The Effect of Exercise on PAI-1 and Other Markers of the Insulin Resistance Syndrome in Overweight & Obese Individuals

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Introduction: Obesity, and in particular central fat accumulation, is associated with a number of metabolic disturbances such as dyslipidaemia and insulin resistance. Such 'clustering' of factors is known as the Insulin Resistance Syndrome (IRS). More recently, hypofibrinolysis as a result of elevated concentrations of PAI-1 at rest has been included in the IRS. Acute exercise in normal weight individuals results in an increase in fibrinolytic capacity due to a rise in t-PA and a reduction in PAI-1 concentrations. The primary aim of the following studies therefore was to determine the effect of acute exercise and exercise training on fibrinolytic markers in obese populations. The secondary aim of this work was to examine the relationships between PAI-1 concentrations and other markers of the IRS to determine a potential role for these factors in the short-term regulation of plasma PAI-1 concentrations.

Methods: Premenopausal female and male overweight and obese volunteers underwent acute bouts of exercise at intensities ranging from 50% to 100% VO_2 max. A group of obese premenopausal women also underwent a graded maximal exercise test to exhaustion before and after 12 weeks of exercise training at 50% or 70% VO_2 max. Venous blood samples were taken at rest, immediately post exercise and up to 2 hours post exercise. Samples were analysed for fibrinolytic factors (t-PA, total PAI-1 & active PAI-1), markers of endothelial damage (vWF) as well as other components of the IRS including lipid profiles, insulin and leptin.

Results: Plasma t-PA concentrations rose with acute exercise at intensities greater than 50% VO_2 max in all study populations with the exception of obese sedentary males. In all cases t-PA returned to baseline levels 30 minutes post exercise. None of the protocols administered were sufficient to lower total PAI-1 concentrations immediately post exercise but exercise at an intensity of 70% VO_2 max and a duration of greater than 30 minutes resulted in elevated PAI-1 concentrations 30 minutes post exercise in the overweight and obese populations. Active PAI-1 concentrations decreased with exercise either immediately or within 30 minutes post exercise at an intensity of 70% VO_2 max and durations greater than 30 minutes. Exercise training at both 50% and 70% VO_2 max increased the t-PA response to maximal exercise but only exercise training at 70% VO_2 max resulted in greater decrease in active PAI-1 with exercise. No factors were consistently associated with PAI-1 throughout the studies although anthropometric measures and blood pressure were regularly associated with PAI-1 at rest.

Conclusions: Overall, exercise at an intensity of 70% VO_2 max for duration of at least 30 minutes in obese populations results in an increased fibrinolytic capacity as shown by elevated t-PA concentrations and decreased active PAI-1 concentrations. It is important to remember however that obese populations still remain hypofibrinolytic with respect to non-obese populations at rest, during exercise and in the recovery period.

Acknowledgements

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Dedicated to my Parents

'For believing that I would get there..... eventually.'

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Introduction

1.1 Obesity

Obesity and Coronary Heart Disease (CHD) are highly prevalent conditions in developed countries (Despres 1994). Recent statistics show that the prevalence of obesity in the UK has tripled since 1980 and will increase further on present trends. 21% of women and 17% of men are now reported to have a Body Mass Index (BMI), [expressed as weight (kg)/ height (m^2)] greater than 30 kg/m^2 the level defined as obesity (National Audit Office report 2001). This increase in obesity contrasts with decreasing energy intakes since 1970 suggesting an increasingly sedentary lifestyle as a key cause (Prentice and Jebb 1995). Obesity is associated with an increased risk of CHD, non insulin dependant diabetes mellitus (NIDDM), cancer, reproductive disorders, sleep apnoea, gall stones, osteoarthritis and psychological disorders (Despres 1994).

Measurements of BMI (>30 kg/m^2) and total body fat (>20% in men and >30% in women) reveal only the cosmetic and weight bearing problems associated with obesity. There is now a large body of evidence to suggest that the regional distribution of body fat is a critically important consideration in the relationship between obesity, metabolism and health (Despres 1994). Obesity can be divided into two main types. Gynoid or gluteal-femoral obesity is the accumulation of excess body fat around the hips and buttocks. This excess fat is metabolically relatively benign and is most commonly found in pre-menopausal women (Despres & Lamarche 1994). The second form of obesity is known as central, visceral or android obesity and is most commonly found in men and post-menopausal women (Despres & Lamarche 1994). Central obesity or even pre obesity (BMI 25 – 30 kg/m^2) is associated with a cluster of metabolic disturbances known as the Insulin Resistance Syndrome (IRS), Plurimetabolic syndrome, Metabolic syndrome or Syndrome X and is characterised by insulin resistance and increased lipolysis leading to dyslipidaemia

(Despres and Lamarche 1994). The IRS has now been defined as having three or more of the following abnormalities: waist circumference ≥ 102 cm in men and 88 cm in women; serum triglycerides of ≥ 1.69 mmol/L, HDL cholesterol of ≤ 1.04 mmol/L blood pressure of ≥ 135/85 mmHg or blood glucose of ≥6.1 mmol/L (National Institute of Health 2001).

The type of obesity can be clinically determined or catagorised using the waist circumference or waist to hip ratio (WHR). A WHR greater than 0.95 in men and 0.80 in women is defined as central obesity and poses an increased risk of the metabolic disturbances associated with accumulation of excess abdominal fat (Despres *et al* 1995). Another measure of excess abdominal adipose tissue is the waist circumference (WC). A WC greater than 1.02m in men and 0.88m in women is associated with increased incidence of the metabolic abnormalities associated with the IRS (Lean 1998).

1.2 The Insulin Resistance Syndrome

In 1988, Reaven first proposed the presence of a cluster of metabolic disturbances associated with a state of insulin resistance. He suggested that insulin resistance associated with compensatory hyperinsulinaemia and possible glucose intolerance are frequently linked to increased triglyceride levels, decreased HDL concentrations and hypertension and, that this cluster of abnormalities may increase the risk of cardiovascular disease. In 1989, abdominal obesity was included in the cluster of risk factors associated with an increased risk of CVD (Jensen *et al* 1989). Since then the metabolic abnormalities associated with CVD have been expanded to include low plasma HDL concentrations, increased small dense LDL concentrations, increased non-esterified fatty acid (NEFA) concentrations, elevated total cholesterol, increased

apolipoprotein B-100, increased fibrinogen concentrations, elevated uric acid concentrations and increased leptin levels.

More recently impaired fibrinolysis due to elevations in plasminogen activator inhibitor –1 (PAI-1) has also been defined as part of the IRS (Juhan-Vague *et al* 1991). In 1985 it was demonstrated that reduced fibrinolytic activity, i.e. low Tissue Plasminogen Activator (t-PA) activity, after venous occlusion was due to elevated levels of PAI-1 activity and that this may have a pathogenic importance in myocardial infarction, particularly in hypertriglyceridaemic patients (Hamsten *et al* 1985).

1.3 Fibrinolysis

Fibrinolysis is the process by which clots formed by the coagulation system are dissolved (see fig 1). Plasmin is a serine protease that catalyses the lysis of the fibrin framework of a thrombus by cleavage of arginine and lysine residues on a number of sites on both fibrin and fibrinogen resulting in successively smaller soluble fragments known as fibrin (ogen) degradation products (Rapaport 1990). Plasmin also catalyses proteolysis on or around cell surfaces in tissues and thus plays an important role in the inflammatory response and in tissue remodeling. An inert circulating precursor of plasmin, plasminogen, is converted to plasmin by cleavage of a single arginine-valine amino acid bond by t-PA and single chain urokinase type plasminogen activator (scU-PA).

1.3.2Tissue Plasminogen Activator (t-PA)

T-PA is the most important activator of plasminogen in humans and is normally present in human plasma in only trace amounts i.e. ~100 pM concentrations. These limited concentrations reflect a balance between limited basal secretion from the vascular endothelium and hepatic tissue and a rapid hepatic clearance. The intravascular half-life of non-complexed t-PA is approximately 2.4 minutes (Chandler

et al 1997) and the half-life of t-PA in complex with PAI-1 is approximately 5 minutes (Rapaport 1990). T-PA concentrations increase rapidly in response to a number of stimuli including venous occlusion, exercise, catecholamines and vasopressin (Streiff & Bell 1994). The activity of t-PA is dependent on the presence of the obligatory cofactor fibrin (van Meijer & Pannekoek 1995). The binding of t-PA to fibrin increases its affinity for plasminogen by two to three orders of magnitude, resulting in a significant acceleration of the conversion of plasminogen to plasmin (van Meijer & Pannekoek 1995). Approximately 90% of circulating t-PA is in complex with its primary inhibitor PAI-1. The remainder circulates either in the active form or in complex with an additional inhibitor C1 (Chandler *et al* 1997). The inhibition of t-PA by PAI-1 occurs in a rapid stochiometric manner resulting in a covalent bond between the two molecules (Bastard & Pieroni 1999). Active PAI-1 acts as a pseudo-substrate for t-PA forming a stable, inactive, covalent 1:1 equimolar complex (van Meijer & Pannekoek, 1995). PAI-1 is consumed in this process and is thus referred to as a "suicide inhibitor".

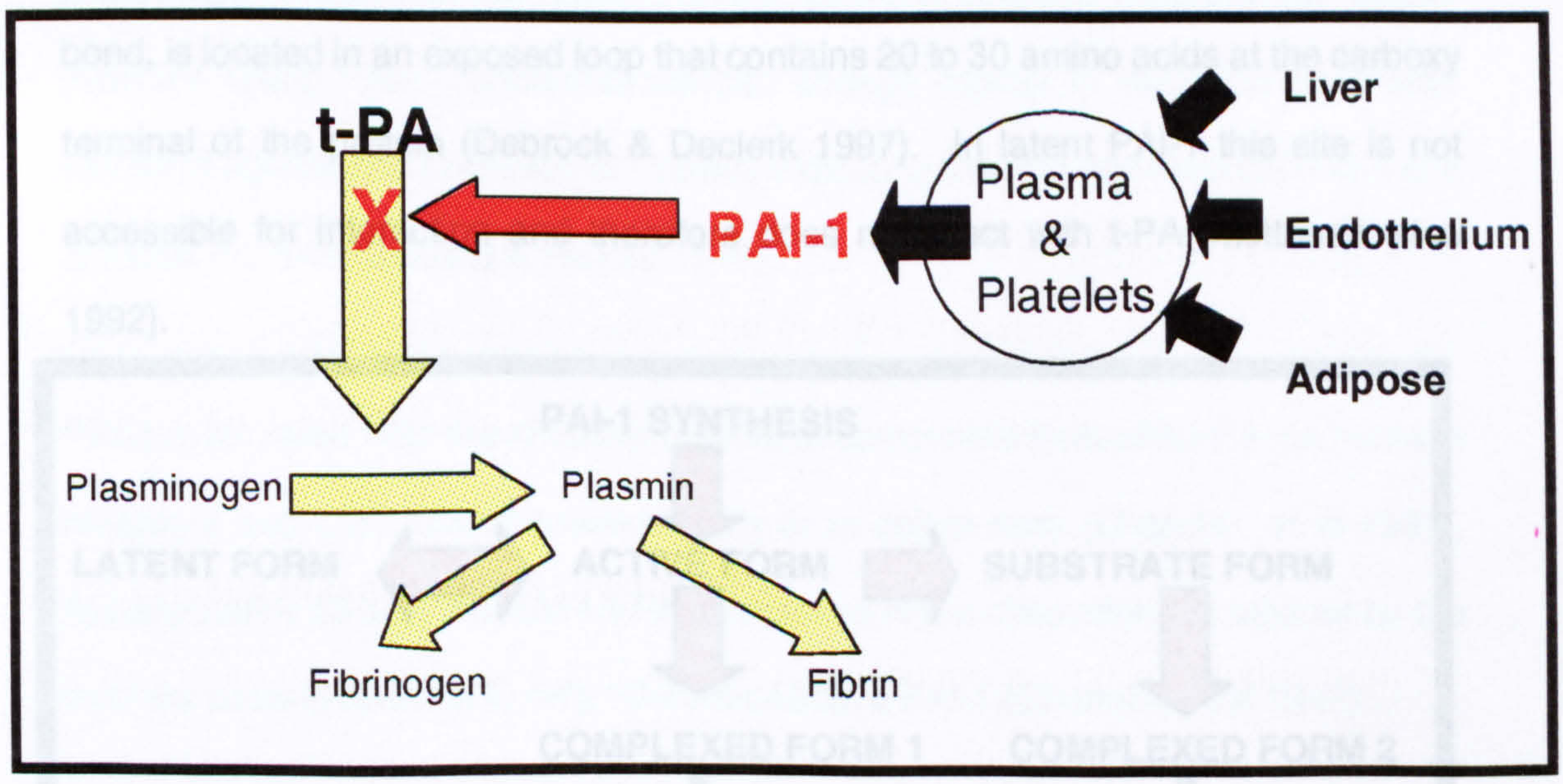


Figure 1.1: A schematic representation of fibrinolysis. T-PA = tissue plasminogen activator, PAI-1 = plasminogen activator inhibitor type 1.

1.3.3 Plasminogen Activator inhibitor (PAI-1)

PAI-1 is a 47 KDa single chain glycoprotein that belongs to the serine protease inhibitor (serpin) family with an *in vivo* half-life of approximately 7.5 minutes (Huber 2001a). It consists of 379 amino acids (Kruithof, 1988) and exists in 3 main forms active, latent and substrate as well in complex with t-PA. Among the serpins, PAI-1 exhibits a unique conformational flexibility. Although it is synthesised in the active conformation, PAI-1 spontaneously converts into the inactive latent form due to a lack of cysteine residues (Huber 2001a). This latent form can however, be partially reactivated by denaturing agents such as sodium dodecyl sulphate (SDS), guanidium chloride and urea, as well as platelet activation (Debrock & Declerk 1997). In plasma, active PAI-1 circulates in a complex with vitronectin, which stabilises this conformation thus increasing its biological half-life (van Meijer & Pannekoek, 1995).

Substrate PAI-1

The third conformation of PAI-1 described reacts as a non-inhibitory substrate towards t-PA (Declerk *et al* 1992). The reactive centre of PAI-1, known as the P_1-P'_1 bond, is located in an exposed loop that contains 20 to 30 amino acids at the carboxy terminal of the protein (Debrock & Declerk 1997). In latent PAI-1 this site is not accessible for interaction and therefore does not react with t-PA (Mottonen *et al* 1992).

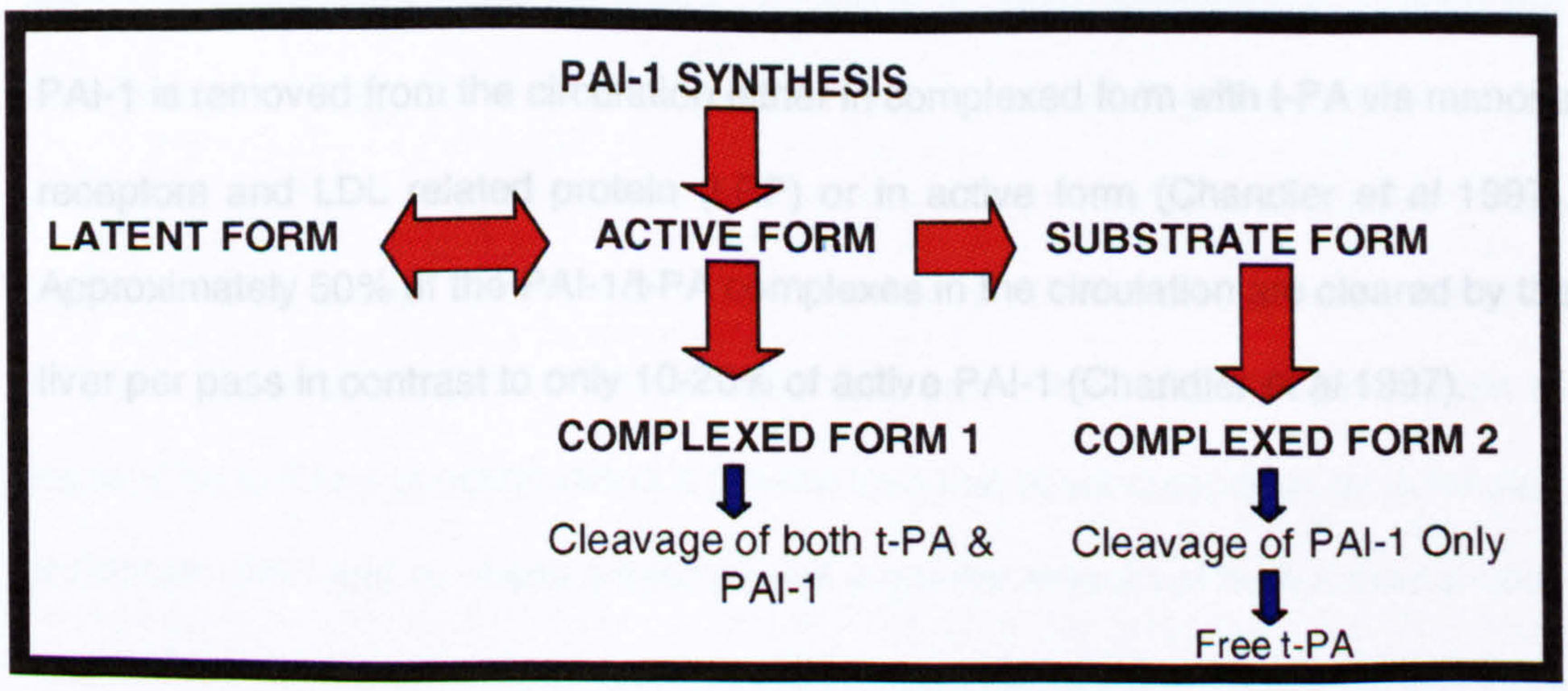


Figure 1.2: A schematic representation of the conformations of PAI-1

In contrast, the non-inhibitory substrate form does react with t-PA resulting in cleavage of the P_1-P'_1 bond but without the formation of a stable t-PA/PAI-1 complex and with the subsequent regeneration of t-PA (Mottonen *et al* 1992). This is in contrast to the active PAI-1 formation of a stable PAI-1/ t-PA complex in which both molecules are cleaved (Debrock & Declerk 1997). This mechanism however is slow and may not be physiologically relevant as the liver clears the substrate PAI-1/ t-PA complexes before dissociation occurs (Mottonen *et al* 1992).

Regulation of PAI-1

PAI-1 has two transcripts the largest of which (3.2kb as opposed to 2.2kb) is less stable and has a shorter half-life (Tremoli *et al* 1993). Both forms of PAI-1 are synthesised in the active form in a number of cells including hepatocytes, endothelial cells and adipocytes (Bastard & Pieroni 1999) and stored in platelets (which harbour 100-200 ng/ml) and in plasma (harbouring ~10ng/ml) (van Meijer & Pannekoek 1995). Normal plasma PAI-1 concentrations range from 6-80 ng/ml and demonstrate a circadian rhythm with the highest levels recorded between midnight and 6 a.m. (Kruithof 1988) PAI-1 concentrations can change rapidly in response to a wide variety of agents and physiological states, indicating that the regulation of PAI-1 is a complex process (Bastard & Pieroni 1999).

PAI-1 is removed from the circulation either in complexed form with t-PA via manose receptors and LDL related protein (LRP) or in active form (Chandler *et al* 1997). Approximately 50% of the PAI-1/t-PA complexes in the circulation are cleared by the liver per pass in contrast to only 10-20% of active PAI-1 (Chandler *et al* 1997).

1.4 Regulation of PAI-1 Secretion and Expression

1.4.1 PAI-1 Secretion and Release by Adipose Tissue

There is a considerable amount of evidence to suggest that PAI-1 is secreted from endothelial cells (Schneider *et al* 1993, Sawdey *et al* 1989) and hepatic tissue (Kooistra *et al* 1989, Alessi *et al* 1988). Adipose tissue, specifically visceral depots, has recently been postulated as a major source of the elevated PAI-1 concentrations in obesity (Janand-Delenne *et al* 1998). Resting PAI-1 Ag and activity have been shown on numerous occasions to positively correlate to BMI, waist to hip ratio, waist circumference and visceral fat mass (Janand-Delenne *et al* 1998, Vague *et al* 1989).

Initial clues for the role of adipose tissue in PAI-1 expression and secretion came from the observation that murine adipose tissue contained significant amounts of PAI-1 mRNA (Samad *et al* 1996, Samad & Loskutoff 1996). Moreover, clinical studies have demonstrated that fat loss due to surgical treatment and diet significantly reduces PAI-1 concentration in obese volunteers (Loskutoff & Samad 1997). The key role of adipose tissue in the biosynthesis of PAI-1 is further emphasised by the production of PAI-1 by adipocytes in response to a number of stimuli as well as localisation of PAI-1 mRNA in fully differentiated mature 3T3 adipocytes (Loskutoff & Samad 1997). Yudkin *et al* (1999) attempted to quantify the contribution of adipose to total PAI-1 secretion by measuring the arterio-venous difference in PAI-1 concentrations across the subcutaneous fat depot in lean subjects. This group determined that the relative contribution of subcutaneous adipose tissue to total PAI-1 concentrations is only 1.6%. As the contribution of visceral fat to PAI-1 concentrations is greater than that of subcutaneous fat (Loskutoff & Samad 1997) and as obese subjects have a greater amount of both subcutaneous and visceral fat than their lean counterparts, we can deduce that adipose tissue does play a significant part in elevated PAI-1 concentrations in obesity although the

contribution of other factors of the IRS to both increased adipose tissue mass and elevated PAI-1 concentrations may make the associations between anthropometric and PAI-1 concentration seem greater.

1.4.2 PAI-1 and Insulin

In vitro studies have demonstrated an increase in PAI-1 secretion and expression in response to insulin in arterial endothelial cells (Schneider *et al* 1993), human hepatocytes (Kooistra *et al* 1989), Hep G2 cells (Alessi *et al* 1988) and 3T3-L1 cultured adipocytes (Samad & Loskutoff 1996). Increases in PAI-1 secretion in response to insulin have also been demonstrated *in vivo* in mice (Samad & Loskutoff 1996). Several studies in human volunteers however, found that hyperinsulinaemia did not modify plasma PAI-1 concentrations (Grant *et al* 1990, Landin *et al* 1991, Potter van Loon *et al* 1990). Studies in humans that have examined the relationship between insulin and PAI-1 secretion *in vivo* directly using either the hyperinsulinemic euglycemic clamp (Landin *et al* 1990, Potter van Loon *et al* 1993) or the minimal model (Apslund-Carlson *et al* 1993) have reported conflicting data. Potter van Loon *et al* (1990) observed that insulin was the major determinant of excess PAI-1 secretion in obesity, when measured against insulin resistance, diastolic blood pressure, BMI and WHR. Using the minimal model, a determinant of insulin resistance however, Apslund-Carlson *et al* (1993) demonstrated that plasma triglycerides and BMI, not insulin concentrations, were the major determinants of PAI-1 secretion in males and in females, HDL cholesterol and 2-hour post-load insulin concentration were the major determinants of PAI-1.

Increased PAI-1 levels however are not always associated with increased concentrations of insulin. For example, non-diabetic patients with Cushing's syndrome have normal PAI-1 levels in spite of hyperinsulinaemia (Vague 1993). In

contrast to the Apslund-Carlson study the majority of experimental data suggests that elevations in PAI-1 are related to insulin resistance rather than hyperinsulinaemia suggesting a mechanism controlled by insulin receptors as opposed to insulin itself. When cells are made insulin resistant (by pre incubation with high doses of insulin to reduce receptor numbers) PAI-1 secretion and expression are enhanced (Vague 1993).

1.4.3 PAI-1 and Lipids

Greig & Rundle first suggested the influence of plasma lipoproteins on PAI-1 in 1956. This group observed an increase in an as yet unknown inhibitor of the fibrinolytic system after the ingestion of a fatty meal. Since then, many groups have described an elevation in PAI-1in hyperlipidemic patients (Epstein *et al* 1970, Vague *et al* 1996) although other markers of the insulin resistance syndrome are often present. A direct relationship between lipids and PAI-1 was demonstrated in patients with type IV hyperlipidemia (Fredrickson's classification) who had no history of cardiovascular disease, hypertension, diabetes or obesity compared to controls (Tremoli *et al* 1993). After a diet regimen only those patients who had reduced their triglycerides by more than 20 % showed a normalisation of PAI-1 levels.

Further *in vitro* work demonstrated that application of VLDL to Hep G2 cells and cultured endothelial cells increased expression and secretion of PAI-1 (Tremoli *et al* 1993) with the greatest increase in secretion from hepatocytes. The greater response from hepatocytes may be due to the expression of a distinct apo E receptor for VLDL binding in hepatic cells as well as the classical apo B/E receptor observed in endothelial cells (Dawson *et al* 1991) Moreover, VLDL from hypertriglyceridaemic patients had a greater effect than VLDL from normolipidemic patients. This may be due to the larger VLDL particle size in hypertriglyceridaemic patients that contains freshly transferred apoE that has a greater affinity for both receptor types (Stiko-

Rahm *et al* 1990). Sironi *et al* (1996) also demonstrated an increase in PAI-1 expression in response to stimulation by VLDL. Interestingly, this group noted that the PAI-1 expressed was the more stable 2.2kb form and that application of insulin further increased PAI-1 secretion. Nilsson *et al* (1999) demonstrated that it is the unsaturated fatty acid component of VLDL that is responsible for increasing PAI-1 secretion in endothelial cells.

The negative correlations between HDL and plasma PAI-1 concentrations observed in numerous epidemiological studies (Vague 1993, Vague *et al* 1995) have been attributed to the close metabolic relationship between triglycerides and HDL particles as HDL concentrations are dependent on rates of triglyceride uptake (Frayn 1999). This was confirmed by Cimminello *et al* (1997) who showed that patients with triglyceride levels > 2.25 mmol/L and HDL concentrations <0.91 mmol/L had increased plasma concentrations of PAI-1 compared to controls and patients with only low HDL cholesterol. There is limited research however to suggest that glycated HDL cholesterol, that occurs in insulin resistance and type II diabetes increases PAI-1 secretion from vascular endothelial cells independently of triglycerides (Ren & Shen 2000). This may explain the few studies that show a negative correlation between PAI-1 concentration and HDL cholesterol despite controlling for triglyceride.

1.4.4 PAI-1 and Cytokines

Tumour Necrosis Factor α (TNFα)

It has been shown that TNFα is a potent inducer of PAI-1 secretion and expression in a wide variety of tissues in a dose dependent manner (Samad *et al* 1996). Cigolini *et al* (1999) demonstrated a TNFα stimulated secretion of PAI-1 from cultured adipose tissue and a dose dependent decrease in PAI-1 expression and secretion in response to TNFα antagonists. TNFα-induced PAI-1 expression has also been

shown in vascular endothelial cells, HepG2 cells and adipocytes (Samad *et al* 1996). Sakamoto *et al* (1999) demonstrated that both superoxides and hydrogen peroxide were potent inducers of PAI-1 and that hydroxyl radical scavengers completely abolished the TNFα induction of PAI-1. This group further demonstrated that TNFα and insulin exert synergistic effects and hypothesised that TNFα stimulates PAI-1 production and this effect is potentiated by insulin.

Transforming Growth Factor β (TGFβ)

TGFβ has also been shown to induce PAI-1 synthesis and secretion in a variety of tissues including the endothelium (Sawdey *et al* 1989), smooth muscle cells (Reilly & McFall 1991), fibroblasts (Lund *et al* 1987), epithelial cells (Thalacker & Nilsen-Hamilton 1987), adipocytes (Samad *et al* 1997) and Hep G2 cells (Westerhausen *et al* 1991). Infusion of TGFβ into mice results in an increase in PAI-1 expression in a number of tissues with the largest increase being observed in adipose tissue (Sawdey & Loskutoff 1991). Furthermore, TGFβ induces a greater increase in PAI-1 expression than TNFα and insulin (Sawdey & Loskutoff 1991). Adipose tissue expression of TGFβ mRNA is also significantly higher in obese mice compared with lean controls and this increase can be attributed to increased expression of TGFβ mRNA in mature adipocytes, stromal cells and vascular cells within the adipose tissue (Samad *et al* 1997). As TGFβ is stored and released from the α granules of activated platelets (Lundgren *et al* 1996) and is known to augment PAI-1 synthesis, this cytokine may provide a positive feedback mechanism inducing further PAI-1 synthesis.

1.4.5 PAI-1 and Leptin

The evidence for a link between PAI-1 and plasma leptin concentrations comes mainly from epidemiological studies. De Mitrio *et al* (1999) found that after

adjustment for fat mass, waist to hip ratio and BMI, PAI-1 Ag correlated only with leptin, insulin and HDL. The correlations between PAI-1 and leptin were thought to result from the strong correlations between insulin and leptin but Soderburg *et al* (1999) demonstrated a positive correlation between PAI-1 and leptin concentrations after adjustment for fasting insulin concentrations in obese male volunteers. Studies in adolescents however, have shown that the increased levels of leptin that occur with puberty do not result in increases in PAI-1 suggesting that leptin is not independently associated with PAI-1 concentrations (Sudi *et al* 2000). This is confirmed by the limited *in vitro* data. Aprath-Husmann *et al* (2001) showed that when supraphysiological concentrations of leptin were applied to mature and preadipocytes no change in PAI-1 secretion was observed. There is still limited cellular data on the role of leptin in PAI-1 secretion and expression. Many more studies on different cell types must be conducted before firm conclusions can be drawn.

1.4.6 PAI-1 and the Renin-Angotensin-Aldosterone System (RAAS)

Obesity is associated with elevated plasma angiotensin, plasma renin activity, tissue-specific angiotensin converting enzyme (ACE) and plasma aldosterone levels (Egan *et al* 2001). Alterations in the renin-angiotensin-aldosterone system (RAAS) are thought to be responsible for the relationship between fat mass and blood pressure and thus may indirectly account for the rise in PAI-1 concentration with increasing fat mass due to increased shear stress induced endothelial damage.

In vivo (Ridker *et al*, 1993, Van Leeuven *et al* 1994) and *in vitro* studies (Vaughan *et al* 1995) however, implicate the RAAS directly in the regulation of PAI-1. Early evidence appears to confirm this link as patients treated with ACE inhibitors show reduced concentrations of PAI-1 (Huber *et al* 2001). These studies have also shown that these changes are not mediated via secondary changes in haemodynamics.

In vitro,(figure 1.3) Angiotensin II has been shown to up-regulate PAI-1 production from both smooth muscle and endothelial cells (Huber 2001a, Brown *et al* 1998) and this suggests an indirect role for ACE in the regulation of PAI-1 secretion and expression. Furthermore, infusion of exogenous angiotensin II has been shown to increase PAI-1 Ag selectively in both normotensive and hypertensive individuals (Brown *et al* 1998). Increased expression of PAI-1 in response to angiotensin II in cultured endothelial cells however, does not appear to be mediated via either angiotensin type I or type II receptors. Rather, angiotensin II is metabolised to the hexapeptide angiotensin IV that acts on the specific AT4 receptor (Kerins *et al* 1995) to up regulate PAI-1 secretion. In contrast, increases in PAI-1 expression observed in rat aorta and heart ventricle appear to be mediated via the angiotensin II/ angiotensin type I receptor pathway (Huber 2001a).

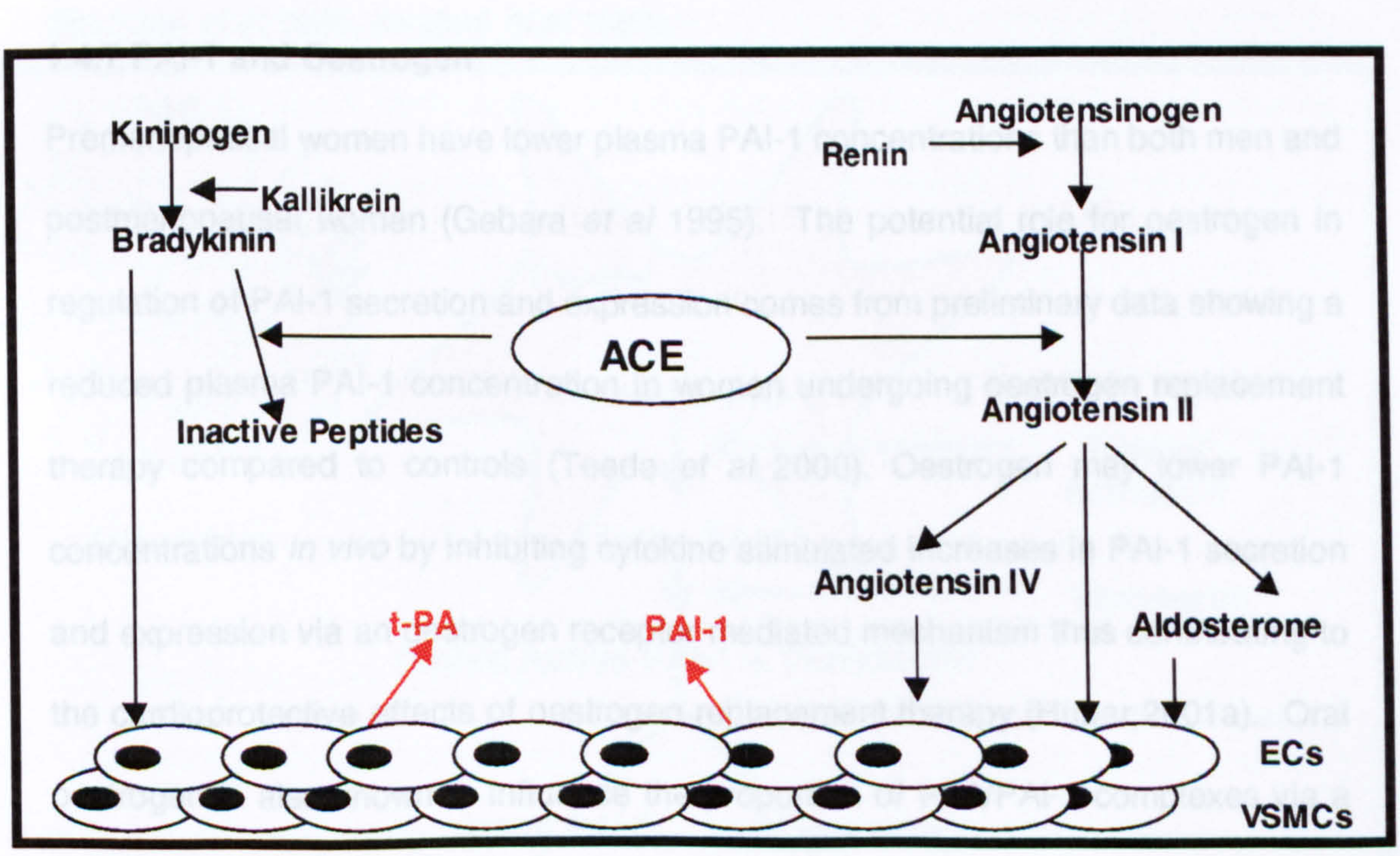


Figure 1.3: Relationship between renin-angiotensin-aldosterone system, kallikrein-kinnogen system and the fibrinolytic system. ACE= angiotensin converting enzyme, ECs= endothelial cells, VSMCs= vascular smooth muscle cells, PAI-1=plasminogen activator inhibitor 1, t-PA= tissue plasminogen activator. Adapted from Vaughan (2001)

Inhibition of ACE therefore, results in reduced formation of both angiotensin II and angiotensin IV leading to an abatement of PAI-1 expression and secretion.

ACE inhibitors not only block the formation of angiotensin II but also inhibit the breakdown of bradykinin. Bradykinin is a potent stimulus for t-PA secretion from endothelial cells as well as being a stimulus for nitric oxide production and therefore vasodilatation (Brown *et al* 1998).

Research in this area is limited but promising and suggests that treatment with ACE inhibitors will not only reduce blood pressure, but will also increase fibrinolytic potential via stimulation of t-PA secretion and expression and inhibition of synthesis and release of PAI-1.

1.4.7 PAI-1 and Oestrogen

Premenopausal women have lower plasma PAI-1 concentrations than both men and postmenopausal women (Gebara *et al* 1995). The potential role for oestrogen in regulation of PAI-1 secretion and expression comes from preliminary data showing a reduced plasma PAI-1 concentration in women undergoing oestrogen replacement therapy compared to controls (Teede *et al* 2000). Oestrogen may lower PAI-1 concentrations *in vivo* by inhibiting cytokine stimulated increases in PAI-1 secretion and expression via an oestrogen receptor mediated mechanism thus contributing to the cardioprotective effects of oestrogen replacement therapy (Huber 2001a). Oral oestrogen is also known to influence the proportion of t-PA/PAI-1 complexes via a stimulation of hepatic t-PA clearance.

1.4.8 Release of PAI-1 by Platelets.

Over 90% of available PAI-1 is associated with platelets and its release has been the subject of a large number of studies (Erickson *et al* 1984, Erickson *et al* 1985, Kruithof *et al* 1986, Kruithof *et al* 1987). PAI-1 is stored in the alpha granules of platelets with approximately 80% present in the latent form (Huber 2001a). This latent PAI-1 is converted to the active confirmation by triggers such as platelet activation and aggregation caused by vessel trauma. Whilst the exact mechanism by which this conversion occurs is unknown, large amounts of active PAI-1 can be released from activated platelets at sites of arterial platelet rich thrombi (Huber 2001a). Stabilisation of PAI-1 within platelets appears to be a function of Ca^{2+} binding rather than formation of PAI-1/vitronectin complexes (Lang & Schleef 1997). The release of PAI-1 from the alpha granules of platelets occurs in parallel with release of vitronectin, phosphokinase A, platelet factor 4 and β thromboglobulin (Erickson *et al* 1985, Kruithof *et al* 1986).

