylglycerol concentration, plasma PAI-1 and t-PA concentrations, and PAI-1 activity, and a significant decrease in t-PA activity during the postprandial period. PAI-1 concentration and activity increased more following the high n-3 LCPUFA compared with the low n-3 LCPUFA beverage. Overall, acute intake of a high n-3 LCPUFA beverage shifted the balance between plasma PAI-1 and t-PA, which might indicate a lower capacity for fibrinolysis. These results are contradictory to the reduction in CVD mortality and morbidity observed in prospective studies following chronic consumption of high amounts of fish oil (Bucher et al., 2002; Whelton et al., 2004). Therefore, with respect to fibrinolytic measurements, acute and chronic intake of fish oil may show opposite effects.

Phang et al. (2009) studied the efficacy of n-3 LCPUFA to inhibit platelet aggregation*in vitro* and found significant gender differences. EPA was found to be more effective in reducing platelet aggregation compared with docosapentaenoic acid (DPA) and DHA. This differential pattern was followed in males only when results were grouped by gender, whereas in females, DHA, DPA and EPA were all equally effective. Between group analyses (n-3 LCPUFA vs. gender) showed that both DHA and DPA were significantly less effective in males compared with females, while EPA was equally effective in reducing platelet aggregation in both groups. Phang et al. (2012 a, b) further studied the acute effects of dietary supplementation with EPA or DHA rich oils on a reduction in platelet aggregation in healthy male and females and found interactions to exist between sex hormones and omega-3fatty acids. Healthy males may benefit more from EPA supplementation while females were found to be more responsive to DHA. This research was extended to include the acute effects of supplementation with EPA- or DHA-rich oils on circulating platelet microparticle levels and activity in healthy subjects. EPA and DHA were found to exert gender dependent effects on platelet aggregation and platelet microparticle activity, but not on platelet microparticle levels (Phang, Lincz, & Garg, 2013). At 24 hours post supplementation both EPA and DHA reduced platelet aggregation relative to placebo whereas only EPA reduced platelet microparticle activity. When grouped by gender, males showed a reduction in both platelet aggregation and platelet microparticle activity following EPA, while females showed significantly reduced platelet aggregation but not platelet microparticle activity after DHA only. Hence males may benefit more from EPA supplementation with respect to thrombotic disease risk. This may explain the lack of effect of DHA supplementation on platelet aggregation in published studies carried out predominantly or exclusively in men (Agren et al., 1997; Nelson et al., 1997).

In general, dietary factors alone or in combination have an effect on PAI-1 production, and may be important to consider when addressing CVD risk management.

6. Effects of Weight Loss on Fibrinolysis

Body fat mass has a major influence on the fibrinolytic activity. Numerous studies investigating the effect of weight loss on PAI-1 levels found a decrease in PAI-1 with decreasing body weight (Calles-Escandon et al., 1996; Marckmann et al., 1998;Lindahl, Nilsson, Jansson, Asplund, & Hallmans, 1999; Mavri et al., 1999; Bastard et al., 2000). Mertenes and Van Gaal, (2002), reviewed data on the association between obesity and fibrinolytic factors and found an improvement in altered fibrinolytic parameters with weight loss. A reduction in plasma PAI-1 concentrations was found to be relative to the extent of weight loss, this effect was seen in children and adolescents (Estelles et al., 2001; Sudi et al., 2001). In addition, visceral fat diameter was found to be a major determinant for PAI-1 concentrations during pronounced weight loss after bariatric surgery (Tschoner et al., 2012).