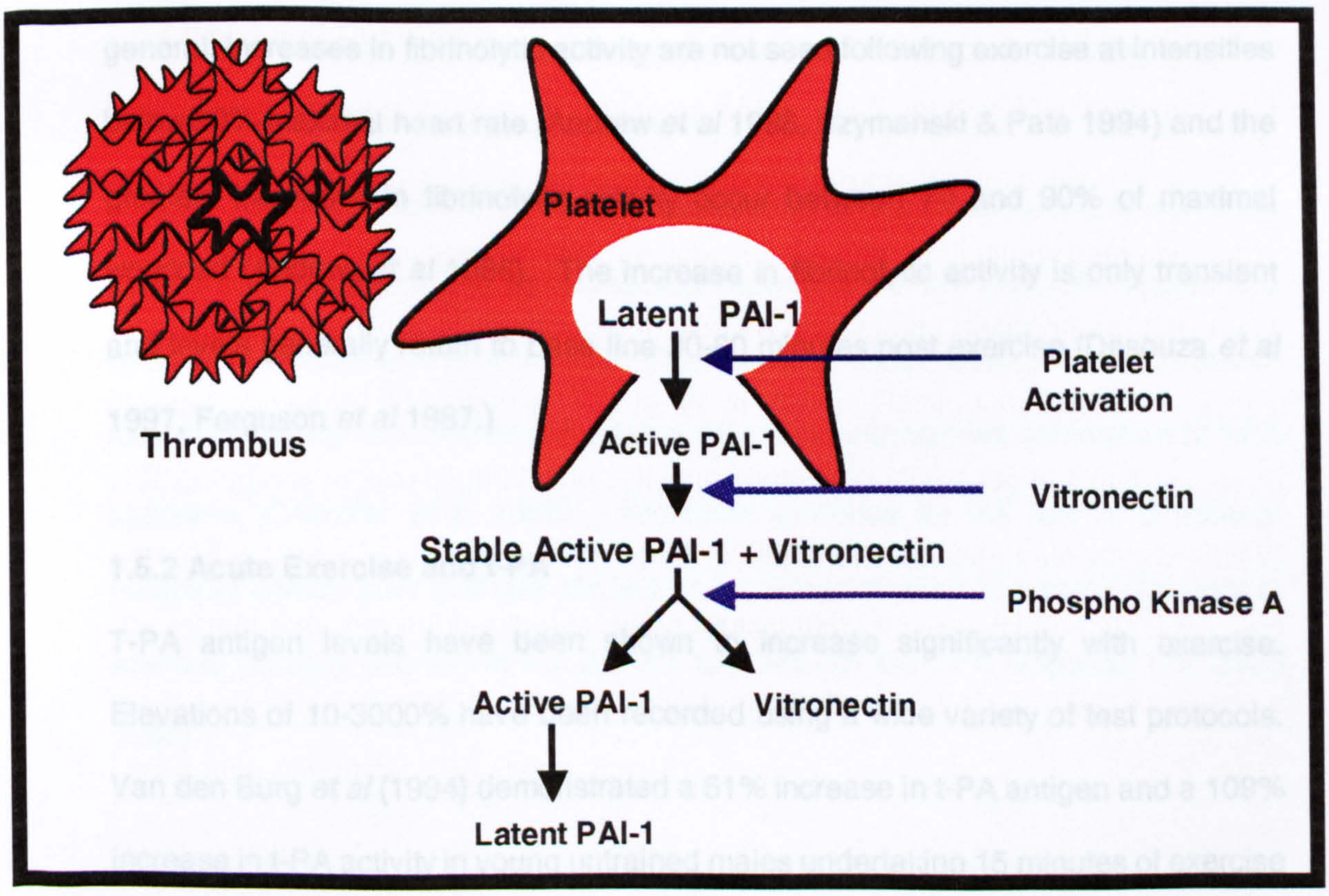


Figure 1.4: Schematic representation of the conformational changes in PAI-1 following platelet activation.

Once released from the alpha granule, if available, the newly activated PAI-1 is stabilised by vitronectin thus increasing its biological half-life (van Meijer & Pannekoek 1995) (figure 1.4). Phosphokinase A (PKA) however is also translocated onto the fibrin network from the surface of platelets (Morgenstern *et al* 2001). Phosphorylation of vitronectin by PKA releases the active form of PAI-1 that 'spontaneously' converts back to the latent conformation thus promoting fibrinolysis.

1.5 Acute Exercise and Fibrinolysis

An increase in fibrinolysis with exercise was first reported by John Hunter in 1794 (El-Sayed 1996) but the exact mechanism by which this occurs still remains to be elucidated. Dilute blood clot lysis time has been documented to decrease between 20 and 83% following strenuous exercise (Streiff & Bell 1994, Bourey & Santoro, 1988) and is dependent on a wide variety of factors including exercise intensity, fitness of subjects, exercise protocol and correction for haemoconcentration. In general, increases in fibrinolytic activity are not seen following exercise at intensities below 50% maximal heart rate (Andrew *et al* 1986, Szymanski & Pate 1994) and the greatest increases in fibrinolytic activity occur between 70 and 90% of maximal workload (Andrew *et al* 1986). The increase in fibrinolytic activity is only transient and levels generally return to base line 30-60 minutes post exercise (Desouza *et al* 1997, Ferguson *et al* 1987.)

1.5.2 Acute Exercise and t-PA

T-PA antigen levels have been shown to increase significantly with exercise. Elevations of 10-3000% have been recorded using a wide variety of test protocols. Van den Burg *et al* (1994) demonstrated a 51% increase in t-PA antigen and a 109% increase in t-PA activity in young untrained males undertaking 15 minutes of exercise on a cycle ergometer at 70% of their respective VO_2 max. Szymanski and Pate (1994) demonstrated that the rise in t-PA activity is dependent on exercise intensity,

with exercise at 80% VO_2 max eliciting a greater t-PA response than exercise at 50% VO_2 max, and time of day with t-PA antigen increased in the evening compared to the morning. There is no reported evidence of an age effect on t-PA concentrations. Van den Burg *et al* (1995) found no correlation between t-PA secretion in response to 25 minutes cycling at 70% VO_2 max, and age in 38 sedentary males aged 20 to 60 years. There is also no reduction of t-PA secretion in response to exercise in older hypertensive men compared to age matched normotensive controls (Desouza *et al* 1997).

There is also evidence of a rise in scU-PA with exercise suggesting that the acceleration in blood fibrinolytic activity also includes activation of scU-PA (Van den Burg *et al* 1994). It should be noted however that peak levels of t-PA and scU-Pa do not coincide in time and magnitude in response to maximal exercise (El-Sayed, 1996). The mechanism behind the increase in t-PA with exercise involves a shift in the balance between t-PA secretion and clearance. T-PA secretion during exercise is linearly related to plasma adrenaline concentrations that act via both α and β receptor types (Chandler *et al* 1997). Stimulation of α adreno-receptors increases plasma t-PA concentrations by decreasing hepatic blood flow that in turn leads to a decreased clearance t-PA by the liver (Chandler *et al* 1995). Stimulation of β adreno-receptors results in an increased plasma t-PA concentration via stimulation of t-PA secretion (Chandler *et al* 1995). Additional evidence for the role of β adreno-receptors comes from exercise studies where administration of propranolol prior to exercise partially decreased but did not fully abolish the t-PA response (El-Sayed 1996).

The relative contributions of increased t-PA secretion and decreased t-PA clearance to plasma t-PA concentrations are dependent on the exercise protocol administered.

Sustained submaximal exercise results in reduced hepatic blood flow and therefore decreased t-PA clearance (Chandler *et al* 1995). T-PA rises gradually during the exercise period in parallel to the reduced hepatic clearance. When exercise ceases hepatic blood flow and therefore t-PA concentrations rapidly return to baseline levels (Booth *et al* 1987).

When a graded exercise protocol to exhaustion, such as a modified Bruce protocol is applied, initially there is little or no change in plasma t-PA concentration. This is followed by an exponential increase in t-PA that peaks at the point of maximal exercise. Whilst a decrease in hepatic blood flow and therefore a increase in t-PA concentration do occur, it is insufficient to account for the rapid rise in plasma t-PA. Instead, increased t-PA secretion is predicted to be the main contributor to elevated t-PA concentrations during graded exercise to exhaustion (Chandler *et al* 1995). The increase in t-PA secretion can be attributed to the exponential increase in plasma adrenaline in response to this type of exercise protocol (Chandler *et al* 1995).

1.5.3 Acute Exercise and PAI-1

PAI-1 antigen has been reported to either be unaffected by acute bouts of exercise or decreased by up to 30% (Streiff & Bell 1994). Szymanski and Pate (1994) showed significant reductions in PAI-1 in response to acute bouts of cycle ergometer exercise at both 50% and 80% VO_2 max. Surprisingly there was no significant difference in the magnitude of the PAI-1 response to exercise intensity, as the majority of studies have reported a decreasing PAI-1 concentration with increasing exercise intensity (El-Sayed 1996). As with t-PA, Szymanski and Pate (1994) demonstrated that PAI-1 reduction with exercise is dependent on time of day and is highest in the morning. No aging (Van den Burg *et al* 1995) or hypertensive effect (Desouza *et al* 1997) has been observed in PAI-1 secretion in response to acute bouts of sub-maximal exercise.

The mechanisms that lead to a reduction in PAI-1 remain to be fully elucidated. Sustained submaximal exercise results in decreased hepatic blood flow (Chandler at al 1995) and may therefore decrease PAI-1 secretion from the liver. Increased PAI-1 secretion from the α granules of activated platelets resulting from increased endothelial damage during exercise may mask the true extent of this effect. PAI-1 however is also cleared from the circulation by the liver and therefore this mechanism would suggest that PAI-1 clearance might also be reduced resulting in a lack of significant change in plasma PAI-1 concentration. Numerous studies have reported a reduction in PAI-1 activity without a reduction in total PAI-1 Ag (Fernhall *et al* 2000, DeSouza *et al* 1997). This suggests that the reduction in PAI-1 activity is a direct result of increased t-PA concentrations and the formation of t-PA/PAI-1 complexes. Further research is needed in this area to determine the effect of exercise on plasma PAI-1 secretion and clearance.

1.5.4 Effectiveness of Increased Fibrinolytic Capacity

How effective the increases in t-PA and scU-PA and the decrease in PAI-1 are on fibrin(ogen)olysis remains a matter of debate. Ferguson *et al* (1987) reported a significant increase in fibrin and fibrinogen degradation products in response to maximal exercise, whether other studies have reported no significant change in fibrinogen concentration in response to a variety of test protocols (El-Sayed *et al* 1999). Particularly, Collen *et al* (1977) found no significant change in the plasma fibrinogen concentration but did observe a shortened half life of radio labeled fibrinogen. El-Sayed *et al* (1999) demonstrated a decrease in plasma fibrinogen concentration in response to both maximal and sub maximal (75%) cycle ergometer exercise in healthy moderately active males. Conversely, several groups have found a rise in fibrinogen concentration in response to acute bouts of exercise but these studies did not correct for plasma volume changes which may account for the increase (Streiff & Bell 1994). The most recent plasma volume adjusted studies

show good evidence to suggest that fibrinogen concentrations do indeed decrease and fibrin (ogen) degradation products increase in response to maximal and sub-maximal exercise (El-Sayed, 1999, Prisco *et al* 1998).

The mechanisms responsible for the observed decrease in plasma fibrinogen concentration are not well understood but may be linked to an enhanced rate of fibrinogen catabolism (hyperfibrinogenolysis) or increased removal of fibrinogen from the plasma into the interstitial spaces (El-Sayed *et al* 1999). Further investigation is required to examine fibrinogen concentration with simultaneous measurements of fibrinogen/fibrin degradation products and thrombin activation to elucidate the mechanisms responsible for exercise induced changes in plasma fibrinogen concentration.

Considering all the available information Streiff & Bell (1994) proposed a model of fibrinolysis in response to acute bouts of exercise. As exercise intensity reaches approximately 50% VO_2 max the release of t-PA from endothelial cells begins. The trigger factor for the release of t-PA remains controversial but increases in regional blood flow, signals of local ischaemia and increasing lactate concentrations have been correlated to t-PA release (Ferguson *et al* 1987). When exercise intensity reaches 70 – 90% VO_2 max large increases in t-PA occur. Available fibrin microdeposits are degraded and elevations in fibrin degradation products can be measured depending on the proportions of soluble and insoluble fibrin present (Ferguson *et al* 1987). Relatively little fibrinogenolysis occurs due to the relative fibrin specificity of t-PA and scU-PA and the presence of PAI-1. With strenuous exercise PAI-1 concentrations decrease thus increasing overall fibrinolytic capacity. After the completion of exercise, fibrinolytic activity rapidly dissipates due to the short half-life of t-PA, scU-PA and plasmin. T-PA antigen concentrations may fall to sub

baseline levels (reflecting a transient exhaustion of endothelial stores) but fibrinolytic activity may remain at baseline levels due to a decrease in PAI-1 concentration.

1.6 Fibrinolysis and Exercise Training

Numerous cross sectional (Kvernmo & Osterund 1997, Ferguson & Guest 1994, Ferguson *et al* 1987, DePaz *et al* 1992) and longitudinal (Traber 1999, Stratton *et al* 1994) studies have shown an increase in fibrinolytic activity with exercise training. Kvernmo & Osterund (1997) found that although there were no significant differences in resting fibrinolytic activity between athletes and controls, the athletes demonstrated an increased fibrinolytic capability with reduced PAI-1 antigen concentrations following a maximal exercise test. DePaz *et al* (1992) also showed that athletes were hyperfibrinolytic compared to controls after a maximal exercise test with an increased release of t-PA antigen and a decreased formation of t-PA/PAI-1 complexes in the athletes. This is in contrast to Ferguson and Guest (1994) and Ferguson *et al* (1987) who observed that fibrinolytic activity both at rest and after acute exercise bouts are based on both current and former physical fitness levels.

Longitudinal studies have shown that favorable changes in the fibrinolytic system can be achieved over a relatively short period. Stratton *et al* (1994) showed an 88% reduction in PAI-1 activity following a 6-month training programme in elderly individuals. Traber (1999) however demonstrated a rise in fibrinolytic activity following a 1 month training programme at 80% maximal heart rate and Bowman *et al* (1996) observed a decrease in PAI-1 activity following a 14 day skiing tour. These results confirm the observations by Tofler *et al* (1990) that the risk of triggering myocardial infarction (MI) by heavy physical activity is reduced in physically fit individuals.

1.7 Exercise as a Trigger for Myocardial Infarction and Sudden Cardiac Death

There is evidence that unaccustomed physical activity acts as a trigger for acute MI and sudden cardiac death (SCD). Tofler *et al* (1990) reported that among the 849 patients or relatives interviewed after acute MI, 49% reported exposure to one or more trigger factors. The trigger factors included emotional upset (19%), moderate physical activity (14%) and heavy physical activity (9%). Similar results were observed in a number of other studies (Sumiyoshi 1986, Behar *et al* 1993). In an effort to quantify the risk associated with physical exertion the TRIMM (Mittleman *et al* 1993) and Onset (Willich *et al* 1993) studies were conducted. Both studies reported a significantly increased risk of acute MI during and up to 1 hour after the completion of heavy physical exertion. Heavy physical exertion was defined in both studies as 6 metabolic equivalents per minute (METS) and included slow jogging, tennis, snow shovelling, fast biking and sexual activity (1MET is equal to the energy expended per minute by a subject sitting quietly and is approximately 3.5 mls O_2 /kg/min). The overall increased risk of MI was 5.9 in the TRIMM study and 2.1 in the Onset study. The results of both studies however, showed massive variation with the usual frequency of physical exertion. The Onset study observed a relative risk of 107 in those persons who exercised less than once a week and a risk of 2.4 in those who engaged in heavy physical exertion five or more times a week. The TRIMM study reported a risk of 6.9 in persons who exercised less than four times a week compared to 1.3 in those who exercised more than 4 times a week.

The exact mechanism by which exercise triggers acute MI remains unknown. A proposed mechanism is the rupture of a vulnerable coronary vessel atherosclerotic plaque in response to the haemodynamic stresses of exercise (Mittleman & Siscovick 1996) for example increased blood pressure and heart rate due to increased sympathetic output (Gibbons *et al* 1980). The haemostatic and vasoconstrictive processes that follow determine whether the formed thrombus occludes the lumen of

the coronary artery. Heavy physical exercise has also been demonstrated to acutely activate platelet function in sedentary persons and those persons with a previous history of MI (Kestin *et al* 1993). Conversely exercise has been demonstrated to increase fibrinolytic activity (Winther *et al* 1992). The net effect of thrombus formation and increased fibrinolysis may be the determining factor in the triggering of acute MI. In persons with the metabolic abnormalities associated with the IRS fibrinolysis is impaired at rest and may be impaired during exercise leading to an increased risk of MI with unaccustomed heavy physical exertion. The response of these patients to exercise requires investigation.

1.8 Aims of the Investigation

The aims of this investigation therefore are to determine the effect of acute exercise and exercise training on plasma PAI-1 concentrations in overweight and obese subjects and to examine the correlations between plasma PAI-1 Ag and other factors of the IRS over a variety of physiological conditions to highlight the role of these factors in the short term regulation of PAI-1 secretion.

Chapter 2

Materials & Methods

2.1 Body Fat Analysis - Air Displacement Plethysmography BODPOD™

(Life Measurement Instruments, Concord CA)

Principle of the Measurement

The system consists of two chambers, one for the subject and one serving as a reference for volume (see fig 2.1). With the subject in one chamber the door is closed and sealed. The pressure is then increased slightly and the diaphragm separating the two chambers is oscillated to slightly alter the volumes of the two chambers by 350ml (Ellis 2000). These volume perturbations lead to small complementary pressure fluctuations (approximately 1cm H_2O) between the two chambers. The pressure before and after the subject enters the chamber are recorded and using Boyle's law ($Pressure_1$ / $Pressure_2$ = $Volume_1$ / $Volume_2$) the volume of the subject is then determined. From the volume of the subject the subject's density can be determined (Volume/Mass = Density) and percentage body fat calculated using the equation of Siri (1961).

Percent Fat = 495 / Density – 450

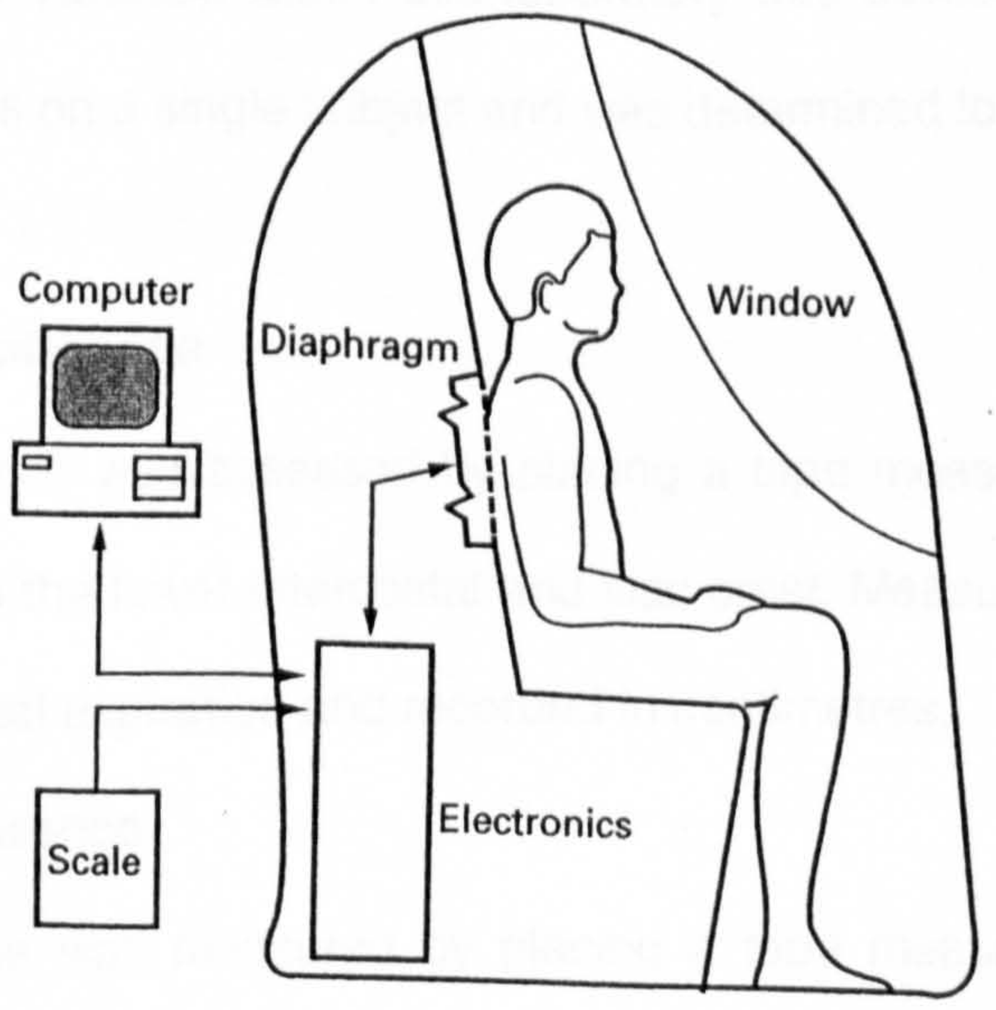


Fig 2.1: Diagrammatic representation of the BODPOD (Dempster & Aitkens 1995)

Procedure

Firstly the BODPOD is calibrated using a 50L cylinder. Subjects were required to remove all clothing except for a close fitting swimming costume or trunks and to put on a close fitting swimming cap. The subject was then weighed. Details of the subject's height, age and sex were then input into the computer. The subject was then required to sit in the BODPOD for two consecutive cycles. If these cycles determined the volume to be different by more than 150ml a third cycle was added. The mean volume of the two cycles closest in agreement was used to determine the percentage body fat.

Limitations

This method has been shown to accurately determine volumes ranging from 25 – 150 L with a sensitivity of 0.001 L (Dempster & Aitkens 1995).

Coefficient of Variation

The coefficient of variation for this method has been reported as 1.7% (McCrory et al 1995) (n=68)

The coefficient of variation within this laboratory was assessed by completing 20 consecutive cycles on a single subject and was determined to be 2.1%

2.2 Waist Circumference

Waist circumference was assessed by placing a tape measure snugly around the midpoint between the lower intercostal and iliac crest. Measurements were taken at the end of a normal expiration and recorded in centimetres.

2.3 Hip Circumference

Hip circumference was measured by placing a tape measure snugly around the maximal posterior extension of the buttocks at the level of the greater trochanter.

2.4 Body Fat Analysis - Tetrapolar Bioelectrical Impedance (Body Stat 1500, Body Stat UK)

Principle of Measurement

Four electrodes are attached to the subject before a weak alternating current is passed through the outer pair of electrodes, while the voltage drop is measured using the inner pair of electrodes from which the body impedance is derived. The method is based on 2 main assumptions firstly, the body can be modelled as an isotropic cylindrical conductor with its length proportional to the subjects height (Ht). Secondly, the reactance (X) term contributing to the body's impedance (Z) is small, such that the resistance component (R) can be considered equivalent to the body impedance. When these two assumptions are combined, it can be shown that the conducting volume is proportional to the term Ht^2/R, called the impedance index. Body fat is then calculated using proprietary equations

Procedure

Subjects rested in the supine position for 15 minutes before measurement. The areas for electrode placement were cleaned thoroughly with alcohol and allowed to dry. Electrodes were placed on the dorsal side of the foot at the midpoint between the medial and lateral malleoli, between the first and second digit of the foot, on the wrist at the midpoint between the medial and lateral styloid processes and between the second and third digits of the hand. The electrodes were then connected to the Body Stat 1500 (Body Stat UK) before a current was passed between the electrodes at a frequency of 50Hz.

Limitations

The human body is not a cylindrical conductor. The tissues are also not electrically isotropic and therefore the reactance component of the body's impedance is not zero.

Coefficient of Variation

The coefficient of variation for this procedure is 3% (n=100) (Body Stat, UK)

The coefficient of variation for this procedure within this laboratory was assessed by performing 20 consecutive measurements on the same subject and was determined to be is 3%

2.5 Body Fat Analysis - Leg to leg Bioelectrical Impedance Body Fat Analysis (Tanita Corp, USA)

Principle of Measurement

The metal sole plates are each divided into two parts. A weak alternating current is passed from the front portion of the two plates, while the voltage drop is measured using back portion of the two plates from which the body is impedance is derived.

As in tetrapolar bioelectrical impedance analysis this the method is based on two assumptions. Firstly, the body can be modelled as an isotropic cylindrical conductor with its length proportional to the subjects height (Ht). Secondly, the resistance component (R) can be considered equivalent to the body impedance (Z). This is then used to determine the body density.

Calculations

Body density (BD) is calculated from height, weight and impedance using the following equations (Jebb et al 2000):

$$BD = 1.100696 - 0.107903 \times Wt \times Z/Ht^2 + 0.00017 \times Z$$

Wt = Weight (kg) Ht = Height (m) Z = Impedance (Ω)

Percentage body fat is then calculated from body density using the equation:

$$\% \text{ fat} = (4.57 / BD - 4.142) \times 100$$

Procedure

Subjects were required to empty their bladders before standing for a minimum of 10 minutes before analysis to minimise potential errors from acute shifts in body fluid distribution. Subjects stood on the metal sole plates of the machine in swimwear to allow accurate determination of body mass before a current was passed between the electrodes at a frequency of 50Hz.

Limitations

This method of body composition analysis is limited by the fact that the current travels through the path of least resistance and therefore only passes through the lower body. Upper body fat therefore must be predicted from lower body fat. As with tetrapolar bioelectrical impedance analysis resistance is dependent on hydration status.

Coefficient of Variation

The coefficient of variation in this laboratory was assessed by completing 30 consecutive measurements on the same subject and was calculated to be 2%

2.6 Height

All measures of height were recorded using a standard wall mounted stadiometer.

2.7 Body Mass

All measures of body mass were recorded using Tanita™ BWB-600 weighing scales.

Subjects were asked to wear minimal clothing for each measurement.

2.8 Submaximal and Maximal Fitness Test Using a Modified Bruce Protocol

Subjects conducted a test of (sub)maximal oxygen uptake using a modified Bruce treadmill protocol (see table 2.1). All tests were performed using a standard motorised treadmill (Cardiosport Powerjog)

Table 2.1: Modified Bruce protocol used for the determination of VO_2 max.

STAGE	SPEED (Km h^{-1})	GRADIENT(%)	TIME @STAGE
0	2.7	0.0	3mins
1	2.7	5.0	3mins
2	2.7	7.5	3mins
3	4.0	10.0	3mins
4	5.4	12.5	3mins
5	6.7	15.0	3mins
6	8.0	17.5	3mins
7	8.8	20.0	3mins
8	9.6	22.0	3mins

Expired air was collected through a mouth piece with nose clip via a plastic tube into Douglas Bags for the last minute of each stage and analysed for percentage oxygen (Servomex 572 Oxygen Analyser), percentage carbon dioxide (Servomex PA404, Carbon Dioxide Analyser), and volume (Harvard Dry Gas Meter). Alternatively, expired air was collected and analysed using an online system (Cortex Meta Max 3B). VO_2 was then calculated using the equation:

$$VO_2 = \text{Volume Expired in 1 minute (L)} = \left(\frac{\%\ O_2 \text{ inspired} - \%\ O_2 \text{ Expired}}{100 - \%\ CO_2 \text{ expired}} \right)$$

All measurements were corrected to standard temperature and pressure using the method of Weir (1949). Heart rate was recorded (Polar Vantage) and ratings of perceived exertion were taken using the Borg scale (see table 2.2.) throughout the last minute of each stage.

For the submaximal fitness test, maximum intensity was achieved when the subjects heart rate reached 80% of predicted maximum. Predicted maximal heart rate was calculated from the following equation:

Predicted Maximal Heart Rate (bpm) = 220 – Subject's Age

For the maximal oxygen uptake test, VO_2 max was achieved when 3 out the 4 maximal criteria were met.

1. A VO_2 that did not vary over 2 consecutive stages
2. A heart rate equal to 220 – Subject's age
3. A rating of perceived exertion using the Borg Scale over 18 for 2 consecutive stages
4. An RER equal or greater than 1.1

At the end of each test subjects were required to walk for 5 minutes at stage 1 of the protocol to act as a cool down.

2.9 Ratings of Perceived Exertion

Ratings of perceived exertion were collected in the last minute of each stage of the oxygen uptake test using the Borg scale of perceived exertion (Borg 1982).

Table 2.2: The Borg scale of perceived exertion

NUMERIC RATING OF YOUR EXERTION	DESCRIPTION OF YOUR EXERTION
6	NONE
7	VERY, VERY LIGHT
8	
9	VERY LIGHT
10	
11	FAIRLY LIGHT
12	
13	SOMEWHAT HARD
14	
15	HARD
16	
17	VERY HARD
18	
19	VERY, VERY HARD
20	

2.10 Blood Sampling Procedures

2.10.1 Venepuncture

All venepuntures were performed after 5 minutes of venous occlusion at 100 mmHg with a standard sphygmometer cuff. Cuff sizes were determined after measurement of arm circumference at the midpoint between the acromomion and the medial epicondyle of the humerous and classed according to the manufacturers instructions. Sampling was performed using a closed vaccutainer system. Sodium citrate samples were withdrawn first followed by no additives samples and then EDTA samples. All venepuncture blood samples were taken following a single venepuncture to standardise for the effect of venous occlusion and venepuncture.

2.10.2 Cannulation

Cannulations were performed using a standard 21G Venflon™ cannula after 5 minutes of venous occlusion at 100 mmHg with a standard sphygmomometer cuff. Prior to each sample a 2.5ml bolus of non-heparinised isotonic saline was administered. A 3 ml blood sample was then withdrawn using a standard 5ml syringe and discarded. Blood samples were then withdrawn using standard 10 ml syringes before a final 2.5ml bolus of non-heparinised isotonic saline was administered. Samples were then divided among vaccutainers with sodium citrate samples being taken first followed by no additives samples and finally EDTA samples. Cannulations were all performed after a single attempt to reduce the effect of venous occlusion and venepuncture.

2.10.3 Mean Arterial Pressure

Mean arterial pressure was calculated using the following equation:

((Systolic Pressure – Diastolic Pressure) + 3) + Diastolic Pressure

2.11 Calculation of Procedure Coefficient of Variation

A single sample was taken from a volunteer and divided into equal aliquots. Samples were then assayed at various points across a sample run, or in various wells within a single ELISA plate and over several different ELISA plates. Coefficients of variation were then calculated as:

Coefficient of Variation = (Standard Deviation / Mean) *100

Data is reported for each procedure including the number of samples

All assays were performed in the laboratories at the University of Hertfordshire unless otherwise stated.

2.12 Total Cholesterol - Beckman Synchron CX Systems Procedure 467825

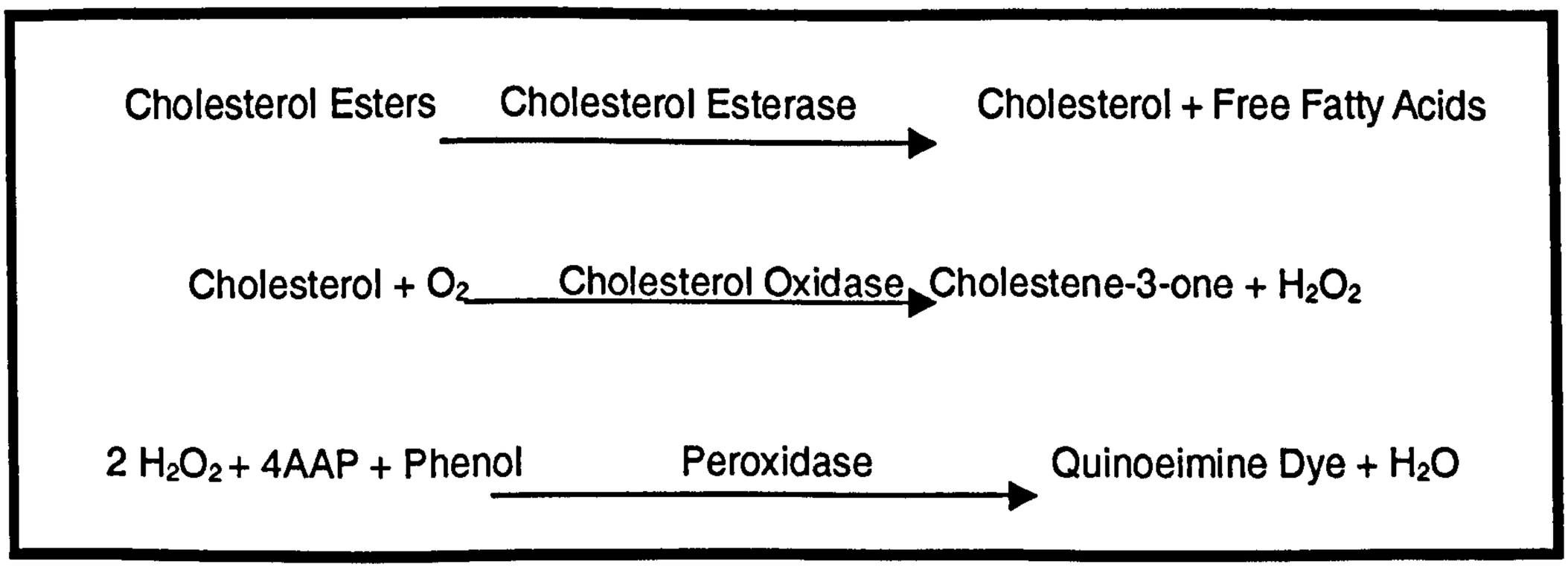
L & D Pathology Department

Procedure

Blood samples (2ml) were collected in tubes containing no additives or anticoagulants. The samples were centrifuged (IEC CL3) for 10 minutes at 3000 rpm and the serum separated. The serum was separated and analysed immediately. This assay was performed and analysed by Synchron CX automated system and was read at a wavelength of 520nm with a blank (isotonic saline) and a standard (7 mmol/l).

Principle of the Assay

Cholesterol esters are enzymatically hydrolysed in a reaction catalysed by cholesterol Esterase (CE) to form cholesterol and free fatty acids. Free cholesterol in the serum and the resultant cholesterol are then oxidised by Cholesterol Oxidase to Cholestene-3-one and Hydrogen Peroxide (H_2O_2). Finally in a reaction catalysed by Peroxidase the H_2O_2 reacts with 4-aminoantipyrine (4AAP) and Phenol to produce a Quinoeimine dye. The absorbance of this dye at 520nm is proportional to the concentration of cholesterol in the sample.



Calculation of Results

The results were calculated as follows:

$$\text{Cholesterol Concentration (mmol/l)} = \left[\frac{\text{Absorbance of Unknown}}{\text{Absorbance of Standard}} \times \text{Standard Concentration}\right] \times 0.0259^{*}$$

* 0.0259 equals the molecular weight of Cholesterol

Limitations

This assay is linear up to 19.4 mmol/l with a sensitivity of 0.01 mmol/l

Coefficient of Variation

The coefficient of variation for this procedure is 3.0 % (n=40) (Beckman Synchron Systems)

The coefficient of variation for this procedure within this laboratory is 1.9 % (n=20)

2.13 HDL Cholesterol (EZ HDL Method) Sigma Diagnostics Procedure 354L

L & D Pathology Department

Procedure

Blood samples (2ml) were collected in tubes containing no additives or anticoagulant. The samples were then centrifuged (IEC CL3) for 10 minutes and the serum separated. This was then analysed immediately. Analysis was performed using a Beckman Synchron Automated system and read at a wavelength of 600nm with a blank (isotonic saline) and a standard (4 mmol/l).

Principle of the Assay

Anti human β-lipoprotein antibody bind to lipoproteins (Chylomicrons, VLDL & LDL) other than HDL. The antigen antibody complexes formed block cholesterol esterase (CE) and cholesterol oxidase (CHO) reactions. When these enzymes are added they react with HDL cholesterol to form cholestenone, fatty acid, and hydrogen peroxide (H_2O_2). H_2O_2 yields a blue colour complex upon oxidase condensation with FDAOS (N-ethyl-N- (2-hydroxy-3-sulphopropyl) -3,5-dimethoxy-4-fluoroanaline, sodium salt) and 4AAP (4-aminoantipyrine) in the presence of peroxidase. The Absorbance of this blue product at 600nm is proportional to the HDL concentration in the sample.

LDL, VLDL, Chylomicrons $\xrightarrow{\text{Anti human β lipoprotein antibody}}$ Ag / Ab complex

HDL Cholesterol + H_2O + O_2 $\xrightarrow{\text{CHE \& CO}}$ Cholestenone + Fatty Acid + H_2O_2

H_2O_2 + FDAOS + 4AAP $\xrightarrow{\text{Peroxidase}}$ Blue Colour Complex

Calculation of Results

The results were calculated as follows:

$$\text{HDL Concentration (mmol/l)} = \left(\frac{\text{Absorbance of Unknown}}{\text{Absorbance of Standard}} \times \text{Standard Concentration} \right) \times 0.0259$$

Limitations

This assay is linear up to 4.66 mmol/l with a sensitivity of 0.026 mmol/l

Coefficient of Variation

The coefficient of variation for this procedure is 0.98 % (n=90) (Sigma Diagnostics)

The coefficient of variation for this procedure within this laboratory is 1.2 % (n=20)

2.14 Triglyceride - (GPO) Beckman Synchron CX Systems Procedure 445850

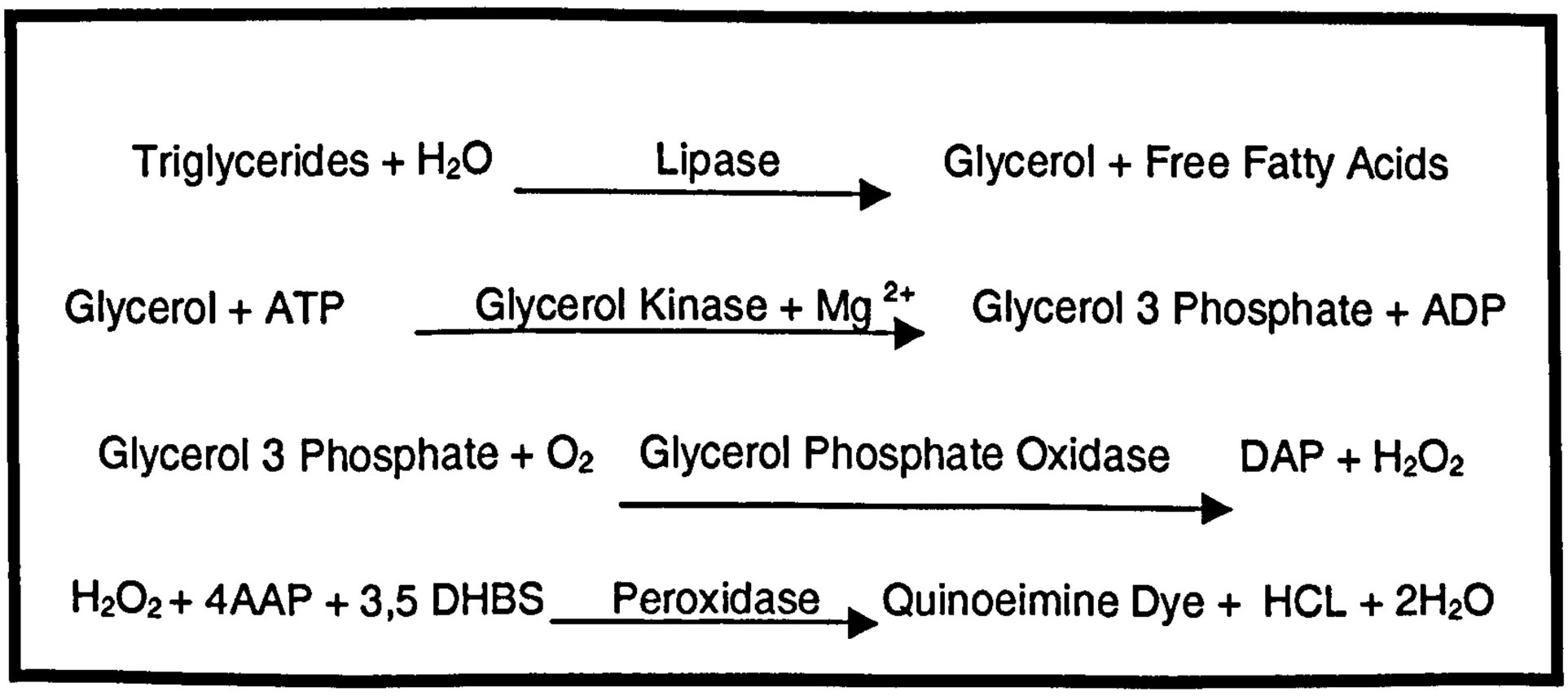
L & D Pathology Department

Procedure

Blood samples (2ml) were collected in tubes containing no additives or anticoagulants. The samples were centrifuged (IEC CL3) for 10 minutes at 3000 rpm and the serum separated. The serum was separated and analysed immediately. This assay was performed and analysed by Synchron CX automated system and was read at a wavelength of 520nm with a blank (isotonic saline) and a standard (2.2 mmol/l).

Principle of the Assay

Triglycerides are enzymatically hydrolysed by lipase to free fatty acids and glycerol. The glycerol is then phosphorylated by ATP in a reaction catalysed by glycerol kinase (GK) to produce glycerol 3 phosphate (G3P) and ADP. The G3P is then oxidised to form dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H_2O_2) catalysed by glycerol phosphate oxidase (GPO). Finally in a reaction catalysed by peroxidase the H_2O_2 reacts with 4-aminoantipyrine (4AAP) and 3,5 dichloro 2 hydroxybenzene sulphonate (3,5 DHBS) to produce a red quinoeimine dye. The absorbance of this dye is proportional to the concentration of triglycerides in the sample.



Calculation of Results

The results were calculated as follows:

$$\text{Triglyceride Concentration (mmol/l)} = \left[\frac{\text{Absorbance of Unknown}}{\text{Absorbance of Standard}} \times \text{Concentration of Standard} \right] \times 0.0113^{*}$$

*0.0113 equals the molecular weight of Triglyceride

Limitations

This assay is linear up to 11.3 mmol/l with a sensitivity of 0.04 mmol/l

Coefficient of Variation

The coefficient of variation for this procedure is 3.0 % (n=40) (Beckman Synchron Systems)

The coefficient of variation within this laboratory is 0.9% (n=20)

2.15 LDL Cholesterol

LDL cholesterol was calculated using the Friedewald equation:

LDL Cholesterol (mmol/L)

$$= \text{Total Cholesterol (mmol/l)} - \left(\frac{\text{Triglyceride (mmol/l)}}{2.19} \right) + \text{HDL(mmol/l)}$$

2.16 Total Plasminogen Activator Inhibitor (PAI-1) Technoclone ELISA

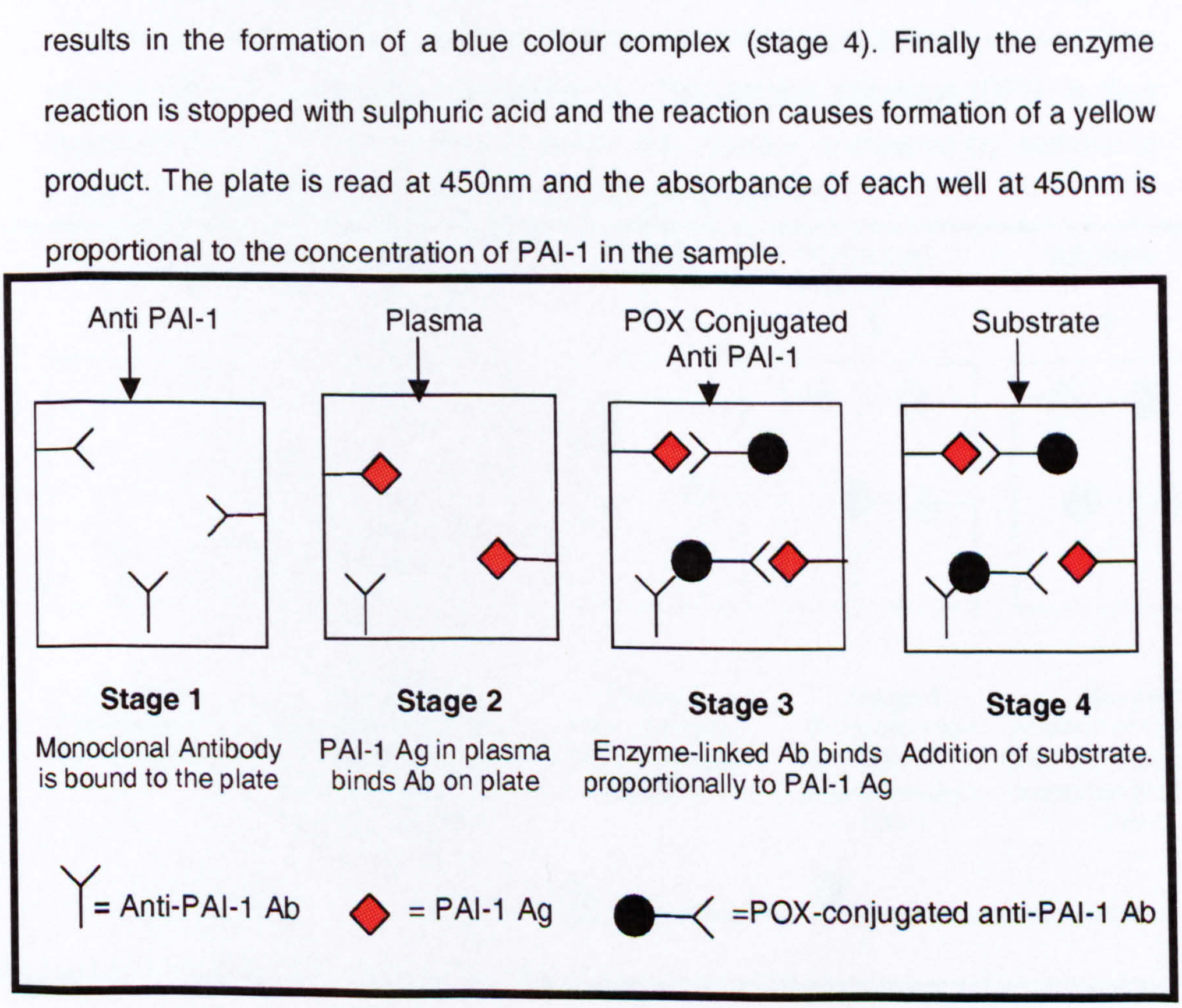
Procedure TC 12075

Procedure

Blood samples (3ml) were collected in tubes containing sodium citrate as an anticoagulant. The samples were then centrifuged (IEC CL3) for 10 minutes at 3000 rpm and the plasma separated and stored in eppendorf tubes at -80°C until analysis. All samples were analysed within 3 hours of thawing and the plate was read at 450nm with a blank and 4 standards (10, 30, 55.5 & 89 ng/ml).

Principle of the Assay

The plate is pre-coated with antihuman PAI-1 monoclonal antibodies (stage 1) to which the plasma PAI-1 antigen binds (stage 2). A peroxidase (POX) linked anti PAI-1 antibody was then added to bind to the plasma PAI-1 antigen (stage 3). The enzyme substrate, Tetramethylbenzidine (TMB) containing H_2O_2 is then added and results in the formation of a blue colour complex (stage 4). Finally the enzyme reaction is stopped with sulphuric acid and the reaction causes formation of a yellow product. The plate is read at 450nm and the absorbance of each well at 450nm is proportional to the concentration of PAI-1 in the sample.



Calculation of Results

Sample concentrations were determined from a graph of standard solutions of PAI-1 concentration Vs absorbance.

Limitations

This method measures total PAI-1 (free, complexed and latent PAI-1).

Coefficient of Variation

The coefficient of variation for this procedure is 5% (Technoclone)

The coefficient of variation for this procedure within this laboratory is 4.3% (n=15)

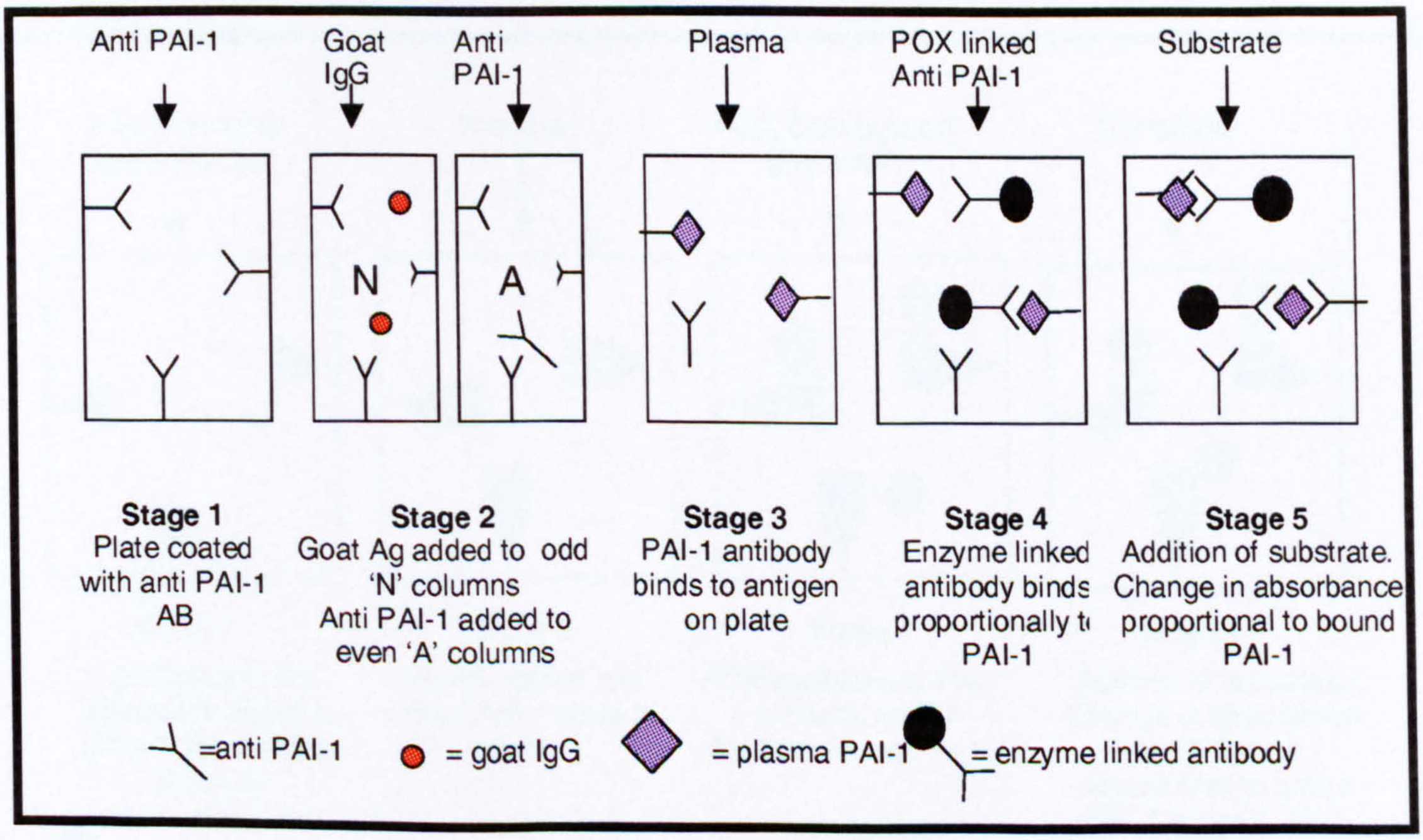
2.17 Imulyse PAI-1 Biopool International ELISA procedure 101005

Procedure

Blood samples (3ml) were collected in tubes containing sodium citrate as an anticoagulant. The samples were then centrifuged (IEC CL3) for 10 minutes at 3000 rpm and the plasma separated and stored in eppendorf tubes at -80°C until analysis. All samples were analysed within 3 hours of thawing and the plate was read at 492nm with a blank and 5 standards (100, 75, 50, 25, 12.5 ng/ml).

Principle of the Assay

This assay utilises the double antibody principle utilising both quenching and normal antibodies as a control for PAI-1 specificity. This excludes false positives which are not uncommon in conventional PAI-1 ELISAs. The plate is coated with goat anti-human PAI-1 immunoglobulin (stage 1) and incubated for 16-18 hours. Normal goat immunoglobulin is added to odd numbered columns (A) and goat anti-human PAI-1 immunoglobulin is added to even numbered columns (N) (stage 2). The plasma samples are then added and the plasma PAI-1 binds to the antibody (stage 3). Horseradish peroxidase labelled goat anti-human t-PA IgG is added and binds to a second site on the plasma PAI-1(stage 4). The enzyme substrate (OPD) is then incubated for 30 minutes (stage 5) before the reaction is stopped by addition of H_2SO_4. Finally, the plate is read at a wavelength of 492 nm.



Calculation of Results

The difference between the "N" value and "A" value is calculated for each standard and sample. This represents the t-PA specific part of the response and is denoted ΔA. The ΔA value of each of the standards is plotted against their known concentration and a straight line is fitted to the points using a minimal least square procedure. The function of this line is used to calculate the PAI-1 concentration of the plasma samples.

Limitations

This assay measures only free and latent PAI-1 (not t-PA/PAI-1 complexes)

The detection limit is 50 ng/ml

The sensitivity of the assay is 0.9 ng/ml

Coefficient of Variation

The coefficient of variation for this assay is 5% (Biopool International)

The coefficient of variation for this assay in our laboratory is 4% (n=20)

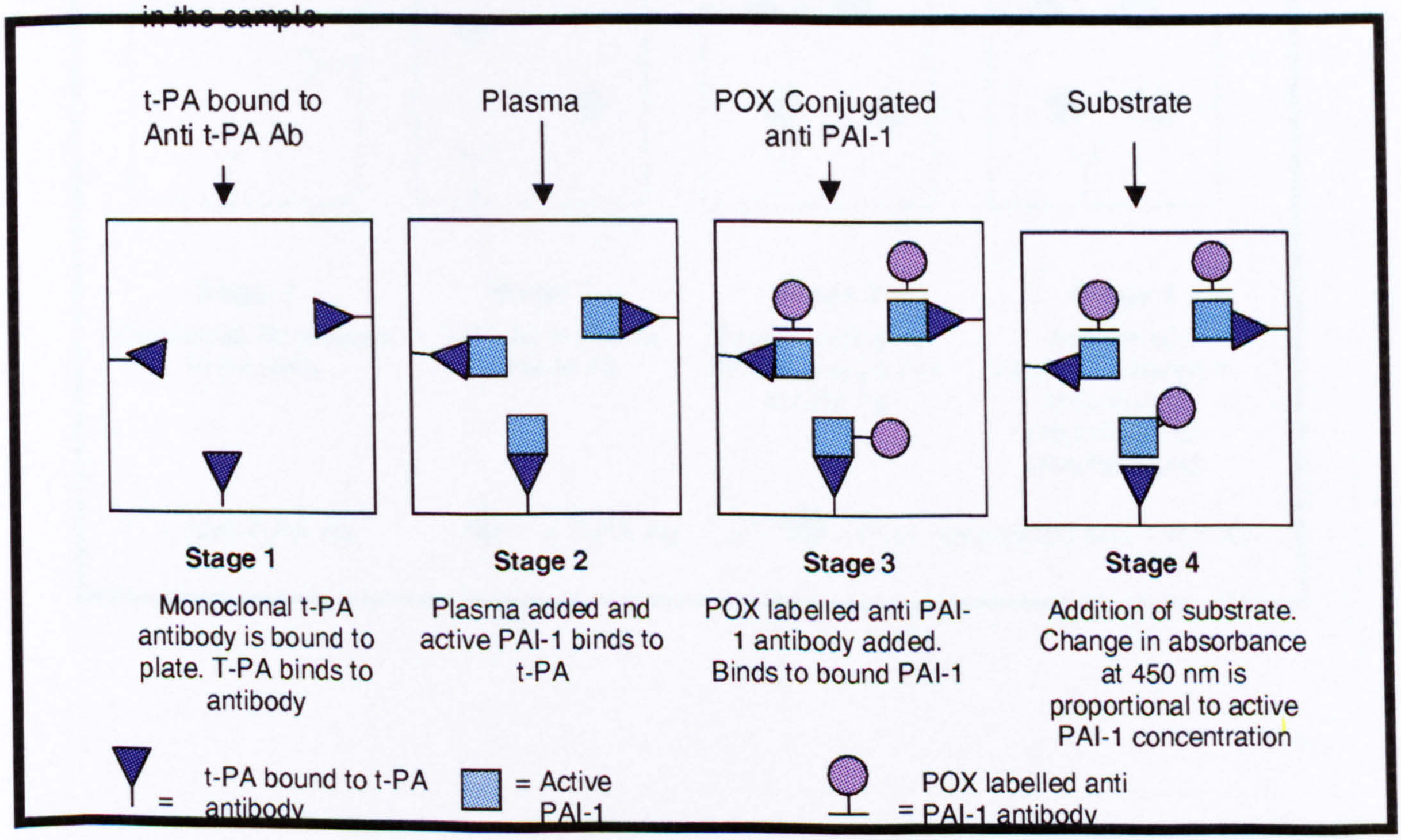
2.18 Active Plasminogen Activator Inhibitor (PAI-1) Technoclone ACTIBIND Procedure TC 16075

Procedure

Blood samples (3ml) were collected in tubes containing sodium citrate as an anticoagulant. The samples were then centrifuged (IEC CL3) for 10 minutes at 3000 rpm and the plasma separated and stored in eppendorf tubes at -80°C until analysis. All samples were analysed within 3 hours of thawing and the plate was read at 450nm with a blank and 4 standards (7, 15, 34, 70 ng/ml). Control samples were used to determine the acccuracy of the procedure.

Principle of the Assay

The Actibind test is a solid phase enzyme immunoassay in which an anti-t-PA monoclonal antibody is utilised to immobilise t-PA onto a 96 well plate (stage 1). The t-PA active site remains exposed and was used to bind active PAI-1 (stage 2). A POX linked monoclonal antibody, which recognises a specific site on the PAI-1 molecule, was incubated simultaneously incubated with the plasma sample (stage 3). Unbound POX labelled PAI-1 was washed away and Tetramethylbenzidine (TMB) containing H_2O_2 was then added resulting in the formation of a blue colour complex (stage 4). Finally the enzyme reaction is stopped with sulphuric acid and the reaction causes formation of a yellow product. The plate is read at 450nm and the absorbance of each well at 450nm is proportional to the concentration of active PAI-1 in the sample.



Calculation of Results

The standard solutions were used to create a graph of active PAI-1 concentration Vs absorbance from which sample concentrations could be determined.

Limitations

This method exclusively measures free, active PAI-1 and is not affected by other forms of PAI-1. The assay range is 1-70 U/ml.

Coefficient of Variation

The coefficient of variation for this procedure is 5% (n=20 Technoclone)

The coefficient of variation for the assay in this laboratory is 5% (n=20)

2.19 Tissue Plasminogen Activator (T-PA) Technoclone ELISA

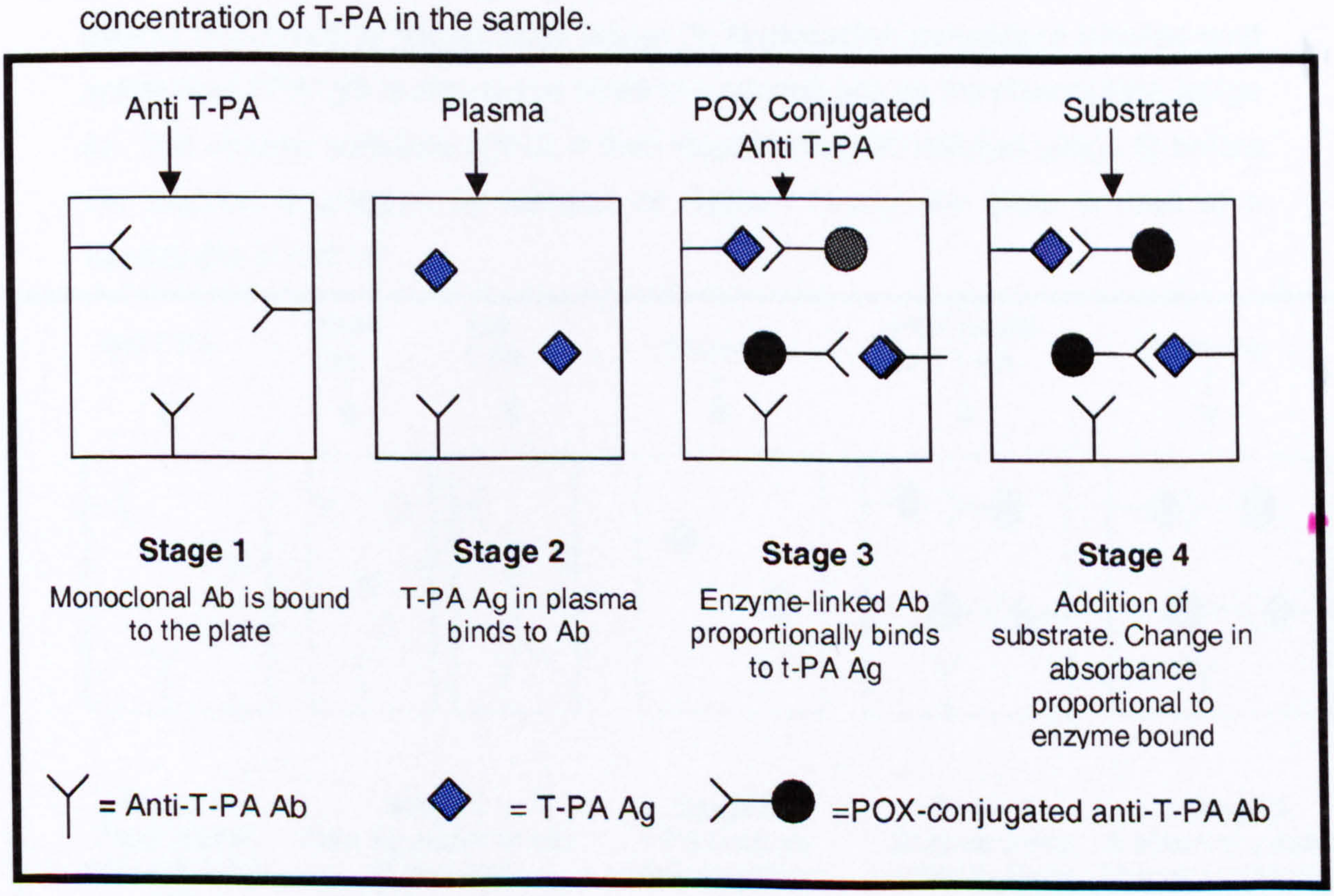
Procedure TC 12075

Procedure

Blood samples (3ml) were collected in tubes containing sodium citrate as an anticoagulant. The samples were then centrifuged (IEC CL3) for 10 minutes at 3000 rpm and the plasma separated and stored in eppendorf tubes at -80°C until analysis. All samples were analysed within 3 hours of thawing and the plate was read at 450nm with a blank and 4 standards (4.5, 10.5, 24.5 & 30 ng/ml).

Principle of the Assay

The plate is pre coated with antihuman T-PA monoclonal antibodies (stage 1) to which the plasma T-PA antigen binds (stage2). A POX linked anti T-PA antibody then added to bind to the plasma T-PA antigen (stage 3). The enzyme substrate, Tetramethylbenzidine (TMB) containing H_2O_2 is then added and results in the formation of a blue colour complex (stage 4). Finally the enzyme reaction is stopped with sulphuric acid and the reaction causes formation of a yellow product. The plate is read at 420nm and the absorbance of each well at 420nm is proportional to the concentration of T-PA in the sample.



Calculation of Results

The standard solutions were used to create a graph of T-PA concentration Vs absorbance and the sample concentrations were read off from there. Control samples were also used to determine the accuracy of the procedure.

Limitations

This method measures total T-PA (free, &complexed).

Coefficient of Variation

The coefficient of variation for this procedure is 5% (Technoclone)

The coefficient of variation for the procedure within this laboratory is 4.2% (n=15)

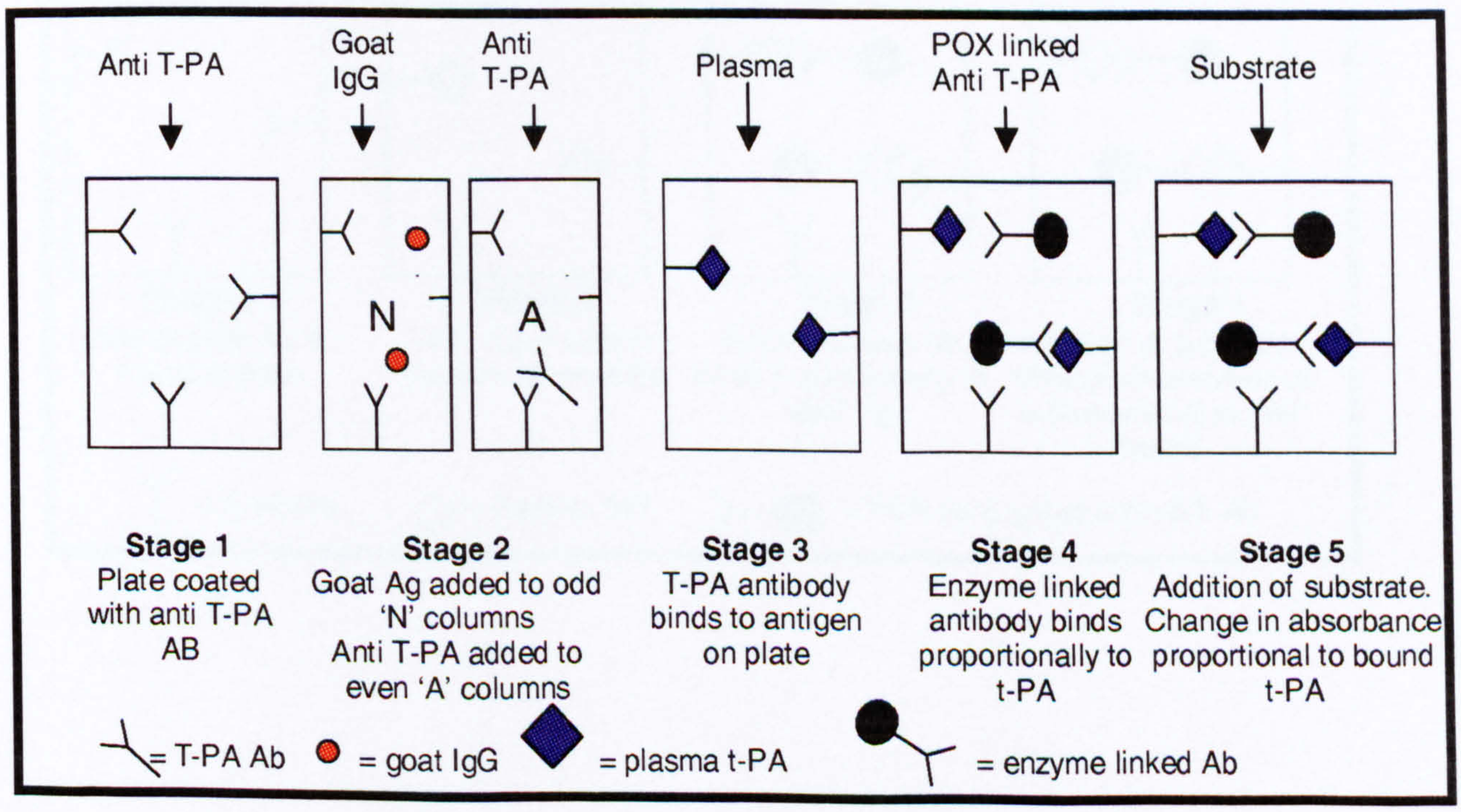
2.20 Imulyse T-PA Biopool International ELISA procedure 101005

Procedure

Blood samples (3ml) were collected in tubes containing sodium citrate as an anticoagulant. The samples were then centrifuged (IEC CL3) for 10 minutes at 3000 rpm and the plasma separated and stored in eppendorf tubes at -80°C until analysis. All samples were analysed within 3 hours of thawing and the plate was read at 492nm with a blank and 7 standards (1.5, 3, 6, 12, 18, 24, 30 ng/ml).

Principle of the Assay

This assay utilises the double antibody principle utilising both quenching and normal antibodies as a control for t-PA specificity this excludes false positives which are not uncommon in conventional t-PA ELISAs. The plate is coated with goat anti-human t-PA immunoglobulin (Ig) (stage 1) and incubated for 16-18 hours. Normal goat Ig is added to odd numbered columns (A) and goat anti-human t-PA Ig is added to even numbered columns (N) (stage 2). The plasma samples are then added and the plasma t-PA binds to the antibody (stage 3). Horseradish peroxidase labelled goat anti-human t-PA IgG is added and binds to a second site on the plasma t-PA (stage 4). The enzyme substrate (OPD) is then incubated for 30 minutes (stage 5) before the reaction is stopped by addition of H_2SO_4. Finally, the plate is read at a wavelength of 492 nm.



Calculation of Results

The difference between the "N" value and "A" value is calculated for each standard and sample. This represents the t-PA specific part of the response and is denoted ΔA. The ΔA value of each of the standards is plotted against their known concentration and a straight line is fitted to the points using a minimal least square procedure. The function of this line is used to calculate the t-PA concentration of the plasma samples.

Limitations

This procedure measures both free and complexed t-PA and has a sensitivity of 1.5 ng/ml. This assay is accurate in the range of 1.5-30 ng t-PA/ml. Samples with a t-PA concentration >30 ng/ml were diluted.

Coefficient of Variation

The coefficient of variation for this procedure is 8% (Biopool International)

The coefficient of variation for this procedure in this laboratory is 6%

2.21 Von Willebrand Factor (vWF) In House ELISA Procedure HCVWF

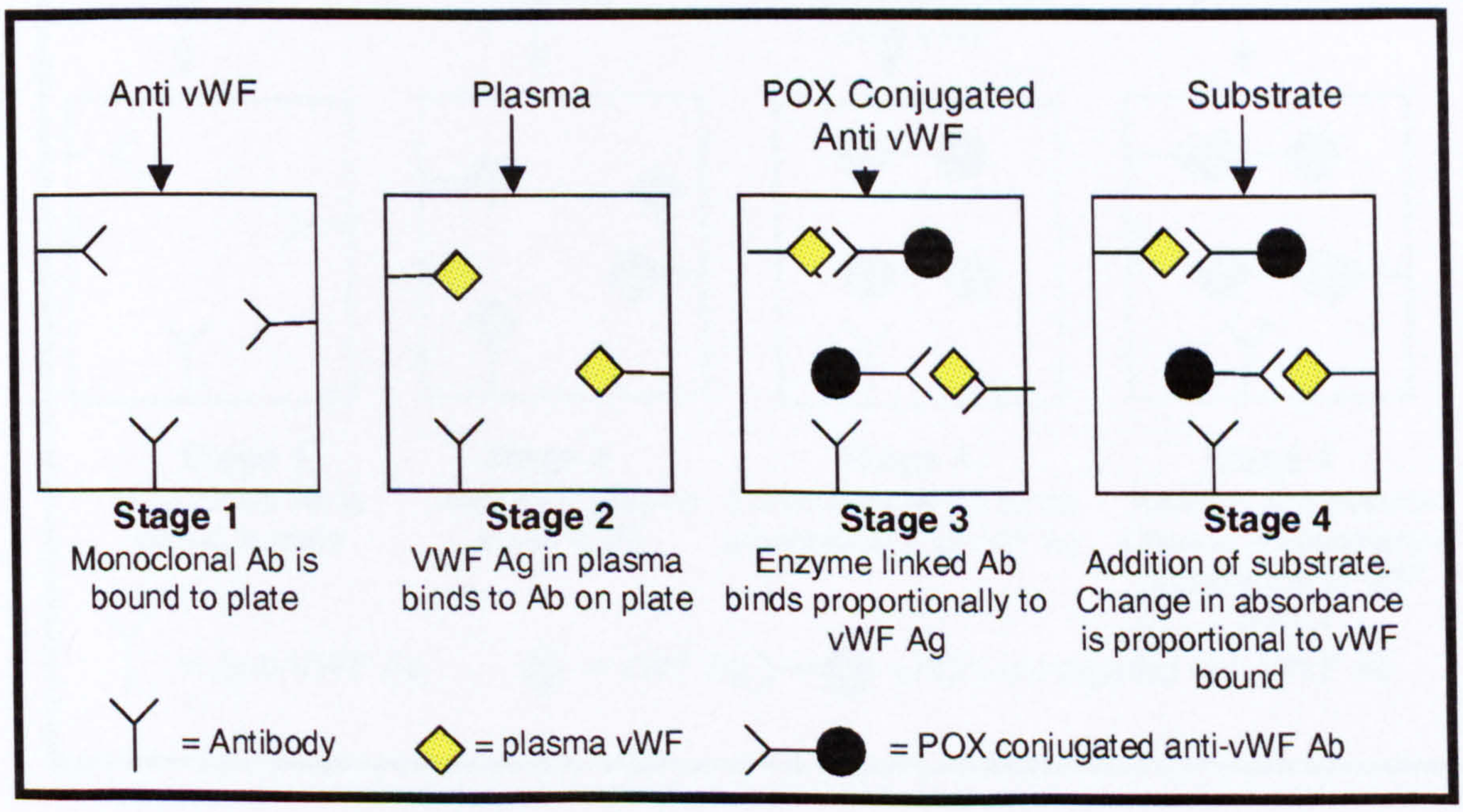
Addenbrookes Hospital Coagulation Department

Procedure

Blood samples (3ml) were collected in tubes containing sodium citrate as an anticoagulant. The samples were then centrifuged (IEC CL3) for 10 minutes at 3000 rpm and the plasma separated and stored in eppendorf tubes at -80°C until analysis. All samples were analysed within 3 hours of thawing and the plate was read at 450nm with a blank and 6 standards (0.2,0.4,0.6,0.8,1.0 & 2.0 IU/ml).

Principle of the Assay

The plate is coated with antihuman vWF monoclonal antibodies (stage 1) to which the plasma vWF antigen binds (stage2). A peroxidase linked anti vWF antibody then added to bind to the plasma vWF antigen (stage 3). The enzyme substrate (OPD) with H_2O_2 is then added and results in the formation of a orange colour complex (stage 4). Finally the enzyme reaction is stopped with sulphuric acid and the reaction causes formation of a yellow product. The plate is read at 420nm and the absorbance of each well is proportional to the concentration of vWF in the sample.