Weight loss of more than 10% of initial weight decreased PAI activity or antigen levels by up to 70% of initial levels (Marckmann et al., 1998). While a more moderate weight loss of 10% of initial body weight decreased PAI-1 levels by 26-54% (Calles-Escandon et al., 1996; Heywood, Mansfield, & Grant, 1996; Willett et al., 1997;

Lindahl et al., 1999; Mavri et al., 1999). It is not known if the decrease in PAI-1 levels is influenced by energy restriction or by weight loss. However, Svendsen et al. (1986) investigated this in postmenopausal women and found PAI-1 activity and antigen levels dropped by 46% and 30% respectively, after twelve weeks of treatment, however results were no longer significant after six months. Whereas, Marckmann et al. (1998) investigated the effects of weight maintenance on PAI-1 levels of obese individuals and found PAI-1 levels to be 34% lower compared to baseline after twenty four weeks of weight maintenance. A review of other studies found mixed findings in relation to changes in weight, BMI or body fat and the changes in PAI-1 levels, with most (Calles-Escandon et al., 1996; Willett et al., 1997; Lindahl et al., 1999), but not all (Primrose, Davies, Prentice, Hughes, & Johnston, 1992) studies finding a correlation. Lindahl et al. (1999) carried out an intensive lifestyle intervention study in subjects with impaired glucose tolerance. The programme consisted of a low-fat, high-fibre diet, regular physical exercise and behaviour modification conducted in a fullboard wellness centre. The study found a greater reduction in PAI-1 activity than a usual care programme. In other studies different types of physical exercise were also shown to significantly improve impaired fibrinolytic activity (Estellés et al., 1989; Gardner, & Killewich, 2002). However, data from these studies do not allow us to decide whether the relationship between weight loss and decrease in PAI-1 levels is causal or indirect. Weight loss is accompanied by an improvement in insulin resistance, and a reduction in plasma triglycerides and glucose levels. These metabolic changes may account in the improvement in fibrinolytic activity. Hence, further studies are needed to elucidate the issue to determine if diet or exercise are causing this effect.

Bariatric surgery has a role in the management of weight loss in obese and morbid obese patients. This treatment has been shown to achieve a sustained weight loss of 40% (Colquitt, Clegg, Loveman, Royle, & Sidhu, 2005), which in turn contributes to a decrease in fat mass and hence PAI-1 levels (Zengin et al., 2002). Tschoner et al. (2012) showed that BMI, visceral fat mass and total fat mass decreased after bariatric surgery. The change in visceral fat mass was associated a decrease in PAI-1 levels. This correlates with previous findings where PAI-1 mRNA increased only in visceral fat during the development of obesity (Shimomura et al., 1996). Hence a reduction in visceral fat mass as a result of bariatric surgery may contribute to a reduction in CVD due to a decrease in PAI-1. Additionally, Pardina et al. (2012) showed that gastric bypass leads to normalisation of the hematological profile and a decrease in PAI-1 levels which in turn results in a decreased risk of thromboembolism in severely obese individuals. Bariatric surgery has also been shown to maintain raised levels of adiponectin, an anti-inflammatory agent (Nijhuis et al., 2007) and resistin which may contribute to a decrease in atherosclerotic events post surgery (Santoro et al., 2008). Hence bariatric surgery may be an effective way of managing obesity and cardiac failure with subsequent knock on effects on the metabolic syndrome, coronary heart disease, and type II diabetes (Ashrafian, le Roux, Darzi, & Athanasiou, 2008) compared to other weight loss therapies.

7. Conclusion

A review of the literature has shown that the fibrinolytic system is involved in the cardiovascular disease process. Haemostatic and fibrinolytic factors have been found to interconnect with obesity, body fat distribution, or chronic low-grade inflammation, all of which contribute to the atherosclerotic process and hence the metabolic syndrome. The biological mechanism underlying these associations is extremely complex, and the fact that these factors sometimes correlate with each other, make it difficult to study the effects and determinants of each separate factor. However, evidence shows that PAI-1 links the triad of pathophysiological situations associated with the metabolic syndrome obesity, cardiovascular events and diabetes. Weight loss, dietary factors and bariatric surgery have been shown to have a beneficial effect on impaired fibrinolysis and may thereby reduce the cardiovascular risk in patients with obesity and or the metabolic syndrome.

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