Calculation of Results

The standard solutions were used to create a graph of vWF concentration Vs absorbance and the sample concentrations were read off from there

Limitations

This method measures total vWF

Coefficient of Variation

The coefficient of variation for this procedure is 6% (Addenbrookes Hospital)

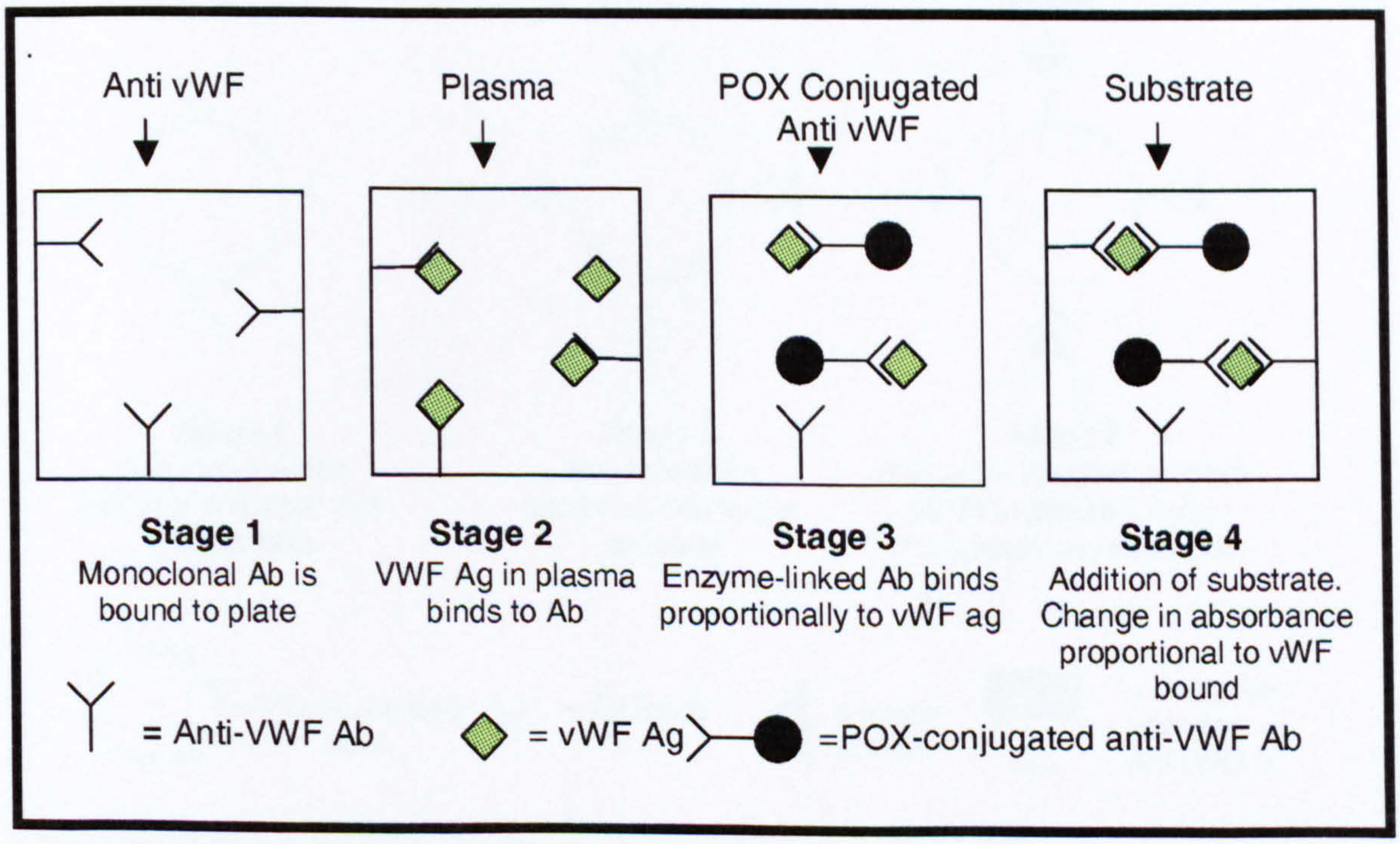
2.22 Immunozym vWF: Ag ELISA. Immuno Ag procedure 5450200

Procedure

Blood samples (3ml) were collected in tubes containing sodium citrate as an anticoagulant. The samples were then centrifuged (IEC CL3) for 10 minutes at 3000 rpm and the plasma separated and stored in eppendorf tubes at -80°C until analysis. All samples were analysed within 3 hours of thawing and the plate was read at 450nm with a blank and 5 standards (0.07, 0.5, 0.9, 1.1 & 1.5 IU/ml).

Principle of the Assay

Plates are first coated with polyclonal anti-vWF antibodies (stage 1). Diluted plasma samples are then incubated in the test wells and plasma vWF binds to the antibodies (stage 2). An anti-vWF-peroxidase conjugate is then added which bind to an alternative site on the plasma vWF (stage 3). Finally H_2O_2 is added as a substrate for the peroxidase (stage 4). The reaction is stopped with sulphuric acid and the plate is read at a wave length of 450 nm.



Calculation of Results

The standard solutions were used to create a graph of vWF concentration Vs absorbance and the sample concentrations were read off from there. Control samples were also used to determine the accuracy of the procedure.

Limitations

This method measures total vWF Ag

The sensitivity of this assay is 0.05 IU/ml

Coefficient of Variation

The coefficient of variation for this procedure is 4% (Immuno AG)

The coefficient for this procedure in this laboratory is 5%

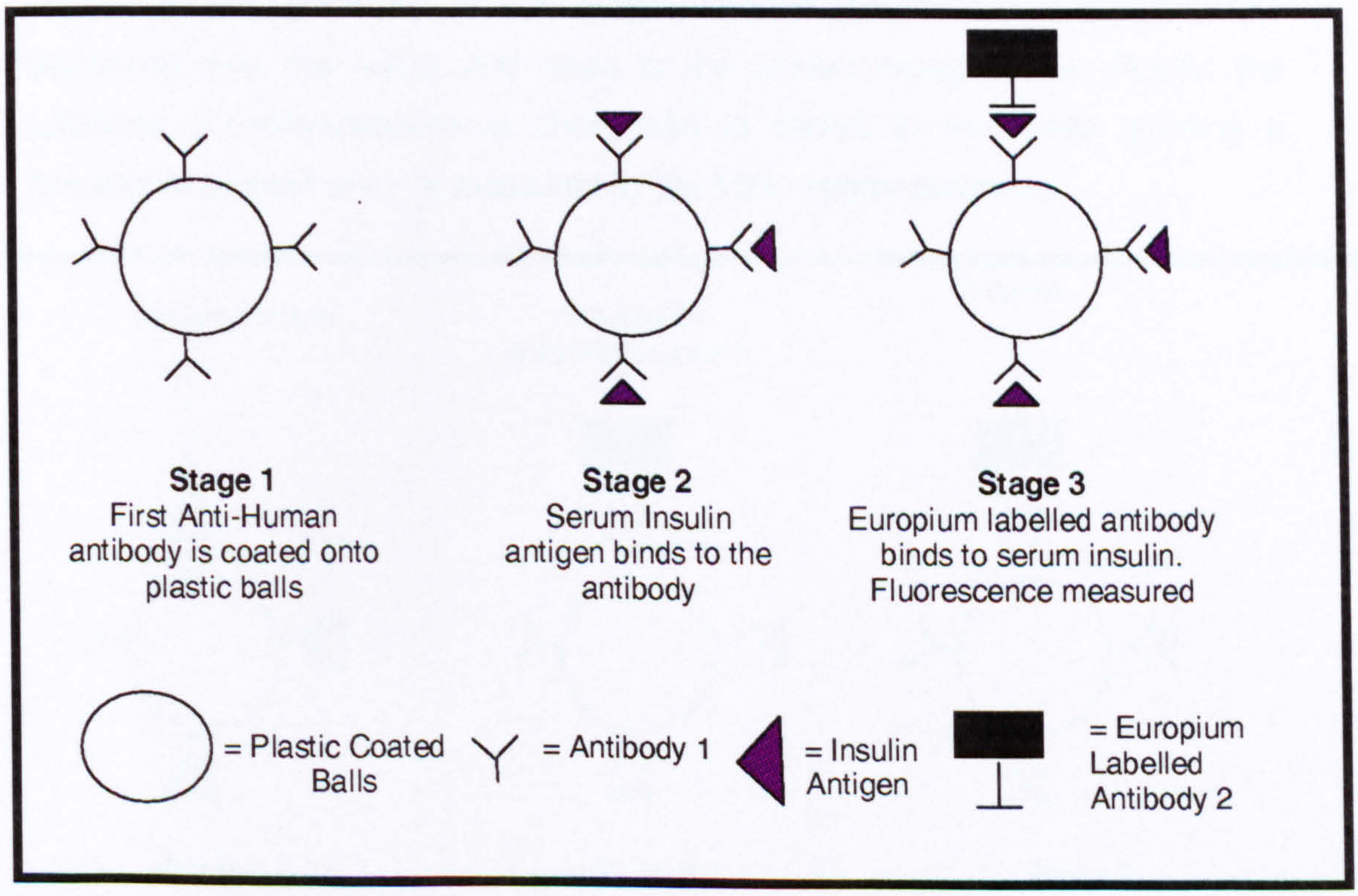
2.23 Insulin In house DELFIA Time Resolved Flurometric Assay, Addenbrookes Hospital Cambridge

Procedure

Blood samples (2ml) were collected in tubes containing no additives or anticoagulants. Samples were then centrifuged (IEC CL3) for 10 minutes at 3000 rpm and the serum separated and stored in eppendorf tubes at -20°C until analysis. All samples were analysed within 3 hours of thawing.

Principle of the Assay

Plastic balls are coated with a first anti human antibody (stage 1). The serum samples are the incubated with coated balls and the insulin antigen binds to the antibody (stage 2). A second antihuman antibody labelled with fluorescent Europium is then incubated with the samples (stage 3). Finally, the fluorescence is measured using a spectro-flurometer (Model 3000, Perkin-Elmer).



Limitations

The limit of detection for this procedure is 1.3 pmol/L

The assay range is 1100 pmol/L

The cross reactivity with proinsulin is 0.0005%

Coefficient of Variation

The coefficient of variation for this procedure is 3.1 % (n=20) (Department of Biochemistry, Addenbrookes Hospital, Cambridge).

2.24 Insulin Microparticle Enzyme Immunoassay (MEIA) Imx Systems

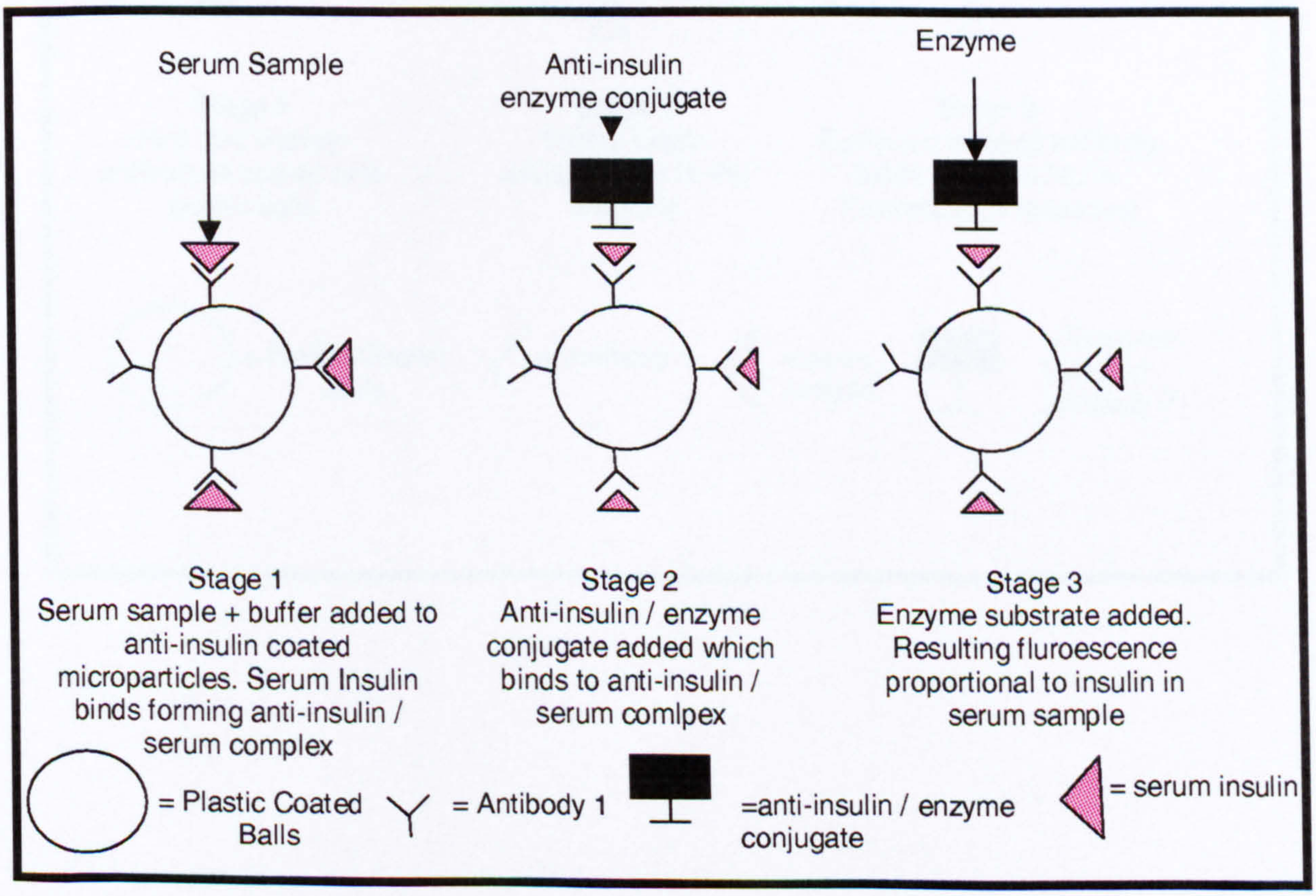
L & D Pathology Department

Procedure

Blood samples (2ml) were collected in tubes containing no additives or anticoagulants. Samples were then centrifuged (IEC CL3) for 10 minutes at 3000 rpm and the serum separated and stored in eppendorf tubes at -20°C until analysis. All samples were analysed within 3 hours of thawing. This is an automated procedure performed at the department of Biochemistry Luton and Dunstable NHS Trust Hospital.

Principle of the Assay

A probe/electrode assembly delivers the sample, anti-insulin coated microparticles and assay buffer into the incubation well of the reaction cell forming an anti-insulin / serum insulin complex. An aliquot of the anti-insulin / serum insulin coated microparticles is then transferred to a glass fibre matrix which is then washed to remove unbound materials. An anti- insulin / alkaline phosphatase conjugate is then dispensed onto the matrix and binds to the coated microparticles. Finally, the substrate (4-methylumbelliferryl phosphate) is added to the matrix yielding a fluroescent product which is measured by the MEIA optical assembly.



Limitations

This assay is linear to 30µU/ml with a sensitivity of 1.0µU/ml. The specificity of the assay is unaffected by triglyceride concentrations ≤1600 mg/dL and haemoglobin <400 mg/dL. The cross reactivity of this assay with proinsulin is 0.005%. there is no detectable cross reactivity with either C-peptide (1000 ng/ml) or glucagon (1,000,000 pg/ml).

Coefficient of Variation

The coefficient of Variation for this procedure is 4% (n= 432,Imx ltd)

The coefficient of variation for this procedure in the Dept Biochemistry, Luton & Dunstable NHS Trust Hospital is 3.5% (n=10).

2.25 Leptin In house DELFIA Time Resolved Flurometric Assay

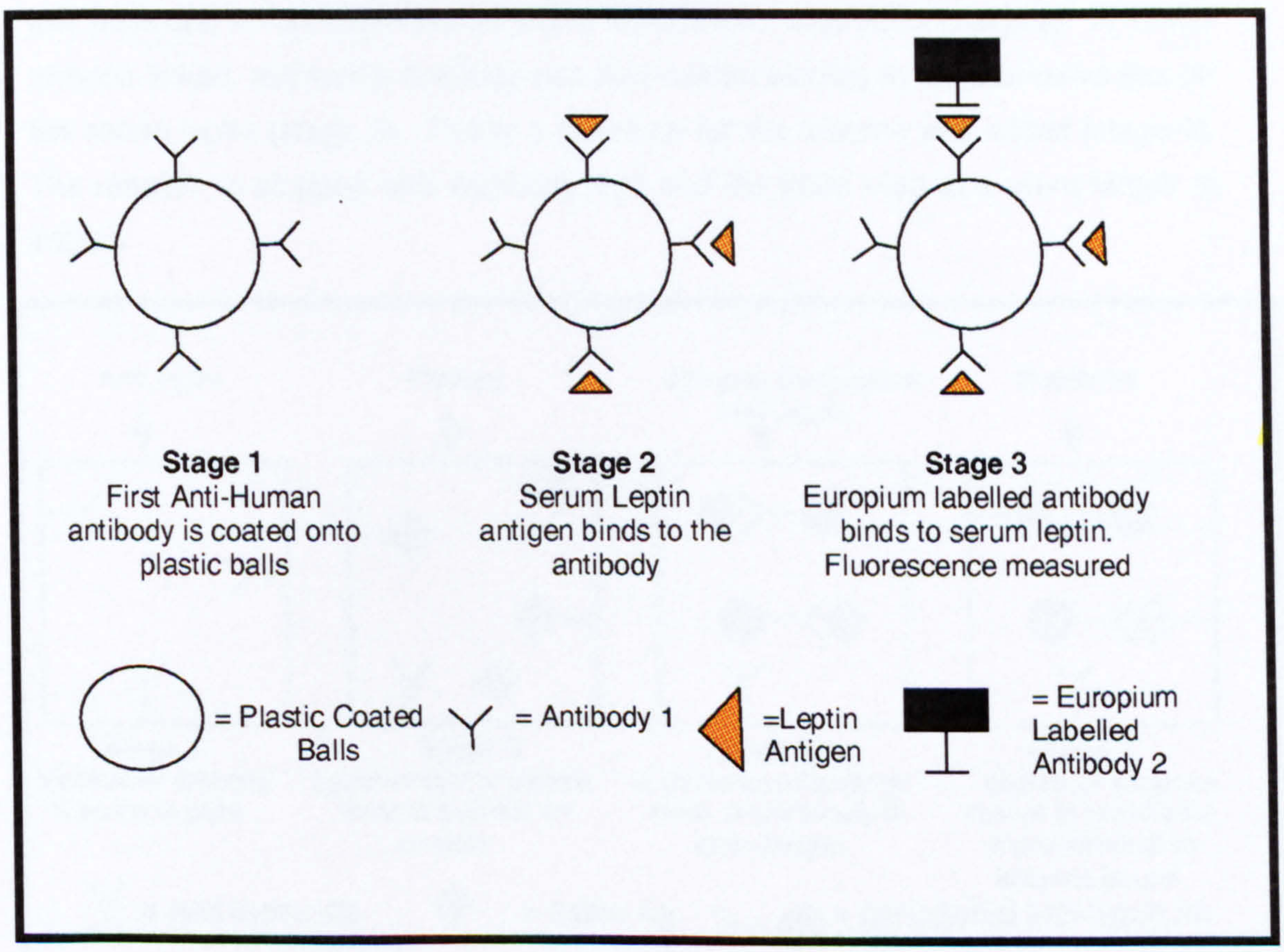
Addenbrookes Hospital Cambridge

Procedure

Blood samples (2ml) were collected in tubes containing no additives or anticoagulants. Samples were then centrifuged (IEC CL3) for 10 minutes at 3000 rpm and the serum separated and stored in eppendorf tubes at -20°C until analysis. All samples were analysed within 3 hours of thawing.

Principle of the Assay

Plastic balls are coated with a first anti human antibody (stage 1). The serum samples are the incubated with coated balls and the leptin antigen binds to the antibody (stage 2). A second antihuman antibody labelled with fluorescent Europium is then incubated with the samples (stage 3). Finally, the fluorescence is measured using a spectro-flurometer (Model 3000, Perkin-Elmer).



Limitations

The limit of detection for this procedure is 0.5 ng/ml

The assay range is 100 ng/ml

Coefficient of Variation

The coefficient of variation for this procedure is 10.2 % (n=50) (Department of Biochemistry, Addenbrookes Hospital, Cambridge).

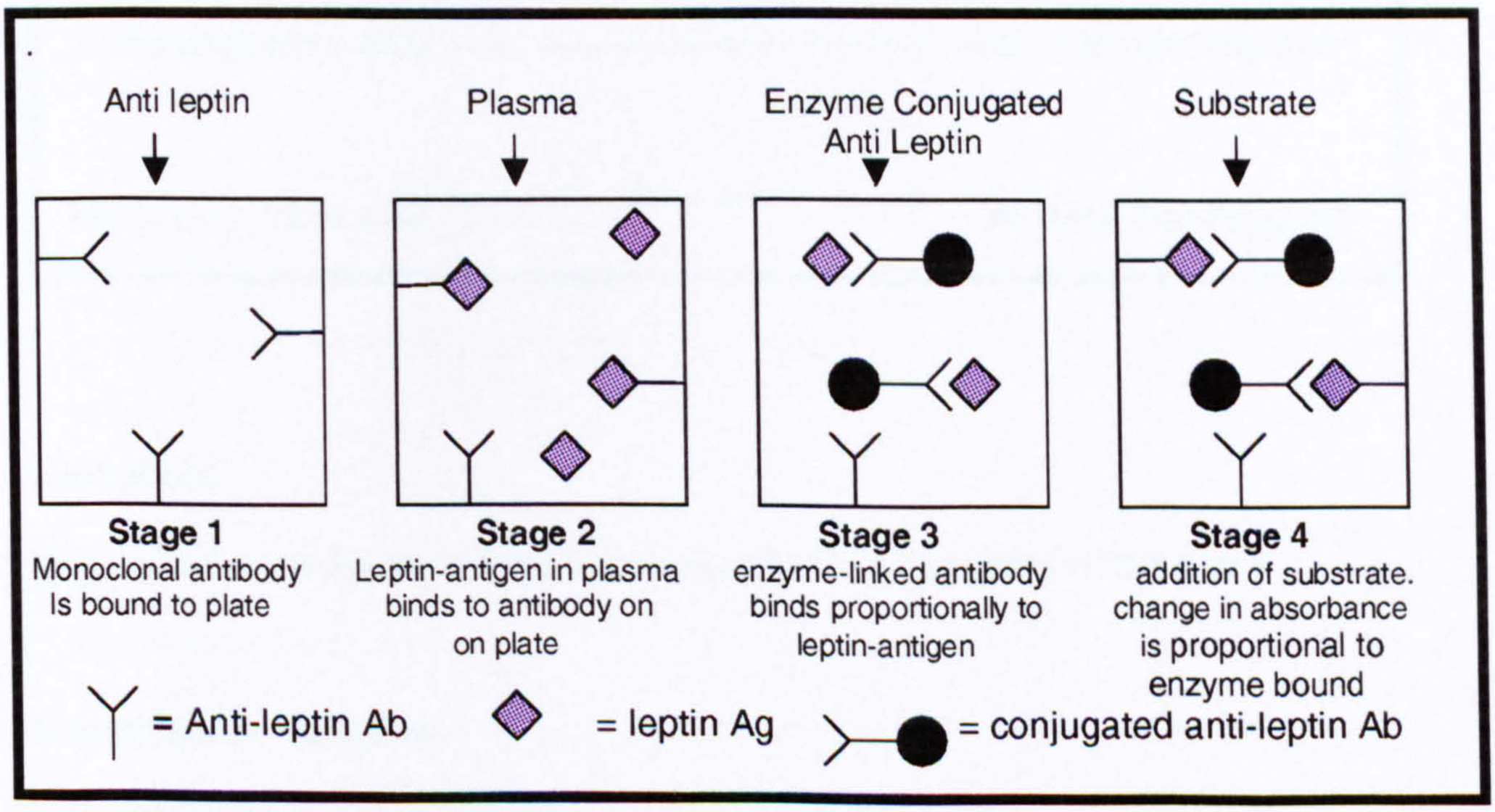
2.26 Quantikine Human Leptin ELISA (Kit No DLP00 R&D Systems)

Procedure

Blood samples (2ml) were collected in tubes containing no additives or anticoagulants. Samples were then centrifuged (IEC CL3) for 10 minutes at 3000 rpm and the serum separated and stored in eppendorf tubes at -20°C until analysis. All samples were analysed within 3 hours of thawing and read at a wave length of 450nm with a blank and 7 standards (15.6, 31.2, 62.5, 125, 250, 500 & 1000 pg/ml).

Principle of the Assay

This assay employs a quantitative sandwich enzyme immunoassay technique. A murine monoclonal antibody specific for leptin was coated onto a standard 96 well microtitre plate (stage 1). 100 X diluted plasma samples were then incubated in the test wells and serum leptin bound to the immobilised antibodies (stage 2). A further enzyme linked, anti-leptin antibody was then added binding to an alternative site on the serum leptin (stage 3). Finally a substrate for the enzyme was added (stage 4). The reaction is stopped with sulphuric acid and the plate read at a wave length of 450nm.



Calculation of Results

The standard solutions were used to create a graph of leptin concentrations Vs absorbance and sample concentrations were read off from there.

Limitations

The sensitivity of this assay is 7.8pg/ml

This assay is linear to 1000 pg/ml

Coefficient of Variation

The coefficient of variation of this assay is 3.2% (R&D Systems ltd) (n=20)

The coefficient of variation for this procedure within this laboratory is 4.2% (n=20)

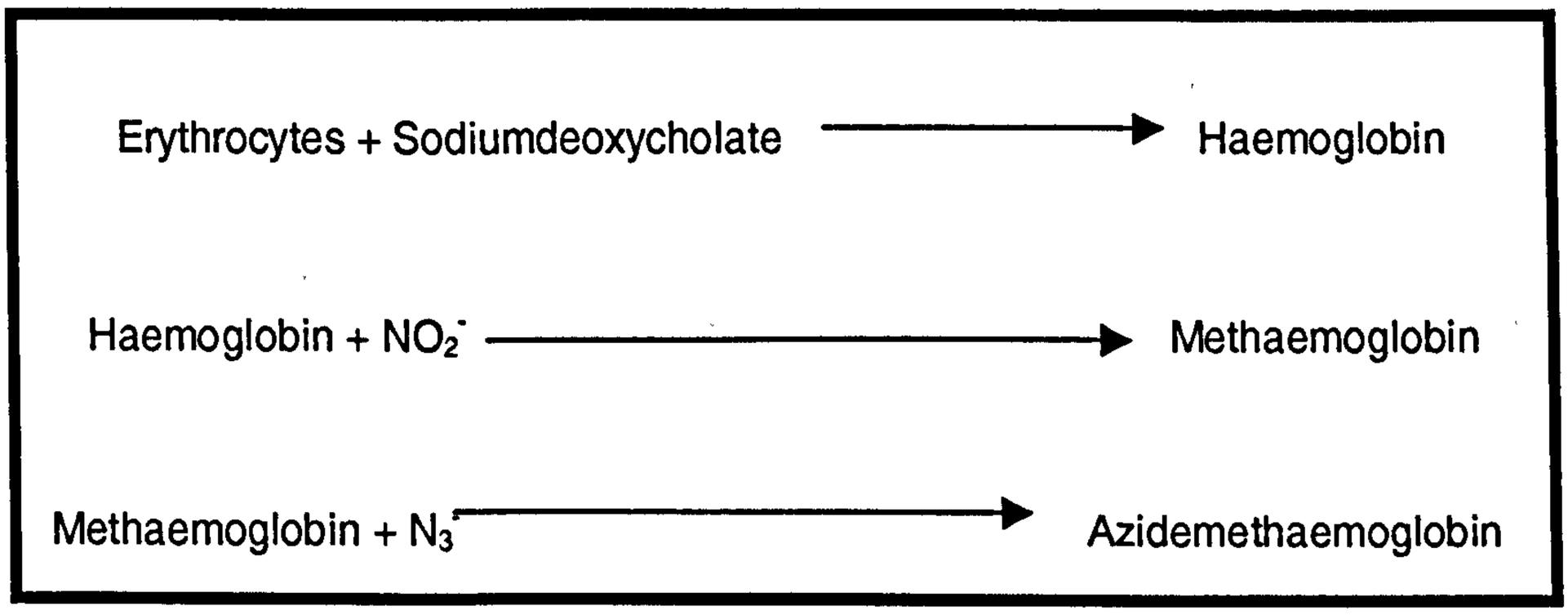
2.27 Haemoglobin (Azidemethaemoglobin Method) Hemocue Sweden

Procedure

Blood samples (2ml) were collected in tubes containing TriPotassium EDTA as an anticoagulant and were well mixed. Whole blood was analysed immediately and read at a wavelength of 570nm. (Hemocue, Angelholm, Sweden).

Principle of the Assay

Haemoglobin is released from the erythrocytes by sodiumdeoxycholate. The haemoglobin is then methylated by nitrite to form methaemoglobin. This is then reacted with sodium azide to form the coloured compound azidemethoglobin. The absorbance of this compound at 570nm is proportional to the amount of haemoglobin in the sample.



Limitations

This procedure is accurate from 0 to 25.6 g/dl with a sensitivity of 0.1 g/dl

Coefficient of Variation

The coefficient of variation for this procedure is 1.5% (Hemocue)

The coefficient of variation for this procedure within our laboratory is 0.98%

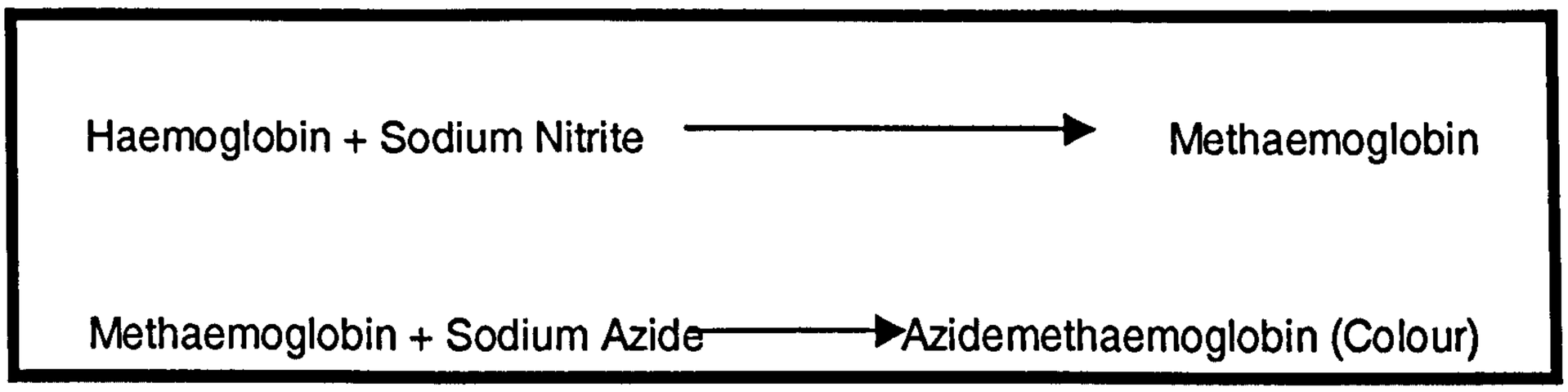
2.28 Haemoglobin (Azidemethaemoglobin Method) GDS Technology Inc UK.

Procedure

Blood samples (2ml) were collected in tubes containing TriPotassium EDTA as an anticoagulant and were well mixed. Whole blood was then analysed immediately and read using 470 nm (Hemosite, GDS, Indiana USA) with a standard of (15 g/dl)

Principle of the Assay

Haemoglobin (Hb) is first methylated to methaemoglobin (mHb) by the actions of sodium nitrite. The methehaemoglobin is then converted to coloured azidemethaemoglobin (AMHb) in a reaction with sodium azide. The absorbance of the azidemethaemoglobin at 470nm is proportional to the haemoglobin concentration.



Calculations

$$\text{Concentration of Hb (g/l)} = \left(\frac{\text{Absorbance of Unknown}}{\text{Absorbance of Standard}}\right) \text{ X Concentration of Standard}$$

Limitations

This procedure is linear from 8.0 g/l to 17.0 g/dl with a sensitivity of 0.1 g/dl.

Coefficient of Variation

The coefficient of variation for this assay is 3.5 % (n=20) (GDS Technology Inc)

The coefficient of variation for this assay within this laboratory is 1.2%

2.29 Haematocrit (Microhaematocrit Method) Hawksley UK

Procedure

Blood samples (2ml) were collected in tubes containing TriPotassium EDTA as an anticoagulant and were well mixed. The samples were then transferred to 3 haematocrit capillary tubes and sealed with Cristaseal (Hawksley, Lancing, Sussex). The samples were then centrifuged at 12,000 rpm for 10 minutes. The separated samples were read using a Microhaematocrit reader (Hawksley, Lancing, Sussex) and the results expressed as the percentage of total volume taken up by erythrocytes.

2.30 Plasma Volume Calculations

Plasma volume was calculated according to the method of Dill & Costhill (1974) which calculates plasma volume changes using both haemoglobin (Hb) and haematocrit (Hct) and thus prevents distortion of the results by changes in red cell volume.

Calculation:

A= New Result

B = Baseline Result

$$\Delta BV\ \% = 100\ (BV_A - BV_B) / BV_B$$

$$\Delta CV\ \% = 100\ (CV_A - CV_B) / CV_B$$

$$\Delta PV\ \% = 100\ (PV_A - PV_B) / PV_B$$

Where:

$BV_B = 100$

$BV_A = BV_B\ (Hb_B / Hb_A)$

$CV_A = BV_A\ (Hct_A)$

$PV_A = BV_A - CV_A$

2.31 Other Methods

Measurements of both TNFα and platelet aggregation were attempted. In both cases the variability in the baseline measurements between subjects and more importantly, within subjects were too great for meaningful results to be gained.

Table 2.3: Coefficient of Variations for platelet aggregation and TNFα

Marker	Between Subject C.V.(%)	Within Subject C.V. (%)	Number of Subjects
Platelet Aggregation	42	32	12
TNF α	31	28	15

Chapter 3

Reliability of body fat assessment: a comparison of 3 commonly used techniques

Chapter 3: Reliability of body fat assessment: a comparison of three commonly used techniques.

3.1 Introduction

There is growing interest in the measurement of body composition, in particular the assessment of fat mass. Body composition is of interest to obesity researchers because of the impact of total body fat and body fat distribution on the metabolic profile. The only direct methods of body fat assessment are by post mortem analysis and neutron activation analysis but many indirect methods have been developed including bioelectrical impedance (BIA), air displacement plethsymography (ADP), computer topography (CT), magnetic resonance imaging (MRI) and dual energy x-ray absorptiometry (DEXA). Indirect methods are by definition based on assumptions and are therefore subject to error. Common sources of error in ADP and BIA are subject hydration status, disease and medication (Jebb et al 2000).

New body composition techniques are frequently compared to 'gold standard' indirect methods such as MRI and underwater weighing as well as undergoing repeatability studies. Little information is available however, on day to day variation in body composition measurements for researchers undertaking intervention trials such as exercise training or caloric restriction studies.

The aim of this study therefore is to assess the variation in body fat measurement as analysed by three different methods on three consecutive days to calculate the error that must be taken into account when analysing body composition data in intervention trials.

3.2 Subjects and Methods.

Ten subjects (5 male & 5 female) reported to the laboratory at 9 am on 3 consecutive days. Subjects were fasted for a minimum of 6 hours and each emptied their bladder immediately prior to the start of measurements.

Height and Weight: Weight was measured with subjects in swimwear to the nearest 10g using a digital scale and height was measured to the nearest 5mm using a wall mounted stadiometer. Height was measured on the first session only and used in all subsequent body composition measurements.

3.2.2 Air displacement plethsymography:

ADP was measured using the BODPOD (Life Measurement Instruments, California USA) The BODPOD was first calibrated according to manufacturer's instructions using a 50 litre cylinder. Subjects were seated in the chamber, wearing close fitting swimwear and swim cap, for two cycles. If the two measures of volume differed by < 0.2% then the result was accepted. If the two results differed by >0.2% then the machine was recalibrated and the measurement repeated (as per manufacturers guidelines). The results were calculated based on predicted rather than measured lung volumes.

3.2.3 Tetrapolar bioelectrical impedance analysis:

Tetrapolar bioelectrical impedance analysis was measured using the Bodystat 1500 system (Bodystat, Dougals, Isle of Man, UK) with electrodes placed at the standard sites on the hand and foot. The location of the electrodes were measured and recorded for accuracy of subsequent measurements. Subjects remained supine for 15 minutes before analysis to minimise potential errors from acute shifts in body water distribution. Body fat was calculated according to the equation provided by the

manufacturer. Exercise levels included in the calculation were standardised as medium for all subjects.

3.2.4 Leg- to-leg bioelectrical impedance analysis:

Leg-to leg bioelectrical impedance analysis was measured using the Tanita-305 body fat analyser (Tanita Corp, IL, USA). Subjects stood on the metal sole plates of the machine in their swimwear. Measurements were made after a 10 min period of standing to minimise potential errors from acute shifts of body water. Fat mass was calculated according to manufacturer's equations. Subjects were standardised as non athletes regardless of exercise habits.

3.2.5 Statistical Analysis:

Reliability of estimating % body fat was determined by calculation of the coefficient of variation (CV) for repeated measures. To determine whether there was a significant difference between CV the non-parametric Wilcoxon sign-rank test was used. The first, second and third trial within each method were compared using a one way analysis of variance. All data is reported as mean $\pm$ SEM

3.3 Results

Ten subjects (5 male & 5 female) of a variety of body weights (range 65.2 –102.7 kg) completed the study. There were no significant differences between days for any of the methods of body composition analysis (Body mass P= 0.882, BODPOD P=0.822, BodyStat P=0.809, Tanita P= 0.778). The between trial CV were: body mass 0.35% ± 0.22 BODPOD, 2.21% ± 0.46%, BodyStat, 2.49% ± 0.64% and Tanita, 2.42% ± 0.43%. This reflects a body fat percentage variation of 0.70% for the BODPOD, 0.77% for the BodyStat and 0.73% for the Tanita. There were no significantly differences in the amount of variation between the three methods of body composition analysis but, there was significantly less variation in body mass (P= 0.02 Body Mass Vs BODPOD, P=0.04 Body Mass Vs Tanita, P=0.04 Body Mass Vs Body Stat).

3.4 Discussion

The reliability of all three methods over the three days was found to be excellent in a variety of subjects (obese and normal weight as well as men and both pre and post menopausal women). The average CV corresponds to trial-to-trial variations of 0.70% fat for BODPOD, 0.77% fat for BodyStat and 0.73% fat for Tanita. Further, the CVs for the body fat measurements were not significantly different from each other indicating that each compares favourably to the others when standard acceptance criteria for reliability are applied. The variations were however significantly greater than the day-to-day variations in body mass reflecting additional sources of error in the composition analysis systems such as hydration status. When conducting intervention trials this small error must however be taken into consideration when reporting changes in body composition.

Chapter 4

Relationships between plasma PAI-1 concentrations and other markers of the insulin resistance syndrome in three obese populations

Chapter 4: Relationships between plasma PAI-1 concentrations and other markers of the insulin resistance syndrome in three obese populations

4.1 Introduction

Since elevated plasma concentrations of plasminogen activator inhibitor (PAI-1) were included as part of the Insulin Resistance Syndrome (IRS) numerous studies have been conducted to establish the nature of the relationships between PAI-1 and the various other markers of the IRS (Vague 1989, Juhan-Vague *et al* 1991, Jannad-Delanne *et al* 1998). These studies provide indications to the possible mechanisms responsible for elevated PAI-1 concentrations that may be confirmed by further *in vitro* or *in vivo* studies.

The results of these studies are conflicting. Jannad-Delanne *et al* (1998) determined that anthropometric measures were the main determinants of PAI-1 concentrations confirming earlier work by Vague (1989). Potter van Loon (1990) however, reported that insulin was the major determinant of excess PAI-1 in obesity when measured against BMI, waist to hip ratio, diastolic blood pressure and insulin resistance. Other groups have reported that plasma triglyceride and BMI (Apslund-Carlson *et al* 1993), VLDL cholesterol (Vague 1993, Vague *et al* 1995) and leptin (De Mitro *et al* 1999) are all responsible for elevated plasma PAI-1 concentrations in obesity.

Reasons for the inconsistency in results include measurement of different markers of the IRS and the use of different methodologies to measure components of the IRS including a number of available ELISA assays for the determination of plasma PAI-1. In most PAI-1 assays the definition of total PAI-1 includes concentrations of the active and latent PAI-1 conformation but does not always include measures of PAI-1 in complex with t-PA.

The primary aim of this study therefore is to determine the nature of the relationships between plasma PAI-1 concentrations and other markers of the IRS in a local population from which volunteers for future studies will be drawn.

4.2 Subjects & Methods

144 (54 premenopausal females, 52 post menopausal females, 38 men) obese volunteers were recruited for participation in the study. Inclusion criteria for the study were BMI >29 kg/m^2 and waist circumference >88 cm in women and 102 cm in men. No subject was taking any prescription or "over the counter" medication for 4 weeks prior to the trial day with the exception of oral contraceptives and hormone replacement therapy.

4.2.2 Experimental Design

All subjects arrived at the Centre for Obesity Research between 9 and 10 am to standardise for the effects of diurnal variation having fasted overnight, abstained from smoking for 8 hours, alcohol for 24 hours and strenuous exercise for 48 hours. Premenopausal women attended the laboratory in the early follicular phase (day 7 – 11) of their menstrual cycle to standardise for the effect of hormonal fluctuation on markers of the insulin resistance syndrome.

On the trial day, subjects underwent measures of height (standard stadiometer), weight (TBF-310, Tanita Corp) waist circumference, hip circumference and blood pressure. Average blood pressure was determined by the mean of three values recorded (Omron 711) 10 minutes apart in a seated position. A 10ml blood sample was taken before blood pressure analysis.

4.2.3 Laboratory Methods

Blood samples were collected in tubes containing no additives and sodium citrate. The no-additives samples were allowed to clot for 20 minutes before being centrifuged at 3000rpm for 15 minutes (IEC Centra-2). The resultant serum was analysed immediately for insulin (MEI, Imx systems) total cholesterol (procedure 467825 Beckman systems), triglyceride (procedure 445850 Beckman systems), and HDL cholesterol (EZHDL Sigma Diagnostics). LDL cholesterol was calculated using the Friedewald equation. Sodium citrate samples were immediately centrifuged at 3000 rpm for 15 minutes (IEC Centra-2) and the resultant plasma stored at -80°C until analysis for PAI-1 Ag by ELISA (procedure 101005 Biopool International). All samples were analysed within 1 hour of thawing.

4.2.4 Statistical Analysis

Data was initially assessed for normality of distribution using a Shapiro-Wilks test. The relationships between PAI-1 and other markers of the IRS were assessed utilising a Spearman's rho correlation test. Data was then log transformed to assume normality before multiple partial correlations were performed. Relationships were considered significant when $P \leq 0.05$.

4.3 Results

4.3.1 Demographic Data

The demographic data for the populations studied is presented in table 4.1. All subjects were Caucasian with the exception of 3 males (2 Asian, 1 Afro-Caribbean) and 2 postmenopausal females (Afro-Caribbean). All subjects were classed as obese (BMI $\geq$ 29 kg/m^2) according to study criteria possessing a waist circumference greater than 102 cm (males) and 88 cm (pre and post menopausal females).

Table 4.1:Demographic features of the population studied. All samples were taken between 9 and 10 am following an overnight fast. Data is shown as median (range)

Marker	Premenopausal Females	Post menopausal Females	Males
Number of patients	54	52	38
Body Mass (kg)	89.20 (72.70–143.60)	84.90 (72.50-125.00)	94.40 (60.80-162.20)
BMI (kg/m^2)	34.37 (29.92-47.90)	34.00 (29.68-44.00)	35.20 (29.00-47.00)
Waist circumference (cm)	98 (90-131)	102 (90- 120)	121 (106-146)
Waist to hip ratio	0.86 (0.76-0.97)	0.85 (0.76-0.92)	1.02 (0.86-1.12)
Systolic blood pressure (mmHg)	126 (97-160)	130 (110-191)	127 (110-161)
Diastolic blood pressure (mmHg)	84 (60-97)	83 (56-99)	87 (63-99)
Insulin (pmol/L)	9.10 (2.50-38.00)	9.20 (1.30-27.20)	19.50 (2.30-60.50)
Total Cholesterol (mmol/L)	5.28 (3.05-7.40)	5.75 (3.90-8.10)	6.00 (4.70-7.18)
Triglycerides (mmol/L)	1.17 (0.50-3.07)	1.55 (0.70-3.66)	2.35 (1.00-5.00)
HDL Cholesterol (mmol/L)	1.21 (0.66-2.03)	1.60 (1.06-2.28)	1.04 (0.76-1.40)
LDL Cholesterol (mmol/L)	3.42 (1.39-4.80)	3.80 (1.90-5.70)	4.00 (2.30-4.52)
PAI-1 (ng/ml)	13.50 (3.70-74.20)	6.85 (3.30-65.10)	24.40 (8.20-98.60)

The relationships between total PAI-1 Ag and other markers of the IRS (table 4.2) were assessed by Spearman's rho correlations. Total PAI-1 Ag showed significant positive relationships with body mass, BMI, waist circumference, systolic blood pressure, insulin and triglyceride concentrations in all three subject groups. Significant negative correlations were also observed between total PAI-1 and HDL cholesterol in all three populations studies. The relationships between waist to hip ratio and PAI-1 as well as total cholesterol and PAI-1 only reached significance in postmenopausal females and the male populations studied. There were no significant correlations between PAI-1 and LDL cholesterol in any of the groups at rest.

Table 4.2: Relationships between total PAI-1 Ag and other markers of the IRS in the three patient groups as assessed by Spearman's rho correlation test. Significant relationships ($P \leq 0.05$) are shown in red.

	Premenopausal Females		Postmenopausal Females		Males	
	R	P	R	P	R	P
Body Mass (kg)	**0.538**	**0.000**	**0.336**	**0.015**	**0.586**	**0.001**
BMI (kg/m^2)	**0.467**	**0.002**	**0.318**	**0.022**	**0.616**	**0.001**
Waist circumference (cm)	**0.486**	**0.001**	**0.396**	**0.004**	**0.655**	**0.000**
Waist to hip ratio	0.189	0.230	**0.343**	**0.013**	**0.495**	**0.009**
Systolic blood pressure (mmHg)	**0.437**	**0.026**	**0.374**	**0.006**	**0.582**	**0.031**
Diastolic blood pressure (mmHg)	-0.178	0.260	-0.074	0.602	0.326	0.097
Insulin (pmol/L)	**0.326**	**0.035**	**0.298**	**0.032**	**0.581**	**0.001**
Total cholesterol (mmol/L)	-0.114	0.473	**0.279**	**0.045**	**0.430**	**0.025**
Triglyceride (mmol/L)	**0.463**	**0.021**	**0.558**	**0.000**	**0.573**	**0.050**
HDL cholesterol (mmol/L)	**-0.220**	**0.042**	**-0.129**	**0.036**	**-0.222**	**0.026**
LDL cholesterol (mmol/L)	-0.019	0.905	0.230	0.100	0.355	0.070

4.3.2 Partial Correlations

In an attempt to determine whether the correlating factors may have a direct influence on total PAI-1 Ag or may exert an indirect influence via other measured factors multiple partial correlations were performed controlling for each factor in turn (tables 4.3, 4.4, 4.5).

No factor was independently associated with total PAI-1 Ag in any of the three subject groups. In all three groups however, body mass, BMI and waist circumference were significantly correlated with total PAI-1 when controlling for waist to hip ratio, blood pressure and blood markers of the IRS. Furthermore, when controlling for each of these factors in turn, no other measured variables significantly correlated with PAI-1. Systolic blood pressure was also significantly correlated with total PAI-1 Ag when controlling for blood markers of the IRS in all three subject groups.

Unexpectedly, insulin, triglyceride and total cholesterol were not independently associated with total PAI-1 Ag when controlling for blood pressure and other blood markers of the IRS in males and, with the exception of insulin when controlling for HDL cholesterol, the same was true in post menopausal females.

HDL cholesterol was not independently significantly associated with total PAI-1 in males when controlling for any of the measured variables. In post menopausal

Partial Correlations

Premenopausal Females

Table 4.3: Multiple partial correlations to assess the relationship between total PAI-1 Ag and other markers of the IRS in premenopausal women. Significant relationships are shown in red. Row labels in blue indicate significant correlations without controlling factors. WC = waist circumference, WHR= waist to hip ratio, Chol = cholesterol, Trigs = triglyceride

	Controlling Factor										
Marker	Body mass Kg	BMI kg/m^2	WC cm	WHR	Systolic BP mmHg	Diastolic BP mmHg	Insulin pmol/L	Total Chol mmol/L	Trigs mmol/L	HDL Chol mmol/L	LDL Chol mmol/L
Body Mass (kg)		R= 0.248 P= 0.134	R= 0.229 P= 0.165	**R= 0.478 P= 0.002**	**R= 0.503 P= 0.001**	**R= 0.507 P= 0.001**	**R= 0.454 P= 0.004**	**R= 0.499 P= 0.001**	**R= 0.515 P= 0.001**	**R= 0.473 P= 0.003**	**R= 0.513 P= 0.001**
BMI (kg/m^2)	R=0.132 P= 0.430		R= 0.182 P= 0.275	**R= 0.444 P= 0.005**	**R= 0.460 P= 0.004**	**R= 0.471 P= 0.003**	**R= 0.408 P= 0.011**	**R= 0.473 P= 0.003**	**R= 0.503 P= 0.001**	**R= 0.441 P= 0.005**	**R= 0.479 P= 0.002**
Waist circumference (cm)	R= 0.086 P= 0.608	R= 0.179 P= 0.283		**R= 0.440 P= 0.006**	**R=0.461 P=0.004**	**R= 0.471 P= 0.001**	**R= 0.415 P= 0.010**	**R= 0.465 P= 0.003**	**R= 0.487 P= 0.002**	**R= 0.452 P= 0.004**	**R= 0.477 P= 0.002**
Waist to hip ratio	R= 0.047 P= 0.808	R= 0.089 P= 0.597	R= 0.070 P= 0.677		R= 0.234 P= 0.158	R=0.235 P= 0.155	R= 0.164 P= 0.325	R= 0.191 P= 0.250	R= 0.221 P= 0.183	R= 0.164 P= 0.324	R= 0.217 P= 0.190
Systolic blood pressure (mmHg)	R=-0.096 P= 0.566	R= 0.037 P= 0.826	R= -0.058 P= 0.729	R=-0.176 P= 0.291		R= -0.121 P= 0.471	**R= 0.351 P= 0.051**	**R= 0.466 P= 0.032**	**R=-0.367 P= 0.021**	**R= 0.399 P= 0.021**	**R= 0.365 P= 0.042**
Diastolic blood pressure (mmHg)	R= 0.110 P= 0.950	R= 0.008 P= 0.961	R=-0.044 P= 0.795	R=-0.133 P= 0.427	R= -0.012 P= 0.943		R= 0.134 P= 0.422	R= -0.097 P= 0.559	R=-0.106 P= 0.527	R=-0.162 P= 0.331	R= -0.108 P= 0.518
Insulin (pmol/L)	R= 0.091 P= 0.587	R= 0.074 P= 0.660	R= 0.118 P= 0.482	R= 0.247 P= 0.136	R= 0.285 P= 0.153	R= 0.299 P= 0.148		R= 0.281 P= 0.147	R= 0.229 P= 0.149	R= 0.201 P= 0.225	R= 0.297 P= 0.151
Total cholesterol (mmol/L)	R=-0.060 P= 0.723	R= 0.126 P= 0.451	R=-0.087 P= 0.606	R=-0.107 P= 0.521	R= 0.118 P= 0.479	R= -0.152 P= 0.364	R= -0.145 P= 0.384		R=-0.141 P= 0.397	R=-0.105 P= 0.530	R=-0.255 P= 0.122
Triglyceride (mmol/L)	R=-0.072 P= 0.668	R=-0.184 P= 0.267	R=-0.122 P= 0.467	R=-0.065 P= 0.697	R= 0.045 P= 0.789	R=-0.070 P= 0.679	R=-0.077 P= 0.645	R=-0.008 P= 0.962		R=-0.112 P= 0.502	R=-0.068 P= 0.687
HDL cholesterol (mmol/L)	R=-0.130 P= 0.436	R=-0.162 P= 0.330	R=-0.196 P= 0.237	R=-0.213 P= 0.189	**R=-0.292 P= 0.054**	**R= -0.289 P= 0.049**	R=-0.164 P= 0.324	R=-0.237 P= 0.151	**R=-0.277 P= 0.043**		R=-0.269 P= 0.102
LDL cholesterol (mmol/L)	R=-0.004 P= 0.980	R=-0.036 P= 0.829	R= 0.005 P= 0.977	R=-0.032 P= 0.848	R= -0.023 P= 0.889	R=-0.061 P= 0.717	R=-0.096 P= 0.565	R= 0.211 P= 0.202	R= -0.054 P= 0.746	R=-0.082 P= 0.623	

women however, HDL cholesterol was significantly correlated with PAI-1 Ag despite controlling for BMI (R= -0.429 P= 0.032) as well as a trend towards a relationship between the 2 factors despite controlling for body mass (R= -0.357 P=0.079). In premenopausal women, HDL cholesterol retained a significant association with total PAI-1 Ag when controlling for blood pressure (systolic R= -0.292, P = 0.054, diastolic R= -0.289 P=0.049) and interestingly, triglyceride (R= -0.277, P-0.043).

Post Menopausal Females

Table 4.4: Multiple partial correlations to assess the relationship between total PAI-1 Ag and other markers of the IRS in postmenopausal women. Significant relationships are shown in red. Row labels in blue indicate significant correlations without controlling factors. . WC = waist circumference, WHR= waist to hip ratio, Chol = cholesterol, Trigs = triglyceride

	Controlling Factor										
Marker	Body mass kg	BMI kg/m^2	WC cm	WHR	Systolic BP mmHg	Diastolic BP mmHg	Insulin pmol/L	Total Chol mmol/L	Trigs mmol/L	HDL Chol mmol/L	LDL Chol mmol/L
Body Mass (kg)		R=-0.008 P= 0.970	R= 0.177 P= 0.398	**R= 0.250 P= 0.045**	**R= 0.276 P= 0.036**	**R= 0.225 P= 0.009**	**R= 0.149 P= 0.048**	**R= 0.201 P= 0.010**	**R= 0.132 P= 0.052**	**R= 0.419 P= 0.037**	**R= 0.195 P= 0.051**
BMI (kg/m^2)	R= 0.131 P= 0.534		R= 0.213 P= 0.306	**R= 0.326 P= 0.011**	**R= 0.314 P= 0.027**	**R= 0.243 P= 0.024**	**R= 0.132 P= 0.052**	**R= 0.306 P= 0.013**	**R= 0.165 P= 0.032**	**R= 0.491 P= 0.013**	**R= 0.280 P= 0.017**
Waist circumference (cm)	R= 0.052 P= 0.805	R= 0.020 P= 0.924		**R= 0.260 P= 0.009**	**R= 0.243 P= 0.024**	**R= 0.179 P= 0.039**	**R= 0.107 P= 0.025**	**R= 0.209 P= 0.031**	**R= 0.099 P= 0.036**	**R= 0.254 P= 0.021**	**R= 0.194 P= 0.035**
Waist to hip ratio	R=-0.209 P= 0.316	R=-0.270 P= 0.192	R=-0.276 P= 0.182		R= 0.207 P= 0.321	R= 0.279 P= 0.176	R= 0.324 P= 0.114	R= 0.105 P= 0.619	R= 0.274 P= 0.185	R= 0.191 P= 0.360	R= 0.131 P= 0.533
Systolic blood pressure (mmHg)	**R= 0.322 P= 0.009**	**R= 0.304 P= 0.045**	R=-0.190 P= 0.364	R= -0.101 P= 0.632		R= -0.513 P= 0.808	**R= 0.224 P= 0.031**	**R= 0.209 P= 0.044**	**R= 0.218 P= 0.044**	**R= 0.179 P= 0.035**	**R= 0.204 P= 0.032**
Diastolic blood pressure (mmHg)	R=-0.137 P= 0.513	R=-0.104 P= 0.623	R=-0.170 P= 0.419	R=-0.257 P= 0.215	R= -0.152 P= 0.469		R=-0.108 P= 0.607	R= 0.077 P= 0.715	R=-0.174 P= 0.404	R= 0.379 P= 0.152	R= 0.197 P= 0.663
Insulin (pmol/L)	R= 0.254 P= 0.221	R= 0.209 P= 0.317	R= 0.283 P= 0.170	**R= 0.401 P= 0.047**	R= 0.310 P= 0.131	R= 0.289 P= 0.162		R= 0.345 P= 0.091	R= 0.255 P= 0.218	**R= 0.379 P= 0.052**	R= 0.331 P= 0.106
Total cholesterol (mmol/L)	R= 0.255 P= 0.219	R= 0.318 P= 0.121	R= 0.308 P= 0.135	R= 0.234 P= 0.259	R= 0.286 P= 0.166	R= 0.313 P= 0.127	R= 0.321 P= 0.118		R= 0.196 P= 0.348	R= 0.287 P= 0.170	R= 0.297 P= 0.149
Triglyceride (mmol/L)	R= 0.175 P= 0.404	R= 0.153 P= 0.465	R= 0.226 P= 0.278	R= 0.324 P= 0.113	R= 0.305 P= 0.138	R= 0.263 P= 0.204	R= 0.190 P= 0.362	R= 0.162 P= 0.438		**R= 0.357 P= 0.049**	R= 0.210 P= 0.312
HDL cholesterol (mmol/L)	R=-0.357 P= 0.079	**R=-0.429 P= 0.032**	R= 0.198 P= 0.342	R= 0.034 P= 0.873	R= 0.215 P= 0.303	R= 0.096 P= 0.648	R= 0.232 P= 0.264	R= 0.045 P= 0.831	R= 0.256 P= 0.218		R= 0.094 P= 0.653
LDL cholesterol (mmol/L)	R= 0.154 P= 0.463	R= 0.220 P= 0.290	R= 0.226 P= 0.277	R= 0.149 P= 0.477	R= 0.051 P= 0.808	R= 0.243 P= 0.243	R= 0.236 P= 0.255	R=-0.255 P= 0.279	R= 0.131 P= 0.532	**R= 0.420 P= 0.037**	

Males

Table 4.5: Multiple partial correlations to assess the relationship between total PAI-1 Ag and other markers of the IRS in males. Significant relationships are shown in red. Row labels in blue indicate significant correlations without controlling factors. . WC = waist circumference, WHR= waist to hip ratio, Chol = cholesterol, Trigs = triglyceride

	Controlling Factor										
Marker	Body mass kg	BMI kg/m^2	WC cm	WHR	Systolic BP mmHg	Diastolic BP mmHg	Insulin pmol/L	Total Chol mmol/L	Trigs mmol/L	HDL Chol mmol/L	LDL Chol mmol/L
Body Mass (kg)		R= 0.028 P= 0.892	R= 0.053 P= 0.796	**R= 0.489 P= 0.011**	**R= 0.477 P= 0.014**	**R= 0.491 P= 0.011**	**R= 0.473 P= 0.015**	**R= 0.459 P= 0.018**	**R= 0.503 P= 0.009**	**R= 0.418 P= 0.033**	**R= 0.446 P= 0.023**
BMI (kg/m^2)	R= 0.176 P= 0.390		R= 0.026 P= 0.900	**R= 0.532 P= 0.005**	**R= 0.504 P= 0.009**	**R= 0.514 P= 0.007**	**R= 0.497 P= 0.015**	**R= 0.483 P= 0.012**	**R= 0.514 P= 0.005**	**R= 0.448 P= 0.022**	**R= 0.458 P= 0.019**
Waist circumference (cm)	R= 0.227 P= 0.265	R= 0.141 P= 0.493		**R= 0.614 P= 0.001**	**R= 0.510 P= 0.008**	**R= 0.530 P= 0.005**	**R= 0.516 P= 0.007**	**R= 0.500 P= 0.009**	**R= 0.556 P= 0.003**	**R= 0.467 P= 0.016**	**R= 0.473 P= 0.015**
Waist to hip ratio	R=-0.146 P= 0.476	R= 0.221 P= 0.279	R=-0.398 P= 0.444		R= 0.088 P= 0.669	R= 0.165 P= 0.420	R= 0.108 P= 0.601	R= 0.115 P= 0.577	R= 0.119 P= 0.564	R= 0.017 P= 0.935	R= 0.124 P= 0.545
Systolic blood pressure (mmHg)	R=0.097 P= 0.638	R= 0.113 P= 0.582	R= 0.039 P= 0.851	R= 0.100 P= 0.627		R= 0.225 P= 0.269	**R= 0.444 P= 0.048**	**R= 0.477 P= 0.039**	**R= 0.435 P= 0.051**	**R= 0.471 P= 0.031**	**R= 0.466 P= 0.041**
Diastolic blood pressure (mmHg)	R= 0.021 P= 0.918	R=-0.005 P= 0.981	R=-0.065 P= 0.753	R=-0.061 P= 0.768	R=-0.159 P= 0.438		R=-0.056 P= 0.788	R=-0.046 P= 0.823	R=-0.065 P= 0.754	R=-0.069 P= 0.738	R=-0.063 P= 0.760
Insulin (pmol/L)	R= 0.082 P= 0.691	R=-0.083 P= 0.687	R=-0.118 P= 0.566	R= 0.130 P= 0.528	R= .0154 P= 0.452	R= 0.179 P= 0.382		R= 0.083 P= 0.689	R= 0.140 P= 0.494	R= 0.236 P= 0.247	R= 0.063 P= 0.760
Total cholesterol (mmol/L)	R= 0.039 P= 0.850	R=-0.016 P= 0.940	R=-0.042 P= 0.838	R= 0.171 P= 0.405	R= 0.212 P= 0.299	R= 0.204 P= 0.318	R= 0.133 P= 0.518		R= 0.171 P= 0.405	R= 0.251 P= 0.215	R=-0.048 P= 0.815
Triglyceride (mmol/L)	R=-0.465 P= 0.422	R=-0.192 P= 0.348	R=-0.232 P= 0.254	R= 0.036 P= 0.861	R= 0.058 P= 0.780	R= 0.123 P= 0.549	R= 0.040 P= 0.845	R=-0.016 P= 0.938		R= 0.040 P= 0.847	R= 0.009 P= 0.966
HDL cholesterol (mmol/L)	R=-0.089 P= 0.666	R=-0.094 P= 0.647	R=-0.104 P= 0.613	R=-0.255 P= 0.208	R=-0.260 P= 0.200	R=-0.302 P= 0.134	R=-0.335 P= 0.095	R=-0.033 P= 0.099	R=-0.280 P= 0.166		R=-0.303 P= 0.133
LDL cholesterol (mmol/L)	R= 0.151 P= 0.462	R= 0.081 P= 0.693	R= 0.069 P= 0.738	R= 0.258 P= 0.208	R= 0.275 P= 0.173	R= 0.279 P= 0.167	R= 0.225 P= 0.270	R= 0.196 P= 0.337	R= 0.253 P= 0.212	R= 0.281 P= 0.164	

4.4 Discussion

4.4.1 Summary of the Study

144 obese volunteers (54 premenopausal women, 52 post menopausal women, 38 men) underwent measures of height, weight, waist and hip circumference and each had a blood sample taken which was subsequently analysed for insulin, total cholesterol, triglyceride, HDL cholesterol, LDL cholesterol and total PAI-1. The assay for total PAI-1 measures the active and latent forms of PAI-1 only. Multiple bivariate and partial correlations were used to assess the relationship between PAI-1 Ag and each of the markers of the IRS measured.

4.4.2 Correlations with PAI-1 Ag.

Total PAI-1 Ag (table 4.2) showed significant positive relationships with body mass, BMI, waist circumference, systolic blood pressure, insulin and triglyceride concentrations in all three subject groups. Significant negative correlations were also observed between total PAI-1 and HDL cholesterol in all three populations studies. Numerous research studies have reported significant correlations between PAI-1 Ag and BMI (Mavri *et al* 1999, Alessi *et al* 2000), waist circumference (Landin *et al* 1990, Mertens & Van Gaal 2002), waist to hip ratio (Mertens & Van Gaal 2002), systolic blood pressure (Poli *et al* 2000), insulin (Giltay *et al* 1998, Abassi *et al* 1999), triglyceride (Mertens & Van Gaal 2002) and HDL cholesterol (Abassi *et al* 1999).

Interestingly, most of these studies (Cigolini *et al* 1996, Alessi *et al* 2000, Mertens & Van Gaal 2002) as with this one, observed that the significant correlations between PAI-1 and the various metabolic variables disappeared after controlling for anthropometric variables, particularly measures of visceral fat mass such as waist to hip ratio and waist circumference. These are however crude measures of visceral adipose tissue mass. Cigolini *et al* (1996) determined the effect of visceral adipose tissue on PAI-1 Ag more precisely utilising computed tomography at the L4-L5 level.

This group concluded that visceral fat mass was an independent predictor of PAI-1 activity. The results of this study, as well as our own, suggest that the associations between PAI-1 and the measured blood pressure and metabolic markers of the IRS are mediated via an increase in adipose tissue mass in particular, visceral fat, possibly via adipose tissue-secreted factors such as leptin or cytokines. Measurement of these variables may further elucidate the mechanism by which increased visceral adipose tissue results in hypofibrinolysis via elevated active and latent PAI-1 Ag. These results combined with significant *in vitro* evidence suggest a role for adipose tissue in the expression and secretion of PAI-1 in response to a number of stimuli including insulin (Samad & Loskutoff, 1996) and triglycerides (Vague *et al* 1986).

The relationships between waist to hip ratio and PAI-1Ag (table 4.2) and total cholesterol and PAI-1 only reached significance in postmenopausal females and the male population studied. Furthermore, the correlations between these factors were weaker in postmenopausal females than males. This may be due to the non exclusion of premenopausal females taking oral contraceptives and postmenopausal females taking hormone replacement therapy. Oral oestrogen is known to decrease PAI-1 Ag directly (Villablanca *et al* 2002), as well as influencing the proportion of PAI-1 in the complexed form via increases in t-PA clearance (Nozaki *et al* 1999, Lansink *et al* 1999). Failing to exclude those patients either taking oral contraceptives or undergoing hormone replacement therapy may have lead to unreliable results and a potential masking of significant relationships.

In all three subject groups systolic blood pressure retained a significant positive relationship with PAI-1 independently of insulin, total cholesterol, triglyceride, HDL cholesterol and LDL cholesterol and in the case of post menopausal women, body mass and BMI. There are two possible explanations for the relationship between

systolic pressure and PAI-1 Ag. The first is release of PAI-1 from endothelial and platelet stores in response to endothelial damage. Elevations in blood pressure increase the shear stresses imposed on the vessel wall thus increasing the likelihood of vessel wall damage and rupture of vulnerable atherosclerotic plaques (Mittleman & Siscovick 19960. Measurement of von Willebrand factor (vWF, a marker of endothelial damage) would indicate if this was a viable mechanism. The Framingham offspring study however, reported that there was no significant relationship between, systolic or diastolic blood pressure and vWF (Poli *et al* 2000). This group however, did not report the time between cannulation and withdrawal of the samples that may have resulted in the lack of a significant relationship.

A second possible explanation is the involvement of the renin-angiotensin-aldosterone system (RAAS). Angiotensin II, angiotensin IV and aldosterone have all been shown to stimulate PAI-1 synthesis and secretion *in vitro* as well and increasing mean arterial pressure (Brown *et al* 1999). Furthermore, inhibition of angiotensin converting enzyme (ACE) leads to reductions in both mean arterial pressure and plasma PAI-1 Ag in a dose dependent manner (Vaughan 2001). In addition, t-PA synthesis and release is also affected by ACE, which metabolises bradykinin, a stimulant of endothelial t-PA secretion, therefore decreasing t-PA secretion. The effect of this system on active and latent PAI-1 Ag may be therefore influenced by the availability of t-PA to form the t-PA/PAI-1 complex.

The lack of a relationship between systolic pressure and plasma PAI-1 when controlling for anthropometric markers of visceral obesity could also be explained by the involvement of the RAAS. Obesity, in particular visceral obesity is associated with elevated plasma levels of angiotensin and renin and increased amounts of tissue specific ACE (Egan *et al* 2001). This has long been thought to be the link between central obesity and hypertension but is beginning to be proposed as a

possible mechanism for elevated PAI-1 concentrations and the resultant hypofibrinolysis.

Finally, an interesting result in this study was the persistence of a relationship between PAI-1 and HDL cholesterol in premenopausal females despite controlling for triglyceride concentration (R= -0.277 P=0.043). Although this may be an artefact of the inclusion of subjects taking oral contraceptives, there is evidence to suggest that insulin resistant patients develop a glycated form of HDL that increases PAI-1 secretion from vascular endothelial cells (Ren & Shen 2000).

Overall this study has confirmed the results of a number of other groups by indicating that anthropometric variables including body mass, and BMI, but in particular, measures of central obesity such as waist to hip ratio and waist circumference are the main predictors of PAI-1 secretion in obese healthy volunteers. This provides additional evidence to support the role of adipose tissue in expression and secretion of PAI-1 in vivo in response to a number of different stimuli.

Chapter 5

The effect acute, moderate intensity exercise on plasma PAI-1 concentration in obese Vs non obese sedentary male volunteers.

Chapter 5: The effect acute, moderate intensity exercise on plasma PAI-1 concentration in obese Vs non obese sedentary male volunteers.

*5.*1 Introduction

Obesity and central fat accumulation in particular are known to be risk factors for coronary heart disease and thrombotic events (Despres *et al* 1990). The hypofibrinolytic state observed at rest in central obesity has been attributed to elevated concentrations of plasminogen activator inhibitor type 1 (PAI-1), which is now considered an independent risk factor in the insulin resistance syndrome (IRS) (De Pergola *et al* 1997).

Regular physical activity is known to reduce resting plasma PAI-1 concentration by up to 58% (Streiff & Bell 1994). Unaccustomed acute bouts of strenuous exercise however, result in an increased risk of myocardial infarction (MI) onset and sudden cardiac death (SCD) in both obese and sedentary populations during and up to one hour post exercise (Mittleman & Siscovick 1996). This increased risk has been attributed to increased platelet number and platelet hyperactivity in these populations (Kestin *et al* 1993). Activation of platelets results in alpha granule secretion of, amongst others, PAI-1. It is therefore possible that hypofibrinolysis due to elevated concentrations of PAI-1 may be responsible for MI onset as a result of exercise.

Acute exercise in non obese subjects at an intensity greater than 50% of maximal heart rate has been shown to significantly decrease plasma PAI-1 concentration with a concomitant increase in tissue plasminogen activator (t-PA), increasing overall fibrinolytic capacity (Streiff & Bell 1994). The largest increases in fibrinolytic activity

occur with exercise intensity greater than 70% of maximal oxygen uptake (VO_2 max) (Streiff & Bell 1994) when the largest reductions of PAI-1 are observed.

Work in non obese patients with markers of the IRS have produced conflicting results with hypertensive subjects showing no significant reduction in fibrinolytic capacity (DeSouza *et al* 1997) and NIDDM patients demonstrating a reduced fibrinolytic capacity (Schneider *et al* 1988) compared to healthy controls both at rest and in response to acute exercise bouts. The fibrinolytic response to an acute exercise bout has not been investigated in an obese population.

The purpose of this present study therefore was to determine the effect of an acute bout of exercise at 70% predicted VO_2 max on plasma t-PA and PAI-1 concentrations in habitually sedentary obese versus sedentary non-obese male volunteers.

5.2 Subjects & Methods

8 obese, sedentary, male volunteers were matched for age (+/- 1 year) and ethnic origin with 8 sedentary non obese males. Obesity was defined as BMI >30 kg/m^2 and waist circumference >102 cm. "Sedentary" was defined as <20 min recreational exercise per week and having a non manual profession.

5.2.2 Experimental Design

All subjects underwent physical examinations and biochemical and haematological screening prior to entry into the study. Maximal oxygen uptake was predicted by means of a submaximal fitness test to 80% predicted maximal heart rate on a motorised treadmill employing a modified Bruce protocol. Expired air was collected

(using the Douglas bag method) for the last minute of each stage and analysed for FEO_2, FECO2, and volume to allow calculation of VO_2 (all volumes were corrected to STPD). A workload corresponding to 70% VO_2 max was then determined during a 30 minute treadmill walking trial in which subjects were familiarised with the protocol. Measures of height, weight and percent body fat (using air displacement plethysmography (BODPOD™)) as well as waist and hip circumferences were also recorded during this visit.

On the trial day, all subjects arrived at the laboratory at 09.00h to standardize for the effects of diurnal variation, after an overnight fast having abstained from smoking for 8 hours, alcohol for 24 hours and strenuous exercise for 48 hours. No subject was taking prescription or "over the counter medication" for 6 weeks prior to the commencement of the study. A 21G cannula was inserted into a suitable forearm vein (during 3 minutes of venous occlusion at 100 mmHg) and a fasting blood sample (VO) was taken. Subjects then consumed a standardised 281 kcal breakfast (62.2g carbohydrate, 2.0g fat, 7.6g protein). Subjects were then required to rest in a seated position for 75 minutes before a resting blood sample (REST) was taken. This was followed by an acute bout of walking exercise on a motorised treadmill for thirty minutes at 70% predicted VO_2 max (preceded by a 3 minute warm up period (2.7 km/h^{-1}, 0% gradient) before a further blood sample (END) was taken. Subjects then performed a 5 minute cool down (2.7 km/h^{-1}, 0% gradient). A final blood sample was taken 30 minutes after the completion of the exercise bout (POST).

5.2.3 Laboratory Methods.

Blood samples were distributed between evacuated sealed test tubes containing sodium citrate or EDTA. The EDTA samples were analysed immediately for concentrations of haemoglobin (azidemethaemaglobin method, Hemocue, Sweden) and haematocrit (microcentrifugation, Hawksley, UK). The sodium citrate samples were centrifuged immediately at 3000 rpm for 15 minutes and the resultant plasma stored at –70°C until analysis by ELISA for total PAI-1 Ag (Kit 12075 Technoclone, Austria), active PAI-1 Ag (Actibind Kit 16075 Technoclone, Austria) and t-PA Ag (Kit 12005 Technoclone, Austria).

5.2.4 Calculations and Statistical Analysis

Plasma volume changes were calculated according to the method of Dill and Costill (1974). All data shown are corrected for plasma volume changes. Data was assessed for normality of distribution using the Shapiro-Wilks test. Comparisons between groups and between time points were performed using Wilcoxon rank tests and Mann-Whitney tests. Differences were considered significant when $P \leq 0.05$.

5.3 Results

5.3.1 Physical Characteristics of the Subjects

Physical characteristics of the subjects are shown in table 5.1. Both groups contained 6 Caucasians and 2 Asians. There were no significant differences in age or VO_2 max between the two groups. The obese group however had a significantly greater body mass, BMI, waist circumference, waist hip ratio and mean arterial pressure than their non obese counterparts.

Table 5.1: Comparison of physical characteristics between the obese and non obese subject groups. Values shown are mean ± SEM for normally distributed data and median (range) for skewed data. N = number of subjects. * = Significantly different from non obese group, $P \leq 0.05$.

Variable	Group: Obese (N=8)	Group: Non Obese (N=8)
Age (years)	43.25 ± 0.56	43.38 ± 0.60
Body Mass (kg)	97.78 ± 2.36*	79.43 ± 3.92
BMI (kg/m^2)	31.6 ± 0.88*	25.0 ± 0.94
Waist Circumference (cm)	112.0 ± 2.09*	88.25 ± 2.07
Waist Hip Ratio	1.05 ± 0.02*	0.86 ± 0.01
VO_2 max (ml/kg/min)	26.51 ± 2.43	26.73 ± 1.88
Mean Arterial Pressure (mmHg)	104.0 ± 1.31*	80.85 ± 1.59
Total t-PA Ag (ng/ml)	14.38 (11.36 to 27.72)	12.32 (9.12 to 33.92)
Total PAI-1 Ag (ng/ml)	125.75 (88.41 to 148.59)*	55.25 (37.47 to 106.87)
Active PAI-1 (U/ml)	33.98 (7.60 to 98.35)*	9.16 (4.10 to 26.78)

Total PAI-1 Ag and active PAI-1 Ag were significantly elevated in the obese group at all time points throughout the trial compared to the non obese group, but there were no significant differences in t-PA Ag between groups at any time point.

5.3.2 T-PA

T-PA Ag (table *5.2*) did not significantly change in the obese group during the trial, although there was a trend towards decreased t-PA Ag 30 minutes post exercise (P=0.093). In the non obese group, there was a significant increase in t-PA Ag as a result of the exercise bout (from 11.26 (8.03 to 29.69) ng/ml to 16.27 (8.86 to 33.83 ng/ml P= 0.03) and a trend towards baseline levels 30 minutes post exercise (P=0.09)

Table 5.2: The effect of an acute exercise bout at 70% VO_2 max on plasma t-PA in obeseVs non obese sedentary male volunteers. VO = venous occlusion, END = immediately post exercise & POST = 30 minutes post exercise. Data is shown as median (range). All data is corrected for changes in plasma volume compared to baseline values. * = significantly different from previous time point $P \leq 0.05$.

Time Point	Obese Group N=8		Non Obese Group N=8	
	Median [t-PA] ng/ml	Range	Median [t-PA] ng/ml	Range
Venous Occlusion	14.38	11.36 to 27.72	12.32	9.12 to 33.92
Rest (Baseline)	15.26	10.27 to 33.69	11.26	8.03 to 29.69
End of Exercise	20.41	10.81 to 31.76	16.27*	8.86 to 33.83
Post Exercise	18.45	11.44 to 25.18	15.95	7.61 to 30.01

5.3.3 Total PAI-1

Total PAI-1 Ag (table 5.3) was significantly elevated in both groups as a result of venous occlusion and cannulation compared to resting values (obese group VO =125.75 (88.41 to 148.59 ng/ml) Vs REST =112.96 (85.58 to 131.79 ng/ml), P=0.05, non obese group, VO=55.25 (37.47 to 106.87 ng/ml) Vs REST = 35.43 (25.65 to 75.84 ng/ml), P=0.01). There were no significant changes however in either group as a result of the exercise bout (REST to END). In addition, there were no significant differences in total PAI-1 Ag concentration between END and POST in the non obese group, but there was a significant increase in total PAI-1 Ag concentration in the obese group (from 110.81 (69.84 to 149.04 ng/ml) to 143.94 (93.16 to 168.51 ng/ml) P=0.04)

Table 5.3: The effect of a 30 minute bout of exercise on total PAI-1 Ag concentration in obese Vs non obese sedentary male volunteers. VO = venous occlusion & cannulation, END = immediately post exercise & POST = 30 minutes post exercise. Data is shown as median (range). All data is corrected for changes in plasma volume. * significantly different from previous time point P≤ 0.05, ** =significantly different from the previous time point P≤ 0.01. $^{+}$ = significantly elevated compared to the non obese group P≤ 0.05, $^{++}$ = significantly elevated compared with the non obese group P≤ 0.01

Time Point	Obese Group N=8		Non Obese Group N=8	
	Median [PAI-1] ng/ml	Range	Median [PAI-1] ng/ml	Range
Venous Occlusion	125.72^{+}	88.41 to 148.59	55.25	37.47 to 106.87
Rest (Baseline)	112.96^{+*}	85.58 to 131.79	35.43**	25.65 to 75.84
End of Exercise	110.81^{+}	69.84 to 149.04	37.06	31.95 to 61.34
Post Exercise	143.94^{++*}	93.16 to 168.51	32.05	17.83 to 78.08

5.3.4 Active PAI-1

Finally, active PAI-1 Ag (table 5.4) decreased from venous occlusion to rest in both the obese group (from 33.98 (7.60 to 98.35 U/ml) VO, to 31.94 (6.05 to 75.66 U/ml) REST P= 0.03) and the non obese group (from 9.16 (5.10 to 26.78 U/ml) VO, to 5.28 (2.14 to 16.81 U/ml) REST P=0.04). In both groups, active PAI-1 further decreased as a result of the exercise bout (Obese group 31.94 (6.05 to 75.66 U/ml) REST, to 23.20 (4.03 to 60.65 U/ml) END P=0.01, Non Obese group 5.28 (2.14 to 16.81 U/ml) REST, 4.26 (0.57 to 13.29 U/ml) END P=0.01). There was no significant change in active PAI-1 Ag in either group following the 30 minute recovery period.

Table 5.4: The effect of a 30 minute bout of exercise on plasma active PAI-1 Ag concentration in obese Vs non obese sedentary male volunteers. VO = venous occlusion & cannulation, END = immediately post exercise &.POST = 30 minutes post exercise. Data is shown as median (range). All data is corrected for changes in plasma volume. . * significantly different form previous time point P≤ 0.05, ** =significantly different from the previous time point P≤ 0.01. $^{+}$ = significantly elevated compared with the non obese group P≤ 0.05, $^{++}$ = significantly elevated compared with the non obese group P≤ 0.01

Time Point	Obese Group N=8		Non Obese Group N=8	
	Median [PAI-1] ng/ml	Range	Median [PAI-1] ng/ml	Range
Venous Occlusion	33.98^{+}	7.60 to 98.35	9.16	4.10 to 26.78
Rest (Baseline)	31.94^{**+}	6.05 to 75.66	5.28*	2.14 to 16.81
End of Exercise	23.20^{**+}	4.03 to 60.65	4.26**	0.57 to 13.29
Post Exercise	19.22^{+}	5.55 to 61.05	4.75	2.84 to 10.95

5.4 Discussion

5.4.1 Summary of the Protocol

8 obese and 8 non obese sedentary male volunteers undertook an acute walking exercise bout at 70% VO_2 max for 30 minutes. Blood samples were taken 100 minutes pre exercise (VO) after 3 minutes of venous occlusion and a standard cannulation, 15 minutes pre exercise (REST) after a standard 190 kcal breakfast, at the end of the exercise bout (END) and thirty minutes post exercise (POST). Samples were analysed for t-PA concentrations, total PAI-1 Ag and active PAI-1 Ag.

5.4.2 Resting Data

At rest, the obese group were hypofibrinolytic compared to the non obese group at rest; shown by both elevated total PAI-1 Ag (112.96 (85.58 to 131.79 ng/ml) obese group Vs 35.43 (25.65 to 75.84 ng/ml) non obese group) and active PAI-1Ag (31.94 (6.05 to 75.66) obese group Vs 5.28 (2.14 to 16.81 U/ml) non obese group) concentrations, with no significant differences in t-PA concentration. The total PAI-1 Ag reported in this study are significantly higher than those reported in the literature. This is because the assay used for determination of total PAI-1 included measurement of complexed, latent and active PAI-1 whereas the assays used in most of the literature only measure latent and active PAI-1 (Vague *et al* 1989, Alessi *et al* 1988*)*. Kruithof (1988) defined a total PAI-1 Ag concentration greater than 100 ng/ml (including complexed PAI-1) as indicative of a hypofibrinolytic state. Furthermore, an active PAI-1 Ag concentration greater than 20 t-PA inhibiting units /ml is also indicative of a reduced fibrinolytic capacity. The results of this study confirm previous reports of a hypofibrinolytic state observed at rest in obese patients (Vague *et al* 1989, Alessi *et al* 1998).

5.4.3 Exercise Data

The main finding of this study is that the hypofibrinolytic state observed at rest in the obese group persisted following the acute bout of exercise at 70% predicted VO_2 max. Total PAI-1 remained unchanged in both groups whilst t-PA significantly increased in the non obese by 31% (P= 0.03), but not the obese group with exercise (median increase 25% P=0.107). Active PAI-1 significantly decreased in both the obese and non obese subject groups. The reduction in active PAI-1 Ag together with unchanged total PAI-1 Ag suggests an increase in the proportion of t-PA bound to PAI-1 at the end of the exercise bout. In the non obese group, t-PA concentrations significantly rose therefore increasing the proportion of free t-PA available. In the obese group however, there were no significant changes in t-PA concentration suggesting that in obese populations 30 minutes exercise at 70% VO_2 max does not have a hyperfibrinolytic effect. A measure of t-PA activity would confirm whether this is the case. The persistence of significantly elevated total and active PAI-1 Ag immediately post exercise with no significant differences in t-PA concentrations compared to non obese individuals, suggests that sedentary obese individuals remain hypofibrinolytic compared to sedentary non obese populations following an acute moderate intensity exercise bout.

The lack of significant change in plasma t-PA concentration with exercise in the obese group however, maybe a result of the large variations in t-PA concentration in this group. As this was the first study to examine the effect of an acute exercise bout on fibrinolysis in an obese population, the power calculations were based on variations in fibrinolytic variables at rest in the obese and the fibrinolytic reactions to exercise in the non obese. Retrospective power calculations have indicated that a population of 22 (11 experimental subjects and 11 controls) would be required to

elucidate with 95% confidence if there were any significant changes in t-PA concentration with acute exercise in a sedentary, obese subject group.

Thirty minutes post exercise, a further reduction in fibrinolytic capacity was observed in the obese group as t-PA Ag showed a trend towards declining concentrations (P=0.093) but total PAI-1 Ag (table 5.4) significantly increased compared to both concentrations recorded at rest and at the end of the exercise bout. (END, 110.81 (69.84 to 149.04 ng/ml) Vs POST 143.94 (93.16 to 168.51ng/ml). Active PAI-1 Ag remained unchanged from those recorded at the end of the exercise bout but were not significantly different from resting values. This suggests, as with the end of the exercise bout, an increased proportion of t-PA bound to the inhibitor PAI-1. These results suggest that sedentary obese populations are hypofibrinolytic immediately, and up to 30 minutes after an acute exercise bout at 70% VO_2 max. These results provide further evidence that hypofibrinolysis may be a potential mechanism in the increased incidence of MI onset in obese and sedentary populations during and up to one hour following an unaccustomed exercise bout (Mittleman & Siscovick 1996).

Although the precise mechanism by which exercise may induce MI onset is unknown, cadaver data suggests that disruption of atherosclerotic plaques and the resultant local activation of prothrombogenic and antifibrinolytic processes may be responsible (Mittleman and Siscovick 1996). These processes include platelet hyperactivity in sedentary subjects (Kestin et al 1993), increased resting fibrinogen concentrations and a hypofibrinolytic state (Vague 1993) contributed to by increased local PAI-1 concentrations as result of elevated PAI-1 gene expression in the sclerotic artery wall (Mittleman & Siscivick 1996). This local increase in PAI-1 may promote the formation and stabilisation of a potentially MI inducing thrombus.

The rise in total PAI-1 Ag accompanied by little or no change in active PAI-1 Ag and decreasing t-PA, suggests increased PAI-1 release from platelets in response to endothelial injury. PAI-1 is stored in the alpha granules of platelets and released in response to a variety of chemical as well as mechanical stimuli (Sprengers *et al 1986)* but only 15-20% of this PAI-1 is in the active form (Loskutoff & Samad 1998) and this rapidly and spontaneously converts to the latent form or binds to t-PA resulting in a t-PA/PAI-1 complex. This may account for the elevation in total PAI-1 without a concomitant increase in active PAI-1. This would need to be confirmed by analysis of a marker of platelet α granule secretion such as platelet factor 4 or β thromboglobulin as well as a marker of endothelial damage such as von Willebrand factor (vWF) and t-PA activity.

In contrast to the obese group, the non obese group demonstrated increased fibrinolytic capacity at the end of the exercise bout from rest as shown by a lack of significant difference in total PAI-1 Ag concentration but significantly decreased active PAI-1 Ag concentration and significantly increased concentrations of t-PA Ag compared to resting levels. Thirty minutes post exercise, active PAI-1 remained unchanged from the end of exercise levels and t-PA declined towards resting levels. The non obese group therefore was hypofibrinolytic thirty minutes post exercise compared to end of exercise levels, but was still hyperfibrinolytic compared to resting values. Similar results have been observed in studies employing a variety of exercise protocols and subject groups (Streiff & Bell 1994).

Our results in the non obese group are also similar to those previously observed in sedentary non obese males performing acute exercise of similar intensity. Desouza

and co workers (1997) reported a 45% rise in t-PA Ag which returned to baseline levels 30 minutes post exercise and a 22% decrease in PAI-1 activity which remained unchanged thirty minutes post exercise following 30 minutes exercise at 65% VO_2 max. The fibrinolytic response to an acute bout of exercise however has not been studied in an obese sedentary population. Work in populations who exhibit other markers of the IRS such as NIDDM (Schneider *et al* 1988) has shown impaired fibrinolytic responses compared with non obese sedentary populations immediately following moderate (65-75 % VO_2 max) intensity exercise. This study also indicates a decrease in the fibrinolytic capacity of obese subjects immediately following an acute exercise bout. The lack of large changes in fibrinolytic capacity with exercise may be in part due to the large intra-group variation of fibrinolytic variables in the obese group. Furthermore, the use of a sub maximal VO_2 predictive exercise test rather than a maximal oxygen uptake test may have resulted in the volunteers exercising at less than 70% VO_2 max, which is considered a threshold for large changes in fibrinolytic variables (Streiff & Bell 1994).

Overall, we have demonstrated the sedentary obese populations are hypofibrinolytic with respect to sedentary non obese population at rest and both immediately and thirty minutes following a moderate intensity (70% predicted VO_2 max) exercise bout. Furthermore, obese populations are hypofibrinolytic compared to rest both immediately following exercise and 30 minutes post exercise and this may explain the increased incidence of MI onset immediately following and up to 1 hour post exercise.

Despite the apparent undesirable effects of acute moderate intensity exercise in sedentary obese male subjects on fibrinolytic processes, it is worth reiterating the

benefits of chronic exercise training. With or without weight loss, regular aerobic exercise training has been shown to reduce resting plasma PAI-1 concentration (Streiff & Bell 1994), decrease platelet reactivity (Kestin et al 1993), improve the lipid profile, and increase insulin sensitivity (Despres et al 1990) therefore reducing the risk of heart disease and MI.

Chapter 6

The relationship between total and active PAI-1 concentrations and other markers of the insulin resistance syndrome at rest, during exercise and post exercise in 16 male volunteers.

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Chapter 6: The relationship between total and active PAI-1 concentrations and other markers of the insulin resistance syndrome at rest, during exercise and post exercise in 16 male volunteers.

6.1 Introduction

Numerous research studies (Alessi *et al* 1988, Jannad-Delanne *et al* 1998) including our own (chapter 4), have demonstrated the close metabolic relationships between plasma PAI-1 concentrations and other markers of the insulin resistance syndrome at rest in obese individuals. The variations in total and active PAI-1 concentrations are a result of variations in secretion of PAI-1, formation of t-PA/PAI-1 complexes, availability of vitronectin and hepatic clearance of both free and complexed formations of PAI-1.

Little is known about the factors involved in the short term regulation of plasma PAI-1 concentrations. Insulin (Kooistra *et al* 1989, Potter van Loon *et al* 1990), triglyceride (Tremoli *et al 1993*, Stiko-Rahm *et al* 1990), HDL cholesterol (Vague 1993, Ren & Shen 2000) and von Willebrand factor (Gibbons *et al* 1990) have all been implicated by both *in vivo* and *in vitro* studies as potential mechanisms in the short term regulation of PAI-1. The aim of this study therefore is to determine if any of these factors has a potential role in the short term regulation of PAI-1 *in vivo*.

6.2 Subjects and Methods

8 obese sedentary males were matched for age (+/- 1 year) and ethnic origin with 8 sedentary non obese males. Obesity was defined as BMI >30 kg/m^2 and waist circumference >102 cm. Non Obese was defined as BMI $\leq$ 27 kg/m^2 and a waist circumference $\leq$ 95 cm. "Sedentary" was defined as <20 min recreational exercise per week and having a non manual profession.

6.2.2 Experimental Design

All subjects underwent physical examinations as well as biochemical and haematological screening prior to entry into the study. Maximal oxygen uptake was predicted by means of a submaximal fitness test to 80% predicted maximal heart rate, on a motorised treadmill, employing a modified Bruce protocol. Expired air was collected (using the Douglas bag method) for the last minute of each stage and analysed for FEO_2, FECO2, and volume to allow calculation of VO_2 (all volumes were corrected to STPD). A workload corresponding to 70% VO_2 max was then determined during a 30 minute treadmill walking trial in which subjects were familiarised with the protocol. Measures of height, weight, percent body fat (using air displacement plethysmography (BODPOD^{TM})), waist circumference and hip circumference were also recorded during this visit.

On the trial day, all subjects arrived at the laboratory at 09.00h to standardise for the effects of diurnal variation having abstained from smoking for 8 hours, alcohol for 24 hours and strenuous exercise for 48 hours. No subject was taking prescription or "over the counter" medication for 6 weeks prior to the commencement of the study. A 21G cannula was inserted into a suitable forearm vein (during 3 minutes of venous occlusion at 100 mmHg) and a fasting blood sample (VO) was taken before subjects consumed a standardised 281 kcal carbohydrate breakfast. Subjects then rested in a seated position for 75 minutes before a resting blood sample (REST) was taken. Subject then performed an acute bout of walking exercise on a motorised treadmill for thirty minutes at 70% predicted VO_2 max before a further blood sample (END) was taken, followed by a 5 minute cool down (2.7 km/h^{-1}, 0% gradient). A final blood sample was taken 30 minutes after the completion of the exercise bout (POST).

6.2.3 Laboratory Methods.

Blood samples were distributed between evacuated sealed test tubes containing no additives, sodium citrate or EDTA. The EDTA samples were analysed immediately for concentrations of haemoglobin (azidemethaemaglobin method, Hemocue, Sweden) and haematocrit (microcentrifugation, Hawksley, UK). The no additives and sodium citrate samples were centrifuged immediately at 3000 rpm for 15 minutes and the resultant serum/plasma stored at –70°C until analysis. Samples were analysed by ELISA for total PAI-1 Ag concentration (Kit 12075 Technoclone, Austria), active PAI-1 Ag (Kit 16075 Technoclone, Austria), t-PA Ag (Kit 12005 Technoclone, Austria) and von Willebrand Factor (In house ELISA) as well as insulin (DELFIA method), leptin (DELFIA method) total cholesterol (Procedure 467825 Beckman systems), triglyceride (Procedure 445850 Beckman systems) and HDL cholesterol (EZ method, Sigma Diagnostics). LDL cholesterol was calculated using the Freidewald equation.

6.2.4 Calculations and Statistical Analysis

Plasma volume changes were calculated according to the method of Dill and Costill (1974). Mean arterial pressure was calculated by the following equation:

(Pulse Pressure / 3) + Diastolic Pressure

All data shown are corrected for plasma volume changes. Data was assessed for normality of distribution by multiple Shapiro-Wilks tests. Univariate correlations between plasma PAI-1 Ag and other markers of the IRS were assessed by Spearman's rho correlation tests. Two tailed significance levels are reported. To assess the effect of variables on total and active PAI-1 Ag independently of other significant correlating variables data was 'normalised' by log transformations before partial correlations were carried out. Finally, a stepwise multiple regression analysis was performed to test the joint effect of different variables on both total and active PAI-1 Ag. Differences were considered significant when $P \leq 0.05$.

6.3 Results

6.3.1 Resting correlations

The resting correlations between total PAI-1 Ag, active PAI-1 Ag and other measured markers of the insulin resistance syndrome are shown in table 6.1.

Table 6.1: Correlations between total PAI-1 Ag, Active PAI-1 Ag and other markers of the Insulin Resistance Syndrome at rest. Significant correlations are shown in red* P=≤0.05, ** P=≤0.01 N=16

	Total PAI-1 (ng/ml) N=16		Active PAI-1 IU/ml N=16	
	R Value	P Value	R Value	P Value
Weight (kg)	0.652	0.006**	0.600	0.014**
BMI (kg/m^2)	0.724	0.002**	0.613	0.012**
Body Fat %	0.702	0.002**	0.482	0.054*
Body Fat (kg)	0.767	0.001**	0.559	0.024*
Waist to Hip Ratio	0.790	0.000**	0.643	0.007**
Waist Circumference (cm)	0.836	0.000**	0.635	0.008**
VO_2 max (ml/kg/min)	-0.145	0.592	-0.270	0.312
Mean arterial Pressure (mmHg)	0.934	0.000**	0.716	0.002**
t-PA (ng/ml)	-0.069	0.590	0.019	0.882
vWF IU/ml	0.376	0.002**	0.169	0.182
Triglyceride (mmol/l)	0.599	0.000**	0.573	0.000**
Total Cholesterol (mmol/l)	0.304	0.014**	0.392	0.001**
HDL- Cholesterol (mmol/l)	-0.589	0.000**	-0.295	0.018*
LDL-Cholesterol (mmol/l)	0.330	0.008**	0.126	0.332
Insulin (pmol)	0.335	0.007**	0.263	0.036*
Leptin (pg/ml)	0.397	0.001**	0.180	0.155
Total PAI-1 (ng/ml)	N/A	N/A	0.565	0.000**
Active PAI-1 (IU/ml)	0.565	0.000**	N/A	N/A

Both total PAI-1 Ag and active PAI-1 Ag were significantly correlated to weight, BMI, kilograms of body fat, waist to hip ratio, waist circumference, mean arterial pressure, triglycerides, total cholesterol, HDL cholesterol and insulin. Interestingly only total PAI-1 Ag concentration was correlated with body fat percentage, LDL cholesterol and leptin. Using a multiple stepwise regression model only MAP entered the model as predictive of both total (R^2 adjusted = 0.863, P = 0.008) and active (R^2 adjusted = 0.512, P = 0.000)

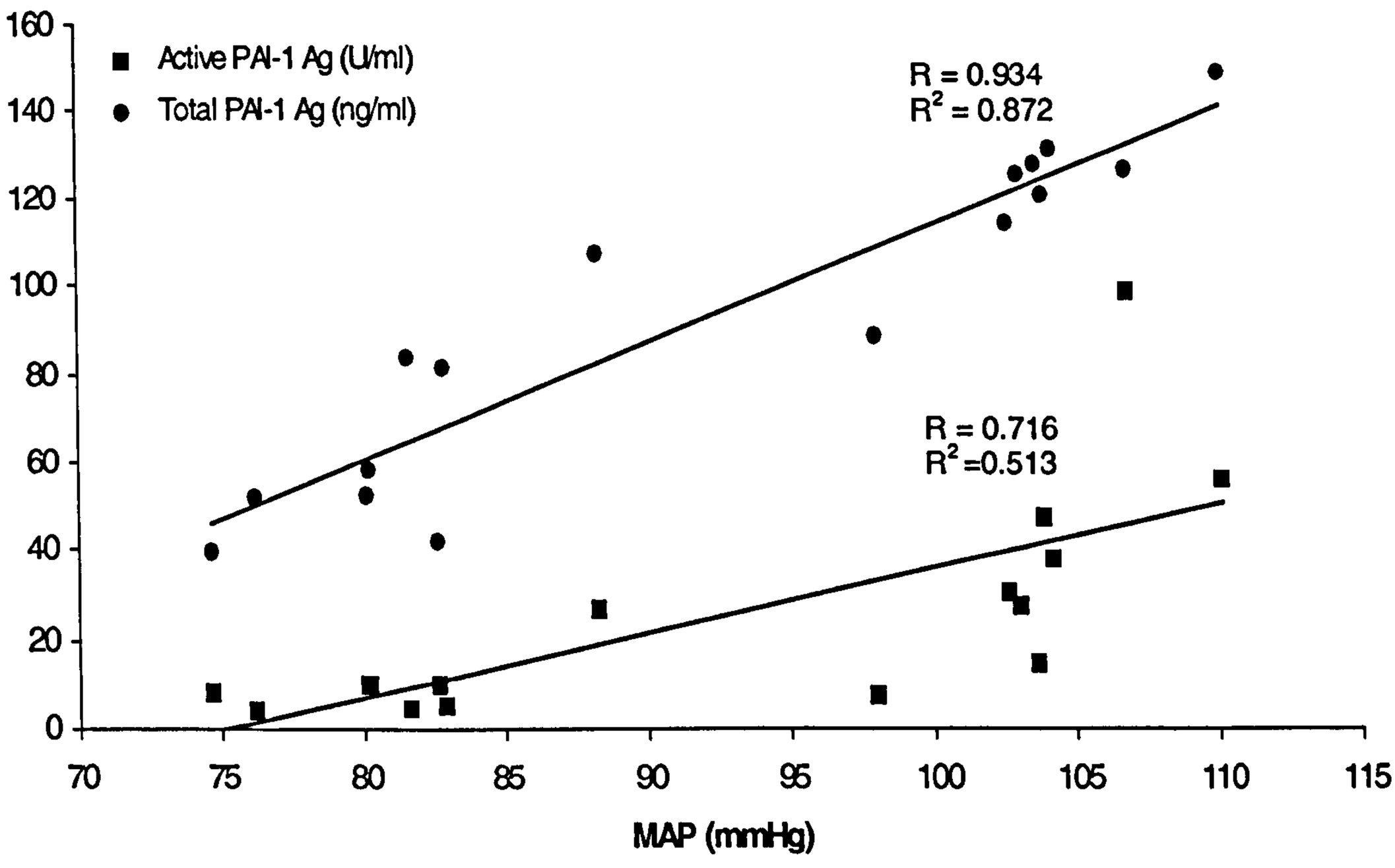

Figure 6.1: Positive correlations between active PAI-1, total PAI-1 and mean arterial pressure at rest in the 16 men. MAP = mean arterial pressure.

To assess whether the correlating factors were directly responsible for changes in total and active PAI-1 Ag or had an indirect influence via other measured factors, multiple partial correlations were performed controlling for each correlating factor in turn (Table 6.2 and 6.3 respectively). Only MAP remained correlated to total PAI-1 Ag irrespective of the other measured factors. Furthermore, controlling for MAP removed all positive correlations between total PAI-1 Ag and the other measured markers of the IRS at rest. No factor positively correlated with active PAI-1 Ag regardless of controlling factors

although, with the exception of waist to hip ratio and waist circumference MAP showed a significant positive correlation or a trend towards a positive correlation at all times. Only waist circumference, waist to hip ratio and MAP removed all significant correlations with active PAI-1 Ag and other measured markers of the IRS.

Table 6.2: Partial correlations between total PAI-1 Ag and other resting measurements in the 16 men. Significant correlations are shown in red $P \leq 0.05$. BF = Body fat, WHR = waist to hip ratio, WC = waist circumference, MAP = mean arterial pressure, Trig = triglyceride concentration & C = cholesterol. N=16

CONTROLLING FACTORS

	NONE	Weight	BMI	BF%	BF kg	WHR	WC	MAP	vWF	Trig	Total-C	HDL-C	LDL-C	Insulin	Leptin
Weight	R=.652 P=.006		R=-.001 P=.997	R=.328 P=.232	R=.044 P=.877	R=-.008 P=.977	R=-.150 P=.595	R=-.327 P=.235	R=.637 P=.011	R=.376 P=.167	R=.678 P=.005	R=.567 P=.027	R=.733 P=.002	R=.506 P=.054	R=.546 P=.035
BMI	R=.724 P=.002	R=.416 P=.123		R=.355 P=.194	R=.091 P=.748	R=.066 P=.814	R=-.234 P=.401	R=-.128 P=.650	R=.743 P=.002	R=.448 P=.094	R=.693 P=.004	R=.601 P=.018	R=.724 P=.002	R=.603 P=.017	R=.629 P=.012
BF %	R=.702 P=.002	R=.462 P=.083	R=.262 P=.346		R=-.265 P=.340	R=.102 P=.718	R=-.138 P=.624	R=.281 P=.310	R=.698 P=.004	R=.429 P=.111	R=.646 P=.009	R=.621 P=.013	R=.671 P=.006	R=.595 P=.019	R=.605 P=.017
BF Kg	R=.767 P=.001	R=.535 P=.040	R=.377 P=.166	R=.495 P=.060		R=.200 P=.473	R=-.100 P=.722	R=.208 P=.458	R=.776 P=.001	R=.514 P=.050	R=.729 P=.002	R=.708 P=.003	R=.750 P=.001	R=.671 P=.006	R=.691 P=.004
WHR	R=.790 P=.000	R=.590 P=.021	R=.464 P=.082	R=.518 P=.048	R=.354 P=.196		R=-.173 P=.536	R=-.296 P=.284	R=.816 P=.000	R=.585 P=.022	R=.754 P=.001	R=.694 P=.004	R=.771 P=.001	R=.708 P=.003	R=.735 P=.002
WC	R=.836 P=.000	R=.699 P=.004	R=.634 P=.011	R=.646 P=.009	R=.525 P=.045	R=.470 P=.077		R=-.033 P=.906	R=.857 P=.000	R=.648 P=.009	R=.809 P=.000	R=.745 P=.001	R=.821 P=.000	R=.772 P=.001	R=.788 P=.000
MAP	R=.934 P=.000	R=.895 P=.000	R=.857 P=.000	R=.876 P=.000	R=.838 P=.000	R=.830 P=.000	R=.758 P=.001		R=.935 P=.000	R=.868 P=.000	R=.924 P=.000	R=.888 P=.000	R=.930 P=.000	R=.910 P=.000	R=.918 P=.000
vWF	R=.376 P=.002	R=-.087 P=.757	R=-.308 P=.265	R=-.170 P=.546	R=-.264 P=.342	R=-.379 P=.0164	R=-.391 P=.150	R=-.236 P=.398		R=.255 P=.359	R=.095 P=.735	R=.307 P=.266	R=.128 P=.651	R=-.229 P=.413	R=.028 P=.923
Trig	R=.599 P=.000	R=.495 P=.061	R=.387 P=.154	R=.430 P=.109	R=.309 P=.262	R=.337 P=220	R=.159 P=.571	R=.166 P=.554	R=.713 P=.003		R=.657 P=.008	R=.589 P=.021	R=.718 P=.003	R=.687 P=.005	R=.653 P=.008
Total-C	R=.304 P=.014	R=.428 P=.112	R=.225 P=.421	R=.019 P=.946	R=.086 P=.761	R=.003 P=.991	R=.025 P=.927	R=.076 P=.787	R=.320 P=.245	R=.147 P=.602		R=.480 P=.070	R=.242 P=.385	R=.319 P=.246	R=.220 P=.430
HDL-C	R=-.589 P=.000	R=-.546 P=.035	R=-.446 P=.094	R=-.528 P=.043	R=-.527 P=.044	R=-.420 P=.118	R=-.347 P=.205	R=-.136 P=.629	R=-.662 P=.007	R=-.481 P=.069	R=.688 P=.005		R=-.600 P=.018	R=-.649 P=.009	R=-.611 P=.015
LDL-C	R=.330 P=.008	R=.508 P=0.53	R=.278 P=.316	R=-.033 P=.907	R=.131 P=.642	R=.020 P=.944	R=.029 P=.920	R=.168 P=.549	R=.235 P=.399	R=.339 P=.216	R=.042 P=.880	R=.093 P=.742		R=.277 P=.318	R=.208 P=.458
Insulin	R=.335 P=.007	R=.187 P=.505	R=-.050 P=.859	R=.221 P=.427	R=.065 P=.818	R=.095 P=.736	R=-.009 P=.974	R=.051 P=.857	R=.514 P=.050	R=.470 P=.077	R=.480 P=.070	R=.524 P=.045	R=.504 P=.056		R=.294 P=.288
Leptin	R=.397 P=.001	R=.198 P=.479	R=.041 P=.960	R=.126 P=.655	R=.013 P=.963	R=-.186 P=.507	R=-.103 P=.715	R=-.175 P=.531	R=.427 P=.113	R=.328 P=.233	R=.375 P=.169	R=.419 P=.122	R=.429 P=.110	R=.191 P=.495	

Table 6.3: Partial correlations between active PAI-1 Ag and other resting measurements in the 16 men. Significant correlations are shown in red $P \leq 0.05$. BF = Body fat, WHR = waist to hip ratio, WC = waist circumference, MAP = mean arterial pressure, Trig = triglyceride concentration & C = cholesterol. N=16

	CONTROLLING FACTORS											
	None	Weight	BMI	BF %	BF (kg)	WHR	WC	MAP	Trig	Total-C	HDL-C	Insulin
Weight	R= .600 P= .014		R= .140 P= .618	R= .422 P= .117	R= .294 P= .288	R= .157 P= .577	R= .165 P= .557	R= .100 P= .724	R= .342 P= .212	R= .645 P= .009	R= .555 P= .032	R= .531 P= .041
BMI	R= .613 P=.012	R= .208 P= .457		R= .435 P= .106	R= .301 P= .275	R= .111 P= .694	R= .086 P= .760	R= .089 P= .753	R= .311 P= .259	R= .564 P= .029	R= .569 P= .027	R= .574 P= .025
BF %	R= .482 P= .054	R= .122 P= .666	R= -.060 P= .831		R=-.295 P= .287	R=-.152 P= .590	R=-.215 P= .443	R= .003 P= .992	R= .105 P= .711	R= .335 P= .222	R= .419 P= .120	R= .384 P= .158
BF kg	R= .559 P= .024	R= .138 P= .623	R= .000 P= .999	R= .427 P= .113		R=-.068 P= .810	R=-.143 P= .611	R= .017 P= .952	R= .188 P= .502	R= .463 P= .082	R= .506 P= .054	R= .478 P= .072
WHR	R= .643 P= .007	R= .327 P= .235	R= .271 P= .329	R= .504 P= .056	R= .388 P= .152		R= .143 P= .611	R= .005 P= .987	R= .317 P= .173	R= .553 P= .033	R= .608 P= .016	R= .587 P= .021
WC	R= .635 P= .008	R= .305 P= .269	R= .228 P= .414	R= .508 P= .053	R= .387 P= .155	R= .054 P= .849		R=-.032 P= .910	R= .312 P= .257	R= .552 P= .033	R= .603 P= .017	R= .579 P= .024
MAP	R= .716 P= .002	R= .496 P=.060	R= .475 P= .074	R = .604 P= .017	R= .539 P= .038	R= .410 P= .129	R= .428 P= .111		R= .500 P= .058	R= .665 P= .007	R= .728 P= .002	R= .674 P= .006
Trig	R= .573 P= .002	R= .388 P= .153	R= .331 P= .228	R= .454 P= .089	R= .368 P= .177	R= .307 P= .265	R= .262 P= .346	R= .230 P= .410		R= .545 P= .035	R=.576 P= .025	R= .587 P= .022
Total-C	R= .392 P= .001	R= .520 P= .047	R= .357 P= .192	R= .276 P= .320	R= .294 P= .287	R= .231 P= .408	R= .264 P= .343	R= .295 P= .285	R= .298 P= .280		R= 473 P= .075	R= .421 P= .119
HDL-C	R=-.295 P= .018	R=-.062 P= .826	R= 049 P= .863	R=-.098 P= .728	R=-.053 P= .852	R= .090 P= .749	R= .133 P= .637	R= .332 P= .226	R= .022 P= .939	R=-.323 P= .241		R=-.243 P= .384
Insulin	R= .263 P= .036	R=-.043 P=.878	R=-.212 P= .448	R= .104 P= .712	R=-.018 P= .948	R=-.063 P= .822	R=-.887 P= .753	R=-.072 P= .799	R= .227 P= .415	R= .288 P= .299	R= .301 P= .276	

6.3.2 Correlations across the Trial

The correlations between total PAI-1 and active PAI-1 and other markers of the IRS throughout the trial are shown in table 5.2. Both total and active PAI-1 correlated with triglyceride concentration (total R = 0.700 P = 0.000, active R = 0.685 P = 0.000), total cholesterol (total R = 0.332 P = 0.007, active (R = 0.375 P = 0.002) HDL cholesterol (R = -0.589 P = 0.000, active R = -0.377 P = 0.002) and insulin (R = 0.373 P = 0.002, active R = 0.367 P = 0.003). Only total PAI-1 correlated with vWF (R = 0.381 P = 0.002), LDL cholesterol (R = 0.267 P = 0.033) and leptin (R = 0.225 P = 0.043) and only active PAI-1 correlated with t-PA (R = 0.225 P = 0.042).

Table 6.4: Correlations between measured markers of the IRS and total and active PAI-1. N=64

	Total PAI-1 (ng/ml) N=64		Active PAI-1 U/ml N=64	
	R Value	P Value	R value	P Value
T-PA (ng/ml)	0.108	0.396	**0.225**	**0.042***
vWF (IU/ml)	**0.381**	**0.002****	0.188	0.138
Triglyceride (mmol/l)	**0.700**	**0.000****	**0.685**	**0.000****
Total Cholesterol (mmol/l)	**0.332**	**0.007****	**0.375**	**0.002****
HDL Cholesterol (mmol/l)	**-0.589**	**0.000****	**-0.377**	**0.002****
LDL Cholesterol (mmol/l)	**0.267**	**0.033***	0.150	0.237
Insulin (pmol)	**0.373**	**0.002****	**0.367**	**0.003****
Leptin (ng/ml	**0.225**	**0.043***	0.200	0.126

Again, to determine whether the correlating factors have direct role in the short term regulation of total or active PAI-1, or had an indirect influence via other measured factors,

multiple partial correlations were performed controlling for each correlating factor in turn (Table 5.5 and 5.6 respectively). Both total and active PAI-1 remained independently correlated with triglyceride. Total PAI-1 also remained independently associated with HDL cholesterol and vWF. Finally, there was a significant positive correlation or a trend towards a correlation (P $\leq$0.10) between insulin and total PAI-1 Ag regardless of controlling factor. The significant correlation between leptin and total PAI-1 was removed when controlling for HDL cholesterol (partial R = 0.188 P = 0.141) and the significant correlations between LDL cholesterol and total PAI-1 Ag were removed when controlling for total cholesterol (partial R = 0.205 P = 0.107), HDL cholesterol (partial R = 0.160 P= 0.211) and insulin (partial R = 0.197 P = 0.122). Finally, the significant correlation between total cholesterol and total PAI-1 Ag was removed when controlling for triglyceride concentration (partial R = 0.151 P = 0.237) and LDL cholesterol (partial R = 0.157 P = 0.219) and was reduced to a trend when controlling for vWF (partial R = 0.227 P = 0.073).

Table 6.5: Partial correlations between total PAI-1 concentration and other significant correlating markers of the IRS for the 16 men across the trial. Trig = triglyceride, C = cholesterol. Significant correlations P $\leq$0.05N are shown in red. N= 64

	CONTROLLING FACTORS							
	NONE	Trigs	Total-C	HDL-C	LDL-C	Insulin	Leptin	vWF
Trigs	R = .700 P = .000		R = .556 P = .000	R = .423 P = .001	R = .598 P = .000	R = .588 P = .000	R = .548 P = .000	R = .619 P = .000
Total-C	R = .332 P = .007	R = .151 P = .237		R = .480 P = .000	R = .157 P = .219	R = .269 P = .033	R = .302 P = .016	R = .227 P = .073
HDL-C	R =-.589 P = .000	R =-.403 P = .001	R =-.668 P = .000		R =-.534 P = .000	R =-.551 P = .000	R =-.502 P = .000	R =-.601 P = .000
LDL-C	R = .267 P = .033	R = .328 P = .009	R = .205 P = .107	R = .160 P = .211		R = .197 P = .122	R = .289 P = .022	R = .254 P = .045
Insulin	R = .373 P = .002	R = .307 P = .014	R = .304 P = .015	R = .230 P = .069	R = .295 P = .019		R = .229 P = .071	R = .259 P = .040
Leptin	R = .225 P = .043	R = .284 P = .024	R = .395 P = .001	R = .188 P = .141	R = .339 P = .007	R = .317 P = .011		R = .303 P = .016
vWF	R = .381 P = .002	R = .417 P = .001	R = .321 P = .010	R = .401 P = .001	R = .273 P = .031	R = .314 P = .012	R = .273 P = .030	

Interestingly, the partial correlation between triglyceride and total PAI-1 Ag was strengthened when controlling for vWF (partial R = 0.619 P = 0.000 Vs R = 0.599 P = 0.000). The same was seen with HDL-C controlling for both total cholesterol (partial R = -.668 P = 0.000 Vs R = -0.589 P = 0.000) and vWF (partial R =-0.601 P = 0.000 Vs R = -0.589 P = 0.000) as well as for vWF controlling for triglyceride (partial R = 0.417 P = 0.001 Vs R = 0.376 P =0.002) and HDL cholesterol (partial R = 0.401 P = 0.001 Vs R = 0.376 P = 0.002).

Active PAI-1 Ag remained independently correlated with total cholesterol as well as triglyceride regardless of controlling factor. HDL cholesterol was significantly correlated with active PAI-1 Ag at all times with the exception of controlling for triglycerides that completely abolished the correlation (partial R = 0.007 P = 0.960). The correlation with insulin observed before controlling factors were applied (R = 0.263 P = 0.036) was reduced to a trend after controlling for triglyceride (partial R = 0.216 P = 0.088) and total cholesterol (partial R = 0.220 P = 0.083) and was non-significant when controlling for HDL cholesterol (partial R = 0.201 P = 0.114).

Table 6.6: Partial correlations between active PAI-1 concentration and other significant correlating markers of the IRS for the 16 men across the trial. Trig = triglyceride, C = cholesterol. Significant correlations P ≤0.05N are shown in red. N= 64

	CONTROLLING FACTOR				
	NONE	Trig	Total-C	HDL-C	Insulin
Trigs	R = .685 P = .000		R = .515 P = .000	R = .515 P = .000	R = .559 P = .000
Total-C	R = .375 P = .002	R = .271 P = .032		R = .457 P = .000	R = .368 P = .003
HDL-C	R =-.377 P = .002	R = .007 P = .960	R =-.383 P = .002		R =-.243 P = .054
Insulin	R = .367 P = .003	R = .216 P = .088	R = .220 P = .083	R = .201 P = .114	

As with total PAI-1 correlations, the correlations between active PAI-1 and total cholesterol were strengthened when controlling for HDL cholesterol (partial R = 0.457 P

= 0.000 Vs R = 0.392 P = 0.001) and for HDL cholesterol when controlling for total cholesterol (partial R = -0.383 P = 0.002 Vs R = -0.295 P = 0.018).

When all the significant correlations with total PAI-1 were entered into a stepwise multiple regression model, triglyceride was the first factor to enter the model (coefficient = 11.861, P = 0.013) followed by vWF (coefficient = 0.346 P = 0.004), HDL cholesterol (coefficient = -93.913 P = 0.000) and total cholesterol (coefficient = 11.157, P = 0.043). This model predicts 59.2% of total PAI-1 Ag (R^2 = 0.592 P = 0.009 constant = 84.316 P = 0.009). Insulin, leptin and LDL cholesterol were removed from the model after controlling for HDL cholesterol.

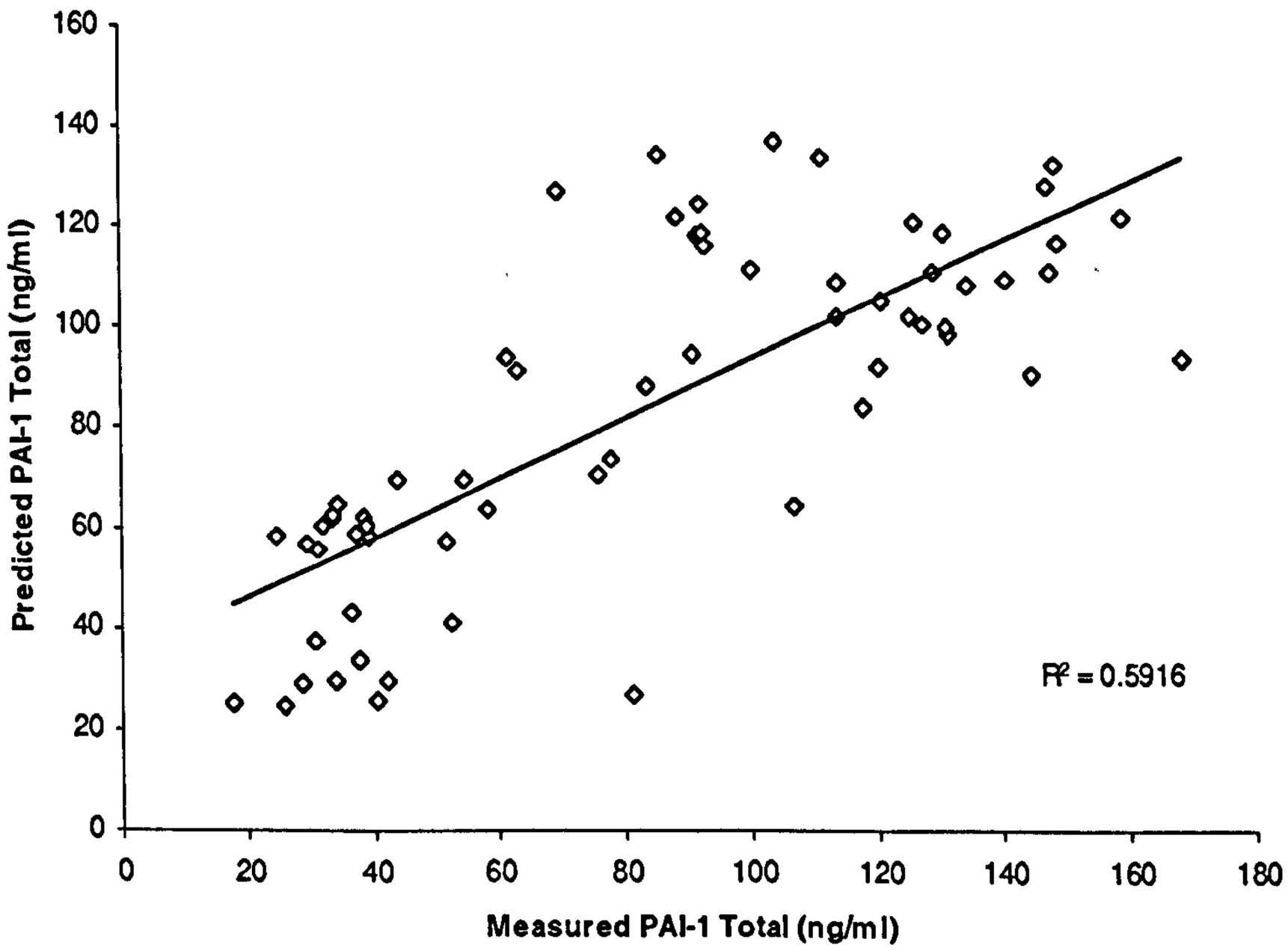

Figure 6.2: Correlation between actual total PAI-1 concentration and PAI-1 concentration predicted by the stepwise multiple regression model. Data is included for all 16 men at all 4 time points

When all the significant correlations with active PAI-1 Ag were entered into a stepwise multiple regression model only triglyceride concentration (coefficient = 9.704 P= 0.000) and total cholesterol concentration (coefficient = 5.909 P=0.032) remained independently associated with active PAI-1 (constant = -26.776). This model however (Figure 6.3) only accounts for 36.3% of active PAI-1 Ag (R^2 = 0.363).

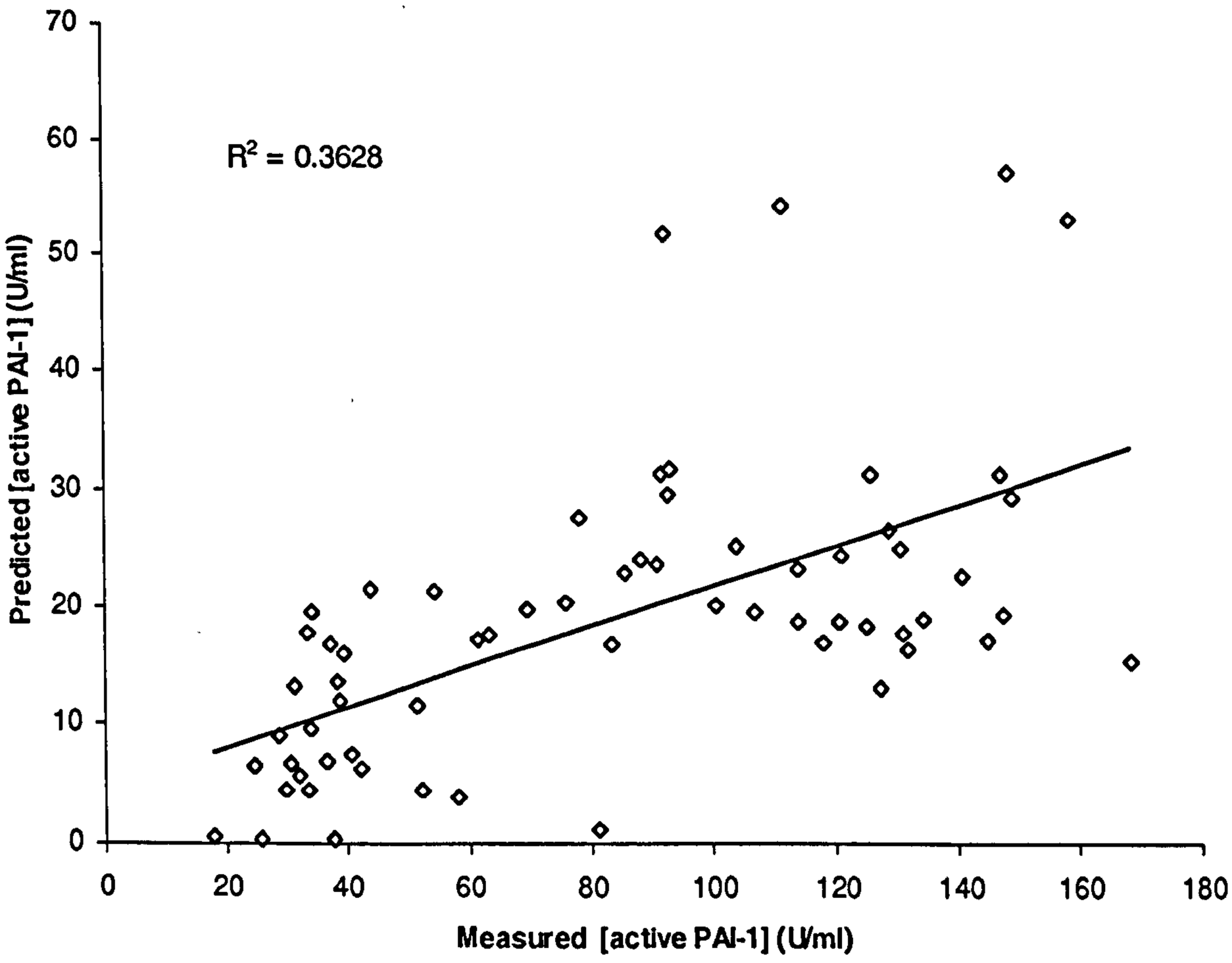

Figure 6.3: Correlation between measured active PAI-1 concentration and active PAI-1 concentration predicted by the stepwise multiple regression model. Data is included for all 16 men at all 4 time points.

6.4 Discussion

6.4.1 Summary of the Protocol

8 obese and 8 non obese sedentary male volunteers undertook an acute walking exercise bout at 70% VO_2 max for 30 minutes. Blood samples were taken 100 minutes pre exercise (VO) after 3 minutes of venous occlusion and a standard cannulation, 15 minutes pre exercise (REST) after a standard 190 kcal breakfast, at the end of the exercise bout (END) and thirty minutes post exercise (POST). Samples were analysed for t-PA, total PAI-1, active PAI-1, insulin, leptin, total cholesterol, LDL cholesterol, HDL cholesterol and triglyceride.

6.4.2 Resting Correlations

Both total and active PAI-1 Ag were positively correlated with total body mass (R=0.652 P=0.006, R=0.600 P=0.014 respectively), BMI (R = 0.724, P=0.002, R=0.613 P=0.012), body fat percentage (R=0.702, P=0.002, R=0.482, P=0.054), kilograms of body fat (R=0.767 P=0.001, R=0.559, P=0.024), waist to hip ratio (R=0.790, P=0.000, R=0.643, P=0.007) and waist circumference (R=0.836, P=0.000, R=0.635, P=0.008). Adipose tissue, specifically visceral depots have recently been postulated as a major source of elevated PAI-1 Ag in obesity (Janand-Delenne *et al* 1998). Resting PAI-1 Ag and activity have been shown on numerous occasions to positively correlated with BMI, waist to hip ratio, waist circumference and visceral fat mass (Janand-Delenne *et al* 1998, Vague *et al* 1989). Moreover, clinical studies have demonstrated that weight loss due to surgical treatment and diet significantly reduces PAI-1 Ag in obese volunteers (Loskutoff & Samad 1997). Yudkin *et al* (1999) however attempted to quantify the contribution of adipose to total PAI-1 secretion by measuring the arterio-venous difference in PAI-1 Ag across the subcutaneous fat depot in lean subjects. This group determined that the relative contribution of subcutaneous adipose tissue to total PAI-1 Ag is only 1.6% and 3.1% to PAI-1 activity.

Although the contribution of visceral fat to PAI-1 Ag is greater than that of subcutaneous fat and the obese have greater amounts of both types of fat, the contribution of adipose tissue to the expression and secretion of PAI-1 is often over exaggerated as a result of correlations between other markers of the IRS, such as triglyceride, and anthropometric measures. Further evidence for this is provided in the present study as when controlling for markers such as triglyceride and mean arterial pressure (table 6.2) the correlations between PAI-1 Ag and anthropometric measures are weakened or abolished. These resting results however are in conflict with those obtained in males in chapter 4, highlighting the complex nature of the insulin resistance syndrome. The discrepancy may be due to the small number of subjects in this study, the inclusion of non obese subjects and the upper BMI limit of 45 kg/m^2.

Total and active PAI-1 Ag was also significantly correlated to triglyceride (R=0.599, P=0.000, R=0.573, P=0.000 respectively), total cholesterol (R=0.304, P= 0.014, R=0.392, P=0.001), HDL cholesterol (R=-0.589 P=0.000, R=-0.295, P=0.018) and insulin (R=0.335, P=0.007, R=0.263, P=0.036). Again, these are correlations observed by numerous studies both *in vitro* (Kooistra *et al* 1989, Tremoli *et al* 1993) and *in vivo* (Potter van Loon *et al* 1993, Cimminello *et al* 1997) and each has been proposed as a mechanism for elevated PAI-1 Ag.

The relationship between mean arterial pressure and total PAI-1 was the closest by far (R = 0.936 P = 0.000) and this relationship was independent of body mass, BMI, fat percentage, fat mass, WHR, WC, insulin, total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, vWF and leptin. Mean arterial pressure therefore was the only factor to enter the multiple regression model. The relationship between total PAI-1 Ag and vWF suggests that increasing mean arterial pressure may result in elevated PAI-1 concentrations via endothelial damage. Endothelial damage results in activation of platelets and release of PAI-1 from the alpha granules as well as release of PAI-1 from

the endothelium and therefore may account for the elevated total PAI-1 Ag. As vWF is also released from the alpha granules of platelets (Best & Taylor, 1993), the correlation between the two suggests the presence of platelet hyperactivity that has previously been described in sedentary populations (Kestin *et al* 1993). An additional marker of platel*et* alpha granule release, such as platelet factor 4 or β thromboglobulin would need to be measured to confirm this.

Another possible mechanism for the relationship between MAP and PAI-1 is the involvement of the renin angiotensin aldosterone system (RAAS). Obesity is associated with elevated plasma angiotensin, plasma renin activity, tissue specific angiotensin converting enzyme (ACE) and plasma aldosterone levels (Egan *et al* 2001). *In vitro* evidence implicates angiotensin II, angiotensin IV and aldosterone in increased secretion of PAI-1 as well as hypertension through sodium retention and vasoconstriction (Huber *et al* 2001a). Increased ACE activity further contributes to a hypofibrinolytic state by reducing the half-life of bradykinin that is known to stimulate t-PA release from endothelial stores as well as promote vasodilatation via the actions of nitric oxide.

Active PAI-1 Ag were significantly correlated with body mass (R = 0.600 P = 0.014), BMI (R= 0.613 P= 0.012), body fat percentage (R=0.482 P=0.054), fat mass (R=0.559 P=0.024), WHR (R= 0.643, 0.007), WC (R=0.635 P=0.008), MAP (R=0.716 P=0.002), triglyceride (R=0.573 P=0.000), total cholesterol (R=0.392 P=0.001), HDL cholesterol (R=-0.295 P=0.018) and insulin (R=0.263 P=0.036). No factor remained independently correlated with active PAI-1 at rest but as MAP had the strongest statistical relationship with active PAI-1 and as this relationship eliminated all other significant relationships between markers of the IRS and active PAI-1 Ag, only MAP entered the multiple regression model. The significant correlation between MAP and active PAI-1 Ag however was removed when controlling for anthropometric variables and triglyceride concentration unlike total PAI-1 Ag which remained independently associated with MAP

regardless of controlling factor. This highlights the complex nature of both the insulin resistance syndrome as a whole and the mechanisms responsible for the regulations of PAI-1 secretion.

The active PAI-1concentration at a given time point is dependent not only on secretion from the endothelium, platelets and other sources but also on free t-PA concentrations and hepatic clearance rates. The significant correlation between total and active PAI-1 (R=0.565 P=0.000) suggests that only 31.9% of the variability of active PAI-1 Ag is explained by total PAI-1 Ag. Additional variables may include variation in latent PAI-1 secretion, availability of vitronectin to stabilise the active PAI-1 conformation and free t-PA concentrations. As these factors were not measured in this study, it is impossible to say whether they influenced active PAI-1 Ag at rest.

6.4.3 Correlations Across the Trial

Variations in PAI-1 release with exercise and recovery are thought to be a result of endothelial damage and subsequent platelet activation and alpha granule secretion (Streiff & Bell 1994). Multiple correlations were performed on both total and active concentrations across the trial to determine the potential role of other measured markers of the IRS in the short term regulation of PAI-1 secretion.

Across the trial total PAI-1 Ag were significantly correlated with vWF (R= 0.381 P= 0.002), triglyceride (R=0.700 P=0.000), total cholesterol (R=0.332 P=0.007), HDL cholesterol (R=-0.589 P=0.000), LDL cholesterol (R=0.267 P=0.033), insulin (R=0.373 P=0.002) and leptin (R=0.225 P=0.043). Only triglyceride concentration, HDL concentration and vWF concentration remained independently associated with total PAI-1 Ag throughout the trial after multiple partial correlations were performed.

The role of triglyceride in the short term regulation of PAI-1 Ag has been extensively studied *in vitro*. Application of triglyceride rich VLDL to cultured endothelial cells and hepatocytes has been shown to increase PAI-1 secretion (Tremoli *et al* 1993). Moreover, the larger VLDL particles, commonly seen in hypertriglycerideamic patients, result in a greater PAI-1 response than the smaller VLDL particles seen in normotriglyceridaemic patients (Stiko-Rahm *et al* 1990). PAI-1 secretion from hepatic and endothelial cells as a result of triglyceride application can be further potentiated by insulin (Sironi *et al* 1996) explaining the persisting relationship between PAI-1 and insulin despite controlling for triglyceride concentration.

The relationship between HDL cholesterol and total PAI-1 Ag has generally been attributed to the close metabolic relationship between HDL cholesterol and triglyceride concentration (Vague 1993, Vague *et al* 1995). This study shows however that although the relationship weakens as a result of controlling for triglyceride, there is a significant independent relationship between the two factors. Research in this area is limited but Ren & Shen (2000) described an increased PAI-1 secretion from endothelial cells in response to glycated HDL cholesterol from patients with type II diabetes mellitus. Furthermore, the level of HDL glycation is proportional to insulin resistance and may therefore explain the lack of a significant relationship between insulin and total PAI-1 Ag after controlling for HDL cholesterol.

The relationship between total PAI-1 Ag and vWF over the trial can be attributed to the co-release of the two factors from the alpha granules of activated platelets and the endothelium in response to damage and exposure of the sub-endothelium (Gibbons *et al* 1990). The damage to the endothelium occurs as a result of increased shear stress on the blood vessel wall. The increased shear stress occurs due to elevated blood pressure

especially during exercise, and may in extreme cases cause the rupture of vulnerable atherosclerotic plaques which may in turn lead to MI onset (Mittleman & Siscovik 1996).

The multiple regression model included triglyceride, vWF, HDL cholesterol and total cholesterol and accounted for 59% of the variation in total PAI-1 Ag. This confirms that the regulation of PAI-1 secretion is a complex process involving a number of different factors including triglyceride, vWF, insulin, HDL cholesterol and other unknown factors. Another consideration is the expression of the 2 PAI-1 transcripts. The larger of the 2 transcripts (3.2 kb Vs 2.2 kb) is less stable and therefore has a shorter biological half life (Tremoli *et al* 1993). The ratio of the 2 transcripts will affect the overall half-life of PAI-1 thus affecting the PAI-1 Ag. Stiko-Rahm *et al* (1990) observed that VLDL molecules from hypertriglyceridaemic patients stimulates the preferential expression and release of the more stable PAI-1 transcript and this may contribute to the increased PAI-1 Ag observed in those with other symptoms of the IRS.

Across the trial active PAI-1 Ag (table 6.6) was significantly correlated with triglyceride concentration (R=0.573 P=0.000), total cholesterol (R=0.392 P=0.000), HDL cholesterol (R=-0.295 P=0.018) and insulin (R=0.263 P=0.036). Following multiple partial correlations, controlling for each significantly correlated factor in turn, only triglyceride concentration and total cholesterol concentration were independently correlated to active PAI-1 levels.

Triglyceride accounted for a similar amount of the variation in active PAI-1 Ag as it accounted for in total PAI-1 Ag (active PAI-1=36%, total PAI-1 = 36%). This provides additional evidence for the role of triglyceride in the short term regulation of PAI-1 secretion. When controlling for triglyceride concentration the relationship between PAI-1 and insulin was reduced to a trend. This still suggests that as with total PAI-1, insulin potentiates the effect of triglyceride on PAI-1 secretion. Unlike the relationship seen with

total PAI-1Ag, controlling for triglyceride abolished the correlation between HDL cholesterol and active PAI-1 Ag (R=0.007, P=0.960). This implies that the association between active PAI-1 Ag and HDL cholesterol is purely a result of the close metabolic relationship between HDL and triglyceride thus confirming the results of Cimminello *et al* (1997) who demonstrated that only patients with both elevated triglycerides and low HDL had elevated PAI-1 compared to those with low HDL cholesterol only.

The independent correlation between active PAI-1 Ag and total cholesterol was not observed with total PAI-1. This association is independent of HDL cholesterol as controlling for this factor strengthens the relationship (R=0.392 without controls, R=0.457 with controls). Controlling for triglyceride concentration weakens the association but does not abolish it suggesting that a single lipoprotein sub-fraction is not the contributing factor. We can postulate therefore that a combination of LDL and VLDL cholesterol is contributing to this association but a direct measure of the all lipoprotein sub-fractions would be needed to confirm this.

The multiple regression model containing both triglyceride and total cholesterol, only accounted for 36% of the variability in active PAI-1 Ag. This suggests that factors that have not been measured in this study are primarily responsible for the short term regulation of PAI-1.

Free t-PA concentration would be a better measure as the t-PA/PAI-1 complex is rapidly formed thus decreasing plasma active PAI-1 Ag. This rapid complex formation may mask the effect of other factors in the short term regulation of active PAI-1 Ag. Another consideration when accounting for the variability of active PAI-1 Ag over time is the availability of vitronectin, which stabilises the active conformation and thus increases its biological half life (Van Meijer & Pannekoek 1995).

Overall this study cannot provide any firm conclusions about the factors involved in the short term regulation of PAI-1 secretion. The large number of variables including PAI-1 secretion rate from different tissues, hepatic clearance rate and availability of such factors as vitronectin and t-PA all contribute to the large variability observed with a study such as this. Another limitation is the assumption that the metabolic markers of the IRS stimulate PAI-1 secretion almost immediately. This research therefore does not account for delayed reactions including larger non measured metabolic pathways or stimulation of PAI-1 expression.

Triglyceride concentration however was the only measured factor to remain significantly correlated to both active and total PAI-1 Ag at rest, during exercise and in the post exercise period despite controlling for a number of markers of the IRS. This suggests a role for triglyceride in the regulation of PAI-1 secretion and expression and confirms both the *in vivo* (Cimminello *et al* 1997, Vague *et al* 1995) and *in vitro* (Stiko-Rahm *et al* 1990, Sironi *et al* 1996) data. Many more studies are needed to assess the effect of different metabolic markers and different environmental conditions on this complex regulation process.

Chapter 7

The effect of a maximal oxygen uptake test on fibrinolytic capacity in sedentary, overweight & obese, healthy, premenopausal, female volunteers

Chapter 7: The effect of a maximal oxygen uptake test on fibrinolytic capacity in sedentary, overweight & obese, healthy, premenopausal, female volunteers.

7.1 Introduction

The results of the study assessing the effect of an acute bout of moderate intensity exercise on obese males volunteers (chapter 5) did not show any significant change in total PAI-1 concentrations suggesting that the decrease in active PAI-1 was merely a result of increased t-PA. Numerous groups have reported a significant decrease in total PAI-1 concentrations in non obese populations following acute bouts of strenuous exercise (El Sayed 1996, Szymanski & Pate 1994).

The hypothesis for this study therefore is that strenuous exercise, in the form of a maximal oxygen uptake test to exhaustion will result in decreased total PAI-1 concentrations.

The results from the previous study also showed that the obese group remained hypofibrinolytic and therefore at increased risk of MI onset compared to the non obese group both during and post exercise. These results suggest that subjecting obese male volunteers to a strenuous maximal oxygen uptake test may further increase the risk of MI onset. In order to avoid this which would present ethical problems for a research project, obese and overweight premenopausal females were used in this study.

Premenopausal females produce lower amounts of PAI-1 than both men and postmenopausal women (Gebara *et al* 1995). Premenopausal women commonly display gynoid obesity (accumulation of fat around the hips and buttocks). This fat is relatively benign in terms of cardiovascular risk and the IRS and as such, premenopausal women display less symptoms of the IRS (Despres & Lamarche

1993). Comparison of these results with those obtained in obese males (chapter 6) may provide a further insight into the mechanisms responsible for elevated PAI-1 concentrations and possible allow prediction of PAI-1 changes with exercise from resting data.

The primary aim of this research study therefore is to determine the effect of a single multi-stage maximal oxygen uptake test on plasma fibrinolytic capacity in overweight and obese premenopausal women. The secondary aim is to determine if the change in fibrinolytic capacity can be predicted from resting anthropometric and blood markers.

7.2 Subjects & Methods

19 (powered to 95% confidence and 20% type II error) overweight and obese premenopausal female volunteers were recruited for participation in the study. Inclusion criteria for the study were BMI >27 kg/m^2 and a waist circumference >88cm. "Sedentary" was defined as <20 minutes recreational exercise per week and having a non manual profession. All subjects were free of any ongoing disease or impairment of gait or balance.

7.2.2 Experimental Design

All subjects underwent a physical examination prior to entry into the study. Volunteers arrived at the laboratory at 09.00h to reduce the effects of diurnal variation having fasted overnight, abstained from smoking for 8 hours, alcohol for 24 hours and strenuous exercise for 48 hours. No subject was taking prescription or "over the counter" medication for 6 weeks prior to the commencement of the study and the test was performed in the early follicular phase (days 7-11) of each volunteer's menstrual cycle.

On the trial day subjects underwent measures of height, weight, percent body fat (by bioelectrical impedance (Body Stat 1500^{TM}) waist circumference, hip circumference and blood pressure. Blood pressure was determined by the average of three values recorded 10 minutes apart (Omron 711). A 21 G cannula was then inserted into a suitable forearm vein (after 3 minutes of venous occlusion at 100 mmHg). Subjects then rested in the seated position for 15 minutes before a 25ml blood sample (PRE) was taken. Maximal oxygen uptake was determined by means of a maximal fitness test on a motorised treadmill employing a modified Bruce protocol. Expired air was analysed on a breath by breath basis using the MetaMax 3B online telemetry system, for FEO_2, $FECO_2$, volume and temperature to allow calculation of VO_2 (all volumes were corrected to STPD) and RER. Heart rate was recorded by the Metamax via a

Polar™ heart rate monitor and perceived exertion was recorded in the last 30 seconds of each stage using 3 of the following 4 criteria to ascertain that subjects had reached maximal oxygen uptake.

1. A heart rate $\geq$ 220 – subjects age
2. VO_2 that remained unchanged over 2 consecutive stages
3. A rating of perceived exertion $\geq$ 18 over 2 consecutive stages
4. RER > 1.1

Subjects then performed a 3 minute cool down period (2.7 km h^{-1}, 0% gradient) before a second 25 ml blood sample (POST) was taken.

7.2.3 Laboratory Methods

Blood samples were distributed between sealed evacuated test tubes containing no additives, sodium citrate or EDTA. The EDTA samples were analysed immediately for haemoglobin concentration (GDS systems) and haematocrit (micro centrifugation Hawksley International). Sodium citrate samples were centrifuged at 3000rpm (IEC Centra-2) for 15 minutes and the resultant plasma was pipetted into eppendorf tubes and immediately frozen at -80°C. The plasma samples were analysed by ELISA for t-PA Ag (Biopool International procedure 101005), Total PAI-1 (Biopool International procedure 101005), active PAI-1 Ag (Technoclone procedure 16075) and vWF (Immunozym procedure 5450200). No additives samples were allowed to clot for 30 minutes before centrifugation at 3000rpm (IEC Centra-2) for 15 minutes and the resultant serum was pipetted into eppendorf tubes and stored at -20°C. The serum samples were analysed for insulin by MEIA (Imx Systems), leptin by ELISA (R&D systems procedure DLP00), total cholesterol (Beckman Synchron CX systems procedure 467825), triglyceride (Beckman Synchron CX systems 445850) and HDL

cholesterol (Sigma diagnostics procedure 345L). LDL concentration was determined using the Friedewald equation.

7.2.4 Calculations and Statistical Analysis

Plasma volume changes were calculated according to the method of Dill & Costhill (1974). All data shown is corrected for plasma volume changes. Normality of distribution determined using a Shapiro-Wilks test. Comparisons between PRE and POST samples were determined using paired T tests for normally distributed data and Mann Whitney test for skewed data. The relationships between measures were determined using Spearman's rho correlation coefficients and multiple stepwise regression models. Two tailed significance levels are reported and differences were considered significant when $P \leq 0.05$.

7.3 Results

7.3.1 Demographic Data

The physical characteristics of the subjects are shown in table 7.1. 5 subjects were overweight (BMI 27-30 kg/m^2) and 14 were obese (BMI $\geq$ 30 kg/m^2). 17 subjects were Caucasian and 2 were Afro Caribbean in origin. All subjects had a waist circumference $\geq$ 88cm which is indicative of elevated cardiac risk.

Table 7.1: The physical characteristics and fibrinolytic variables of the 19 subjects.

Variable	Mean $\pm$ SEM	Median (Range)
Body Mass (kg)		87.34 (72.70 to 110.00)
BMI (kg/m^2)		32.12 (27.50 to 38.10)
Body Fat (%)		41.84 (30.00 to 49.70)
Body Fat (kg)		36.90 (22.20 to 51.92)
Waist Circumference (cm)		94.11 (88.00 to 108.00)
Waist to Hip Ratio		0.83 (0.74 to 0.94)
VO_2 max (ml/kg/min)		18.94 (10.66 to 25.90)
Systolic Pressure (mmHg)		132.83 (120.00 to 160.00)
Diastolic Pressure (mmHg)		85.86 (60.00 to 96.00)
t-PA Ag (ng/ml)	7.87 $\pm$ 0.72	
Total PAI-1 Ag (ng/ml)	11.28 $\pm$ 1.27	
Active PAI-1 Ag (U/ml)	5.99 $\pm$ 1.00	

Concentrations of the other measured blood markers of the insulin resistance syndrome are shown in table 7.2.

Table 7.2: Concentrations of the measured blood markers of the Insulin Resistance syndrome at rest. N=19

Variable	Mean ± SEM	Median (Range)
Total Cholesterol (mmol/L)		5.17 (3.7 to7.4)
Triglyceride (mmol/L)		1.30 (0.55 to 2.98)
HDL Cholesterol (mmol/L)		1.33 (0.78 to2.68)
LDL Cholesterol (mmol/L)		3.26 (2.0 to4.3)
Insulin (pmol/L)	6.41 ± 0.69	
Leptin (ng/ml)	28.64± 6.85	
VWF (IU/ml)	0.42 ± 0.05	

7.3.2 Fibrinolytic Factors with Maximal Exercise

T-PA Ag (fig 7.1) significantly increased by 62% from (mean (SEM)) 7.87 ± 0.72 ng/ml to 12.74 ± 1.58 ng/ml (P=0.02) as a result of the maximal exercise test. Total PAI-1 Ag and active PAI-1 Ag did not change significantly (P=0.996 and P=0.848 respectively).

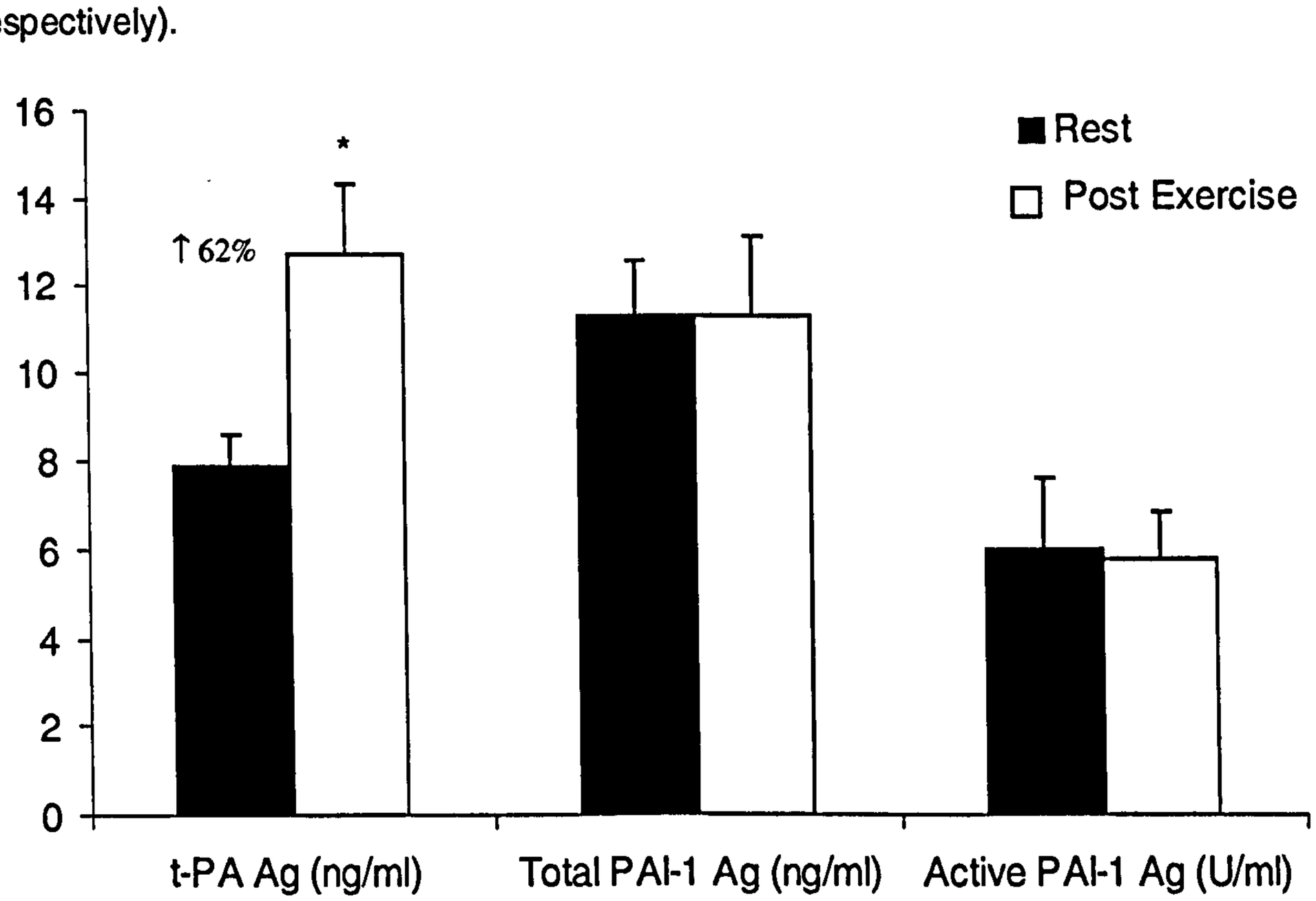

Figure 7.1: The effect of a maximal exercise test on t-PA Ag, total PAI-1 Ag and active PAI-1 Ag. Data is shown as mean ± SEM. All data is corrected for changes in plasma volume. * = P≤ 0.05.

To determine whether 'responders' and 'non-responders' to maximal exercise can be identified from resting data, subjects were grouped according to response to exercise. Those subjects whose total or active PAI-1 concentration decreased with exercise were termed "responders" and those subjects in whom total or active PAI-1 increased with exercise were termed "non-responders".

Concerning total PAI-1 Ag, the women studied included 11 'responders' to exercise (in whom total PAI-1 Ag decreased with exercise) and 8 'non-responders' to exercise (in whom total PAI-1 Ag increased with exercise). Group comparisons showed that

the 'responders' had a significantly higher VO_2max (P = 0.025) than the 'non responders', as well as higher resting t-PA concentration and lower resting total PAI-1 concentrations.

Concerning active PAI-1 concentrations, the women studied included 10 "responders" to exercise (active PAI-1 concentrations decreased) and 9 non-"responders" to exercise (active PAI-1 concentrations increased with exercise). Comparisons between the two groups revealed that the responder group had a significantly higher VO_2 max (P=0.043) and non significant trends towards lower resting total cholesterol concentrations (P = 0.066) and lower resting triglyceride concentrations (P = 0.079).

7.3.3 Total PAI-1 Ag

To assess the possible contribution of other markers of the insulin resistance syndrome in the short-term regulation of fibrinolytic variables over the trial, multiple correlations were performed (tables 7.3 & 7.4). Total PAI-1 Ag was significantly positively correlated with total body mass, BMI, body fat percentage, fat mass and waist circumference.

Table 7.3: Correlations between total PAI-1 Ag concentrations and other anthropometric and blood measures of the Insulin Resistance Syndrome at rest and at the end of the exercise bout. N=19.* = P=≤0.05, ^{T} = P ≤0.10

Marker	R Value	P Value
Body Mass (kg)	**0.517**	**0.023***
BMI	**0.465**	**0.045***
Fat Percentage (%)	**0.455**	**0.050***
Fat Mass (kg)	**0.554**	**0.014***
Waist Circumference (cm)	**0.447**	**0.050***
Waist to Hip Ratio	-0.135	0.583
VO_2 max (ml/kg/min)	**-0.470**	**0.043***
Systolic Pressure	0.034	0.890
Diastolic Pressure	0.209	0.390
Rest Insulin (pmol/L)	0.147	0.380
Rest Total-C (mmol/l)	-0.036	0.830
Rest Triglyceride (mmol/L)	0.121	0.469
Rest HDL-C (mmol/L)	-0.218	0.188
Rest LDL-C (mmol/L)	0.015	0.927
Rest Leptin (pg/ml)	0.096	0.565
Rest vWF (IU/ml)	0.038	0.819
Rest t-PA (ng/ml)	0.131	0.434
Total PAI-1 (ng/ml)	-	-
Active PAI-1 (ng/ml)	0.188	0.259

Unlike the previous study in a male population (chapter 6) however, no blood markers were significantly correlated to total plasma PAI-1 concentrations. Also, unlike the previous study total PAI-1 was negatively correlated with VO_2 max.

7.3.4 Active PAI-1 Ag

Finally, unlike the previous trial active plasma PAI-1 Ag was not significantly correlated to any of the measured factors.

Table 7.4: Correlations between active PAI-1 Ag concentrations and other anthropometric and blood measures of the Insulin Resistance Syndrome at rest and at the end of the exercise bout. N=19

Marker	R Value	P Value
Body Mass (kg)	0.128	0.601
BMI	0.131	0.593
Fat Percentage (%)	0.186	0.445
Fat Mass (kg)	0.171	0.485
Waist Circumference (cm)	-0.032	0.896
Waist to Hip Ratio	-0.144	0.556
VO_2 max (ml/kg/min)	0.072	0.770
Systolic Pressure	0.356	0.134
Diastolic Pressure	0.281	0.245
Rest Insulin (pmol/L)	0.091	0.586
Rest Total-C (mmol/l)	-0.108	0.519
Rest Triglyceride (mmol/L)	-0.062	0.711
Rest HDL-C (mmol/L)	-0.157	0.347
Rest LDL-C (mmol/L)	-0.035	0.835
Rest Leptin (pg/ml)	0.058	0.730
Rest vWF (IU/ml)	0.001	0.997
Rest t-PA (ng/ml)	0.213	0.199
Total PAI-1 (ng/ml)	0.188	0.259
Active PAI-1 (ng/ml)	-	-

To test whether any of the resting variables were predictive of the actual changes or percentage changes in total and active PAI-1 a stepwise multiple regression analysis was performed.

When the dependent variable in the model was percentage change in total plasma PAI-1 Ag, WHR was the first variable to enter (t = 7.502, P = 0.000). Systolic blood pressure (t = 7.425, P = 0.000), resting total PAI-1 Ag concentration (t = -2.194 P = 0.012) and waist circumference (t = -2.890, P = 0.000) were the only other variables that remained independently associated with percentage change in total PAI-1 concentration (R^2 = 0.898, F = 25.518, P= 0.000).

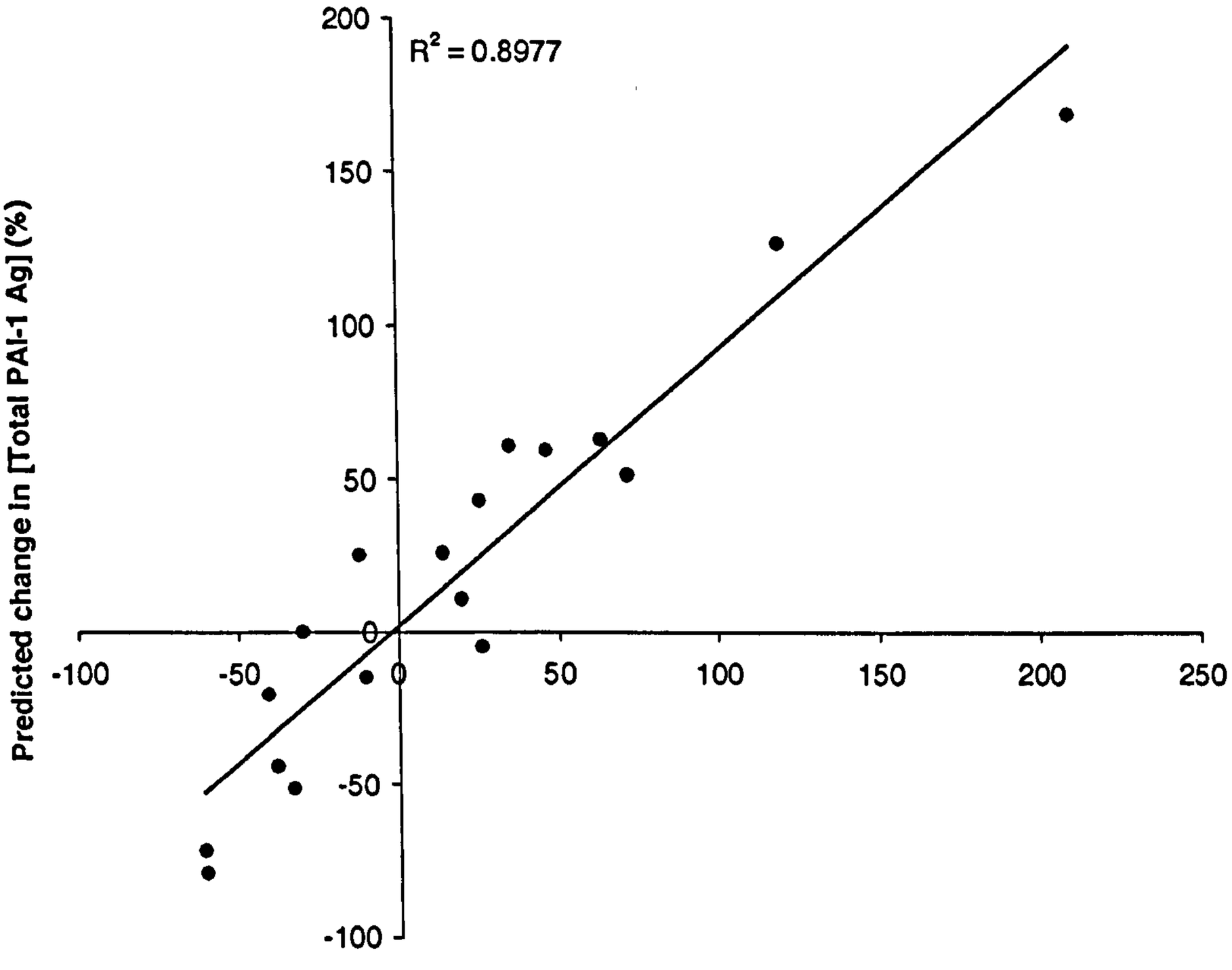

Figure7.2: Correlation between observed percentage change in [Total PAI-1 Ag] and change in [Total PAI-1 Ag] as predicted by the multiple regression equation. N=19

When the dependent variable in the model was actual change in PAI-1 concentration (ng/ml) systolic blood pressure was the first resting variable to enter the model (t = 10.021, P = 0.000) followed by resting total PAI-1 Ag concentration (t = -6.163 P = 0.000), WHR (t = 6.830 P = 0.000), fat mass (t = -4.159 P = 0.001) and resting triglycerides (t = 2.250 P = 0.044) (R^2 = 0.947, F = 42.638).

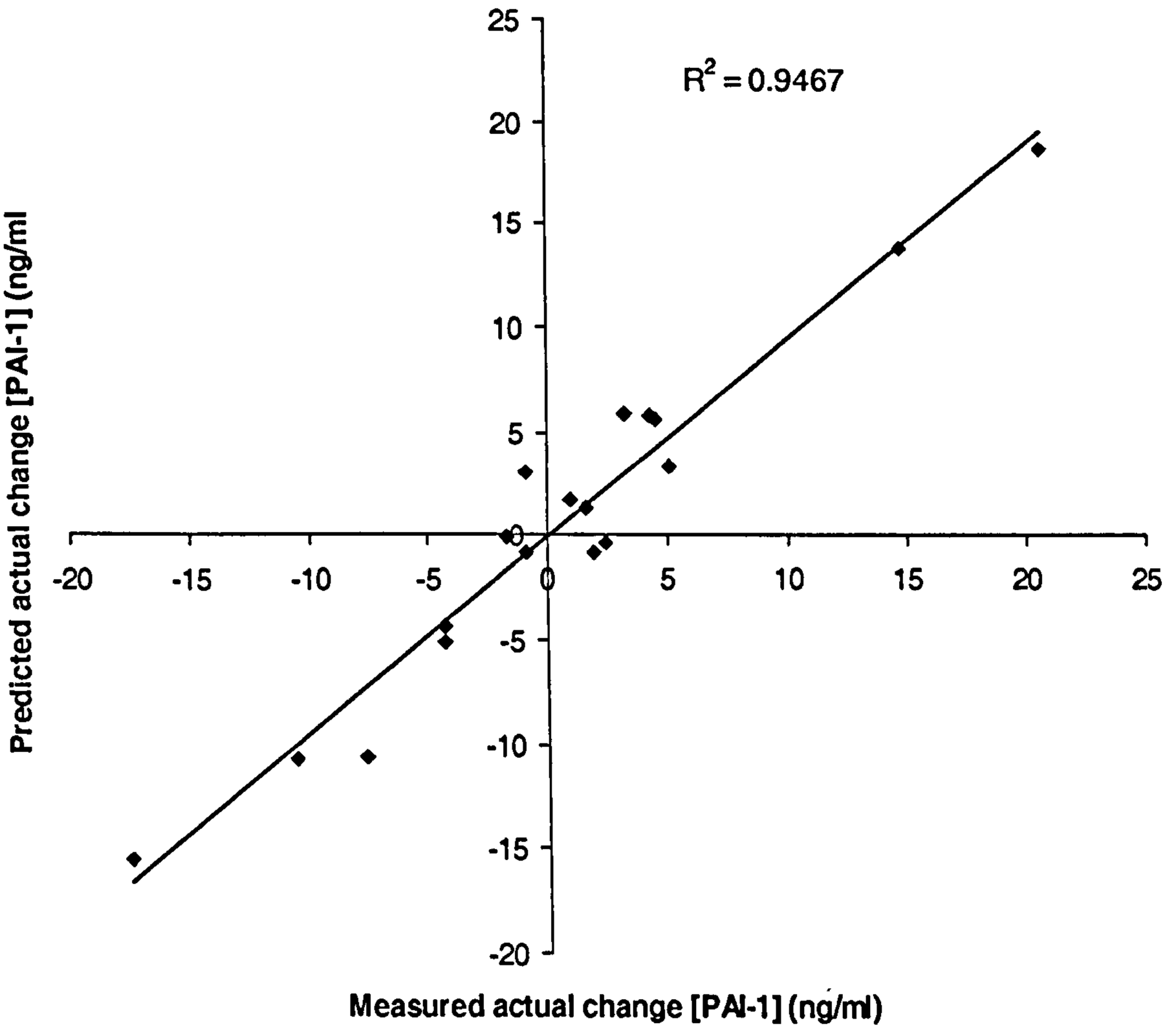

Figure 7.3: Correlation between observed change in [Total PAI-1 Ag] and change predicted by the multiple regression equation. N=19.

No factors were significantly correlated with either percentage change or actual change in active PAI-1 Ag concentration so multiple regression equations could be calculated.

7.4 Discussion

The primary aim of this research study was to determine the effect of a single multi-stage maximal oxygen uptake test on plasma fibrinolytic capacity in overweight and obese pre menopausal women. The secondary aim was to determine if the change in fibrinolytic capacity could be predicted from resting anthropometric and blood markers.

7.4.2 Summary of the Protocol

19 overweight and obese premenopausal females undertook a graded maximal oxygen uptake test on a motorised treadmill. Blood samples were taken at rest and immediately following the exercise bout. Samples were analysed for t-PA, total and active PAI-1, insulin, leptin, total cholesterol, triglyceride, HDL cholesterol, LDL cholesterol and vWF. Measurements of height, weight, body fat and waist and hip circumference were recorded before the exercise bout.

7.4.3 Resting Results

At rest, the 19 women studied exhibited t-PA Ag concentrations within the normal range (Mean $\pm$ SEM, 7.87 $\pm$ 0.72 ng/ml, normal range = 3-10 ng/ml) as well as normal range total PAI-1 Ag (11.28 $\pm$ 1.27 ng/ml, normal range 4-43 ng/ml) and active PAI-1 Ag concentrations (5.99 $\pm$ 1.00 U/ml, normal range 1-7 U/ml). This group of patients did not show a reduced fibrinolytic capacity (Total PAI-concentration >50 ng/ml & Active PAI-1 concentration >20 IU/ml) despite a waist circumference of >88 cm which is indicative of the metabolic disturbances associated with an increased risk of coronary heart disease (Han et al 1995) and a mean waist to hip ratio > 0.80 which is also indicative of an increased susceptibility to atherosclerosis (Despres et al 1995).

The absence of an impaired fibrinolytic capacity was also evident in all subjects despite total cholesterol concentrations of up to 7.4 mmol/L, triglyceride

concentrations of up to 2.98 mmol/L, and LDL cholesterol concentrations of up to 4.3 mmol/L. HDL cholesterol, insulin concentration and leptin concentrations remained within the normal healthy range for all subjects. This suggests that the hypofibrinolytic state associated with an increased waist circumference occurs after the development of a dyslipidaemic profile in the population. The lack of a hypofibrinolytic profile in this group of subjects despite a waist circumference >88cm, adds evidence to the postulated role for oestrogen in the regulation of PAI-1 secretion and expression (Huber 2001a, Teede et al 2000).

7.4.4 t-PA with Exercise

Consequent to the exercise test, t-PA Ag rose by 62% (from 7.87 $\pm$ 0.72 ng/ml to 12.74 $\pm$ 1.58 ng/ml). T-PA Ag has been reported to rise by 10-3000% with exercise of various intensities and durations. The reported exercise intensity threshold for increases in t-PA secretion and expression is approximately 50% VO_2 max with the largest increases occurring at intensities $\geq$ 70% VO_2 max (Streiff & Bell 1994). The increase in plasma t-PA concentrations observed in this study are modest in comparison to a number of other studies at intensities $\geq$ 65% VO_2 max. These studies, in hypertensives and sedentary populations, observed increases of 180% (Womack et al 2001) and 225% (Gleerup et al 1995) respectively. Szymanski & Pate (1994) demonstrated that exercise at 80% VO_2 max elicited a 50% greater response in t-PA concentration when compared to exercise at an intensity of 50% VO_2 max. The results of our investigation suggest that insufficient time was spent exercising at an intensity $\geq$ 70% VO_2 max to elicit the maximal t-PA response or, as with obese males, local changes in fibrinolytic factors are not reflected at the cannulation site until the post exercise recovery period.

7.4.5 PAI-1 with Exercise

As with the previous study in a male population, there were no significant changes in either total PAI-1 or active PAI-1 concentrations after a maximal exercise bout. The majority of studies have reported reductions in total PAI-1 concentration and PAI-1 activity from 30% of resting values (Rocker et al 1990) to undetectable levels (Speiser et al 1988), following an acute bout of exercise at an intensity greater than 70% VO_2 max (Strieff & Bell 1994) unlike t-PA, which has been reported to significantly increase with exercise at an intensity $\geq$ 50% VO_2 max (Streiff & Bell 1994). In this study, due to that nature of a modified Bruce protocol maximal oxygen uptake test, it may be that insufficient time was spent exercising at an intensity of $\geq$ 70% VO_2 max. Also, as the blood sample was taken immediately following the cessation of exercise, local changes in PAI-1 concentration resulting from the short period of exercise at $\geq$ 70% VO_2 max, may not yet have been reflected at the cannulation site. An additional blood sample post exercise would have indicated if this subject group reacted to exercise the same way as the male population by demonstrating significant changes in both total PAI-1 concentration and active PAI-1 concentration post exercise. Finally, the lack of a statistically significant change in total or active PAI-1 concentrations with a maximal exercise test may be due to the equal distribution of "responders" and non-"responders" to exercise within the group ('responders' show a decreased PAI-1 concentration following exercise whereas the 'non-responders' show an increased PAI-1 concentration following an exercise bout).

To determine whether 'responders' and 'non-responders' to exercise can be identified before undertaking an exercise bout, subjects were grouped according to PAI-1 response and an analysis of variance performed on all anthropometric and resting blood marker data to determine the differences between groups. Subjects in the responder group for both total and active PAI-1 had a significantly greater VO_2

max than the subjects in the non-responder group. There were no other differences between the two groups suggesting that physical fitness is an important contributor to the PAI-1 response to exercise.

Numerous longitudinal (Koenig & Ernst 2000, Streiff & Bell 1994) and cross sectional studies (Kvernmo & Osterud 1997, Earl et al 1987) have recorded significantly lower concentrations of both active and total plasma PAI-1 in volunteers undertaking regular exercise. Furthermore, Muller et al (1996) recorded a lower incidence of MI onset following heavy physical activity in those patients that undertook regular physical exercise. Active plasma PAI-1 'responders' (in which a decrease in PAI-1 was observed) to a maximal oxygen uptake test also had a significantly increased resting t-PA concentration compared to the non-responder group. This further highlights the importance of regular physical exertion in increasing both resting fibrinolytic capacity and the fibrinolytic response to exercise.

Overall the results suggest that there was an improvement in fibrinolytic capacity as shown by a significantly increased t-PA concentration with no significant change in either total PAI-1 or active PAI-1 concentrations. This suggests that an unaccustomed exercise bout may decrease the risk of MI onset and SCD in the premenopausal overweight and obese population studied. This response is contrary to response seen in an obese male population (chapter 5). Pre menopausal females display less of the metabolic disturbances associated with obesity as a result of the deposition of fat around the buttocks and thighs. The fat in this area is relatively benign whereas central fat accumulation, as seen in post menopausal women and men is more metabolically active and is strongly associated with the cluster of metabolic abnormalities that make up the insulin resistance syndrome and thus increase the risk of cardiovascular disease (Despres *et al* 1994).

7.4.6 Relationships between PAI-1 and other markers of the IRS

As with the previous study in an obese male population (chapter 5), total plasma PAI-1 Ag was significantly positively correlated with total body mass, BMI, body fat percentage, fat mass and waist circumference. Unlike the previous trial however, total PAI-1 Ag was not significantly correlated with systolic, diastolic or mean arterial blood pressure nor blood markers of the insulin resistance syndrome but was significantly negatively correlated with VO_2 max. As very few subjects showed any biochemical features of the IRS such as increased insulin concentrations and hypertension this is not entirely unexpected. Non obese populations do not demonstrate the same correlations between markers of the insulin resistance syndrome as do obese populations (Landin et al 1990) and as the population in this study ranged from overweight to obese, it may be that insufficient obese patients were included to allow for significant correlations to be observed.

Numerous studies have however reported correlations between resting total PAI-1 Ag (active & latent PAI-1) and anthropometric measures in premenopausal females. De Pergola et al (1997) observed a significant correlation between PAI Ag and waist circumference in obese and lean premenopausal females. This finding was confirmed by Janand-Delenne et al (1998) who showed that this relationship is due to a further significant correlation between PAI-1 Ag and visceral as opposed to subcutaneous fat depots. The relationship of PAI-1 Ag to anthropometric measures such as waist circumference and body fat percentage irrespective of insulin and triglyceride concentrations further supports the in vitro work (Yudkin et al 1999, Samad et al 1996) on the role of adipose tissue in the expression and secretion of PAI-1.

7.4.7 Predicting the PAI-1 response to Maximal Exercise

Finally, from the analysis of PAI-1 concentrations during this study in premenopausal females, we have identified two distinct groups of patients (i.e. 'responders' and 'non-responders' to exercise). We have also demonstrated that there are no anthropometric or blood markers at rest that directly indicate to a physician which category a patient is likely to be in. In view of this, multiple regression analysis was performed to determine whether actual changes (e.g. ng/ml) or percentage changes in plasma t-PA Ag, active PAI-1 Ag or total PAI-1 Ag could be predicted from measures taken at rest in an effort to predict those patients 'at risk'.

As mentioned earlier, VO_2 max was the only way in which the PAI-1 'non-responders' differed from the 'responders'. A stepwise multiple regression equation was therefore performed to determine whether percentage or actual change in total PAI-1 Ag could be predicted from resting markers of the metabolic syndrome. When percentage change in plasma PAI-1 concentration was considered as the dependent variable, the stepwise multiple regression equation incorporated waist to hip ratio, systolic blood pressure, resting total PAI-1 Ag and waist circumference. The predicted values for % change in total PAI-1 Ag concentration with a maximal oxygen uptake test were significantly correlated with the values recorded at the end of the exercise bout ($R^2 = 0.898$, $P = 0.000$). This suggests that percentage change in total PAI-1 Ag with maximal exercise can be predicted with an accuracy of 90%. Only 1 of the 19 patients tested was classified as a responder when in fact, the measured PAI-1 showed that they were as a non-responder and 1 patient was classified as a non-responder when the data showed them to be a responder. In both cases the % response to exercise was small ($\leq$ 20%).

Similarly, actual change in total PAI-1 (ng/ml) following a maximal oxygen uptake test can be predicted with an accuracy of 95% ($R^2 = 0.947$, $P = 0.000$), using the

equation provided by the multiple stepwise regression model which incorporates systolic blood pressure, resting total PAI-1, WHR, fat mass and resting triglyceride concentration. Using this model 3 of the 19 patients were wrongly categorised but again the change in total PAI-1 concentration with exercise was very small (≤3 ng/ml).

If this model could be validated against a large population of lean and overweight subjects it may prove to be invaluable in the prediction of a hypofibrinolytic state following strenuous exercise and the risk of MI onset following an unaccustomed exercise bout in these groups.

Overall from the data we can conclude that overweight and obese premenopausal females exhibit an increase fibrinolytic capacity following a multistage maximal oxygen uptake test, as shown by significantly increase t-PA Ag concentrations with no significant changes in PAI-1 concentration. This population however can be subdivided into two groups of those who show a decreased concentration of PAI-1, and therefore an increased fibrinolytic potential, following exercise and those in whom an increased PAI-1 concentration is observed. These groups can only be directly defined by a VO_2 max assessment but a multiple regression equation can predict both % changes and actual changes in PAI-1 concentration from resting anthropometric and blood markers with an accuracy of ≥ 90%. This formula, if validated in a larger population, may help to identify those at greatest risk from MI onset with unaccustomed exercise and may therefore encourage those patients affected to undertake regular submaximal physical activity that is known to increase fibrinolytic capacity both at rest and following exercise.

Chapter 8

The effect of exercise intensity on fibrinolytic capacity in sedentary overweight and obese healthy premenopausal women.

Chapter 8: The effect of exercise intensity on fibrinolytic capacity in sedentary overweight and obese pre menopausal women.

8.1 Introduction

The model proposed by Streiff & Bell (1994) for changes in fibrinolytic factors in lean active volunteers with exercise states that as exercise intensity reaches approximately 50% VO_2 max small increases in t-PA concentrations can be recorded. When exercise reaches 70% VO_2 max the largest increases in t-PA are recorded and active PAI-1 concentrations decrease. The primary aim of this study therefore is to determine if this model also applies in overweight and obese healthy premenopausal females performing moderate (70% VO_2 max) and low intensity (50% VO_2 max) exercise for duration equivalent to the expenditure of 300 Kcals..

Furthermore, the study in obese male volunteers (chapter 5) showed an elevation in total PAI-1 concentrations thirty minutes post exercise. This study aims to establish whether this result is reproducible in premenopausal females. If so, the extension to the post exercise period may allow an estimation of the time to recovery of PAI-1 to baseline levels.

Finally, following on from the results of the maximal exercise test (chapter 7) in premenopausal females the secondary aim of this study is to determine whether the changes in total and active PAI-1 with exercise at either 50% or 70% VO_2 max can be predicted from anthropometric and other blood markers of the insulin resistance syndrome taken at rest.

8.2 Subjects and Methods

14 overweight and obese pre menopausal females agreed to participate and 6 completed the study. Inclusion criteria were BMI $\geq$ 28 kg/m^2, waist circumference $\geq$ 88cm, participation in less than 20 minutes recreational exercise per week and having a non-manual profession.

8.2.2 Experimental Design

All subjects underwent physical examinations and biochemical and haematological screening prior to entry into the trial. Maximal oxygen uptake was determined by means of a maximal oxygen uptake test on a motorised treadmill employing a modified Bruce protocol. On a separate day, workloads corresponding to 50% and 70% VO_2 max were determined during thirty minute practice trial in which subjects were familiarised with the protocol. Measures of height (standard stadiometer), weight (Tanita^{TM} TBF-310), percentage body fat using bioelectrical impedance analysis (BodyStat^{TM} 1500), waist circumference and hip circumference were also recorded during this visit.

On trial days subjects arrived at the laboratory at 09:00h having fasted for a minimum of 8 hours. All subjects had abstained from smoking for a minimum of 8 hours, alcohol for 24 hours and strenuous exercise for 48 hours. No subject was taking prescription or 'over the counter' medication for 6 weeks prior to the commencement of the study. All visits were completed in the early follicular (day 7-11) phase of each volunteer's menstrual cycle.

On arrival at the laboratory a 21G cannula was inserted into a suitable forearm vein (after 3 minutes of venous occlusion at 100 mmHg). Subjects then rested in a seated

position for 15 minutes before a blood sample (T-30) was taken. After a further 30 minutes of rest a second blood sample (T0) was taken before exercise commenced which consisted of a three minute warm up stage (2.7 km h^{-1}, 0% gradient) and then an increase in speed and gradient to elicit either 50% or 70% VO_2 max. This study was of a random cross over design. Expired air was analysed on a breath-by-breath basis for FEO_2 and $FECO_2$ t, volume, temperature and pressure (Metamax 3B^{TM}) to allow calculation of VO_2, RER and caloric expenditure (by indirect calorimetry). Time to expend 300 Kcals was predicted by averaging the caloric expenditure over a 10 minute period, following 20 minutes of exercise at the given intensity. When the 300 kcal target was achieved subjects performed a 3 minute cool down period (2.7 km h^{-1}, 0% gradient) before a further blood sample was taken (END). Finally, subjects rested in a seated position for a further 2 hours. During this time, blood samples were taken 30 minutes (T+30), 60 minutes (T+60) and 120 minutes (T+120) post exercise.

8.2.3 Laboratory Methods

Blood samples were distributed between sealed evacuated test tubes containing no additives, sodium citrate or EDTA. The EDTA samples were analysed immediately for haemoglobin concentration (GDS systems) and haematocrit (micro centrifugation Hawksley International). Sodium citrate samples were centrifuged at 3000rpm (IEC Centra-2) for 15 minutes and the resultant plasma was pipetted into eppendorf tubes and immediately frozen at -80°C. The plasma samples were analysed by ELISA for t-PA Ag (Biopool International procedure 101005), Total PAI-1 Ag (Biopool International procedure 101005), active PAI-1 Ag (Technoclone procedure 16075) and vWF (Immunozym procedure 5450200). No additives samples were allowed to clot for 30 minutes before centrifugation at 3000rpm (IEC Centra-2) for 15 minutes and the

resultant serum was pipetted into eppendorf tubes and stored at -20°C. The serum samples were analysed for insulin by MEIA (Imx systems), leptin by ELISA (R&D systems procedure DLP00), Total cholesterol (Beckman Synchron CX systems procedure 467825), triglyceride (Beckman Synchron CX systems 445850) and HDL cholesterol (Sigma diagnostics procedure 345L). LDL concentration was determined using the Freidewald equation.

8.2.4 Calculations and Statistical Analysis

Plasma volume changes were calculated according to the method of Dill & Costhill (1974) and all data was corrected for plasma volume changes. Normality of data distribution was assessed by means of a Shapiro-Wilks test. Data that displayed a normal distribution was assessed by means of a one way analysis of variance (ANOVA) followed by Tukey's post hoc analysis. Data for these variables is expressed as Mean $\pm$ standard deviation. Skewed data is presented as Median (range) and was assessed by means of Krushkal-Wallis tests followed by multiple Mann-Whitney paired data tests. To assess the relationships between variables and fibrinolytic factors Spearman's rho coefficients were performed. Two tailed significance levels are reported and differences were considered significant when $P \leq 0.05$.

8.3 Results

8.3.1 Resting Variables

The anthropometric measures and resting blood variables of the 6 women are shown in table8. 1. All 6 women were Caucasian in origin. 5 of the women were classed as obese (BMI $\geq$ 30 kg/m^2) and 1 was classed as overweight (BMI 27-29 kg/m^2). All 6 women also had a waist circumference greater than 88cm that is predictive of the metabolic abnormalities associated with the IRS (Despres & Lamarche 1993)

Table 8.1: The physical characteristics and fibrinolytic variables of the 6 women. Resting concentrations of blood variables are averaged over the two trial days. Normally distributed data is shown as mean $\pm$ S.D and skewed data is shown as median (range).

Marker	Mean $\pm$ S.D	Median (Range)
Body Mass (kg)	82.07 $\pm$ 8.12	
BMI (kg/m^2)	30.33 $\pm$ 1.29	
Body fat (%)	40.10 $\pm$ 4.44	
Fat Mass (kg)	32.94 $\pm$ 5.95	
Waist to hip Ratio		0.79 (0.74 to 0.96)
Waist circumference (cm)		88 (88 to 96)
VO_2 max (mls/kg/min)	18.89 $\pm$ 5.93	
t-PA (ng/ml)	5.40 $\pm$ 1.21	
Total PAI-1 (ng/ml)		8.70 (3.40 to 18.50)
Active PAI-1 (U/ml)		3.50 (0.70 to 11.50)

Resting concentrations of other measured blood markers of the insulin resistance syndrome are shown in table 8.2. There were no significant differences in any of the variables measured at rest between the two trials.

Table 8.2: Concentrations of the other measured blood markers of the Insulin Resistance syndrome at rest. N=6. Normally distributed data is presented as mean ± S.D. and skewed data is presented as median (range). Data is taken from resting values of both the 50% and 70% trials

Marker	Mean (S.D)	Median (range)
Total Cholesterol (mmol/L)		4.29 (4.00 to 5.57)
Triglyceride (mmol/L)	0.81 ± 0.30	
HDL Cholesterol (mmol/L)	1.20 ± 0.42	
LDL Cholesterol (mmol/L)	3.01 ± 0.70	
Insulin (pmol/L)	6.28 ± 3.28	
Leptin ng/ml)		58.68 (31.10 to 86.36)
VWF (IU/ml)		0.69 (0.24 to 1.77)

8.3.2 T-PA with Exercise

T-PA (figure 8.1) increased significantly by 101% following exercise at 70% VO_2 max (P=0.006) returning to baseline levels 30 minutes post exercise (P=0.047). T-PA concentrations remained unchanged in the following 90 minutes (T+30 to T+120) (P=1.000). No significant alterations in t-PA concentration were observed throughout the trial with exercise at an intensity of 50% VO_2 max (P= 0.911, 1.000, 0.998, 0.969)

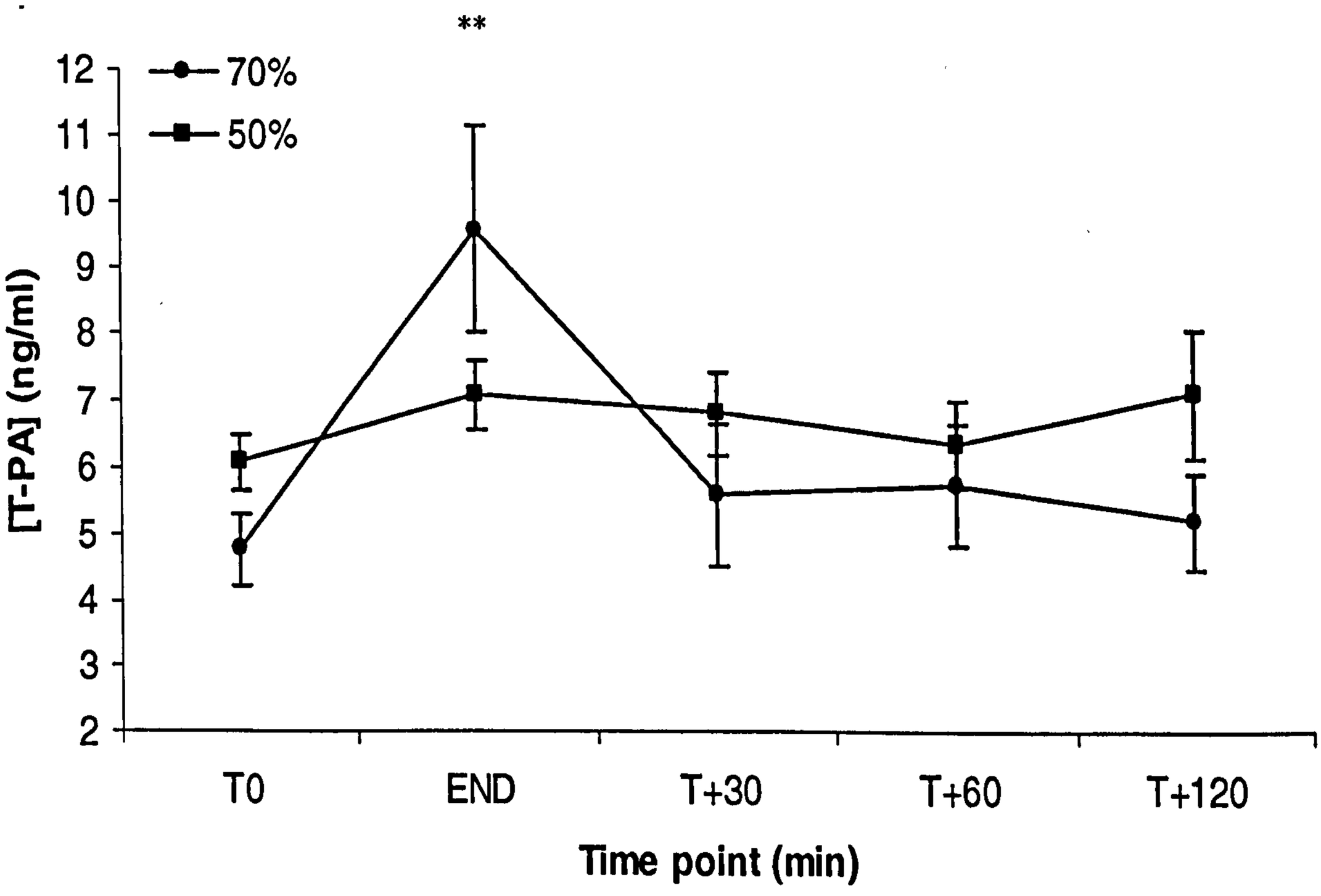

Figure 8.1: The effect of exercise at 50% and 70% VO_2 max on plasma t-PA concentrations. Data is shown as mean ± S.E.M. * = significant different from previous time point $P \leq 0.05$ ** = significantly different from previous time point $P \leq 0.01$. N=6 in each group.

There were no significant differences in t-PA concentration between the two groups at any point during the trial. Also no significant differences between resting t-PA concentrations and those observed post exercise were observed in either group.

8.3.3 Total PAI-1 Ag with Exercise

Total PAI-1 Ag (table 8.3) did not significantly change with exercise at an intensity of either 70% (P = 0.637) or 50% (P=0.307) VO_2 max. Thirty minutes post exercise (T+30) however, total PAI-1 Ag increased significantly by 161% (P=0.03) in the 70% group and remained unchanged during the following 90 minutes (T+30 to T+60 P= 0.637, T+60 to T+120 P=0.875).

In the 50% group there was a trend towards increased total PAI-1 concentrations at 30 minutes post exercise but this did not reach significance (P=0.099). No significant variations in total PAI-1 Ag were observed at any of the 5 time points with low intensity exercise at 50% VO_2 max.

Table 8.3: The effect of 70% and 50% VO_2 max intensity exercise on total plasma PAI-1 concentrations. Data is shown as median and range. **= Significantly different from previous time point $P \leq 0.01$.

	70% VO_2 max Trial		50% VO_2 max Trial	
Time Point (min)	Median [total PAI-1] (ng/ml)	Range	Median [total PAI-1] (ng/ml)	Range
T0	10.40	5.10 – 14.70	7.05	3.40 –18.50
END	9.93	2.78 - 18.26	7.11	3.23 – 9.10
T+30	12.88**	7.48 – 72.68	9.06	3.07 – 30.82
T+60	12.42	3.00 – 56.10	10.44	2.26 – 14.14
T+120	12.87	3.38 – 76.22	11.19	5.88 – 37.94

Despite the significant increases in total PAI-1 concentrations 30 minutes post exercise there were no significant differences between the two groups at any time point throughout the trials. There was however a non-significant trend towards increased total PAI-1 concentrations with higher intensity exercise at the end of exercise (P=0.099) and 60 minutes post exercise (P=0.099) compared with exercise at 50% VO_2 max.

Interestingly, with exercise at 70% VO_2 max, 3 women were classed as responders (total PAI-1 Ag decreased with exercise) and 3 women were classed as non responders (total PAI-1 Ag increased with exercise). With exercise at 50% VO_2 max 4 women were classed as non responders and were classed as responders

8.3.4 Predicting change in PAI-1 with Exercise

Using a stepwise multiple regression model percentage change in total PAI-1 Ag with exercise at 70% VO_2 max could not be predicted from the variables measured at rest. With exercise at 50% VO_2 max however (figure 8.2), only resting active PAI-1 Ag entered the model (coefficient = -15.348, constant = 30.071) the model accounts for 84% of the variability in total PAI-1 concentration with exercise (R^2 = 0.843 P =0.002). Actual (ng/ml) changes in PAI-1 Ag (figure 8.2) with exercise at 70% VO_2 max could be accounted for with 99.8 % accuracy (constant 76.101 R^2 = 0.998 P=0.002) from BMI (coefficient = -1.853), resting vWF (coefficient = -8.087) and resting t-PA (coefficient = -2.573).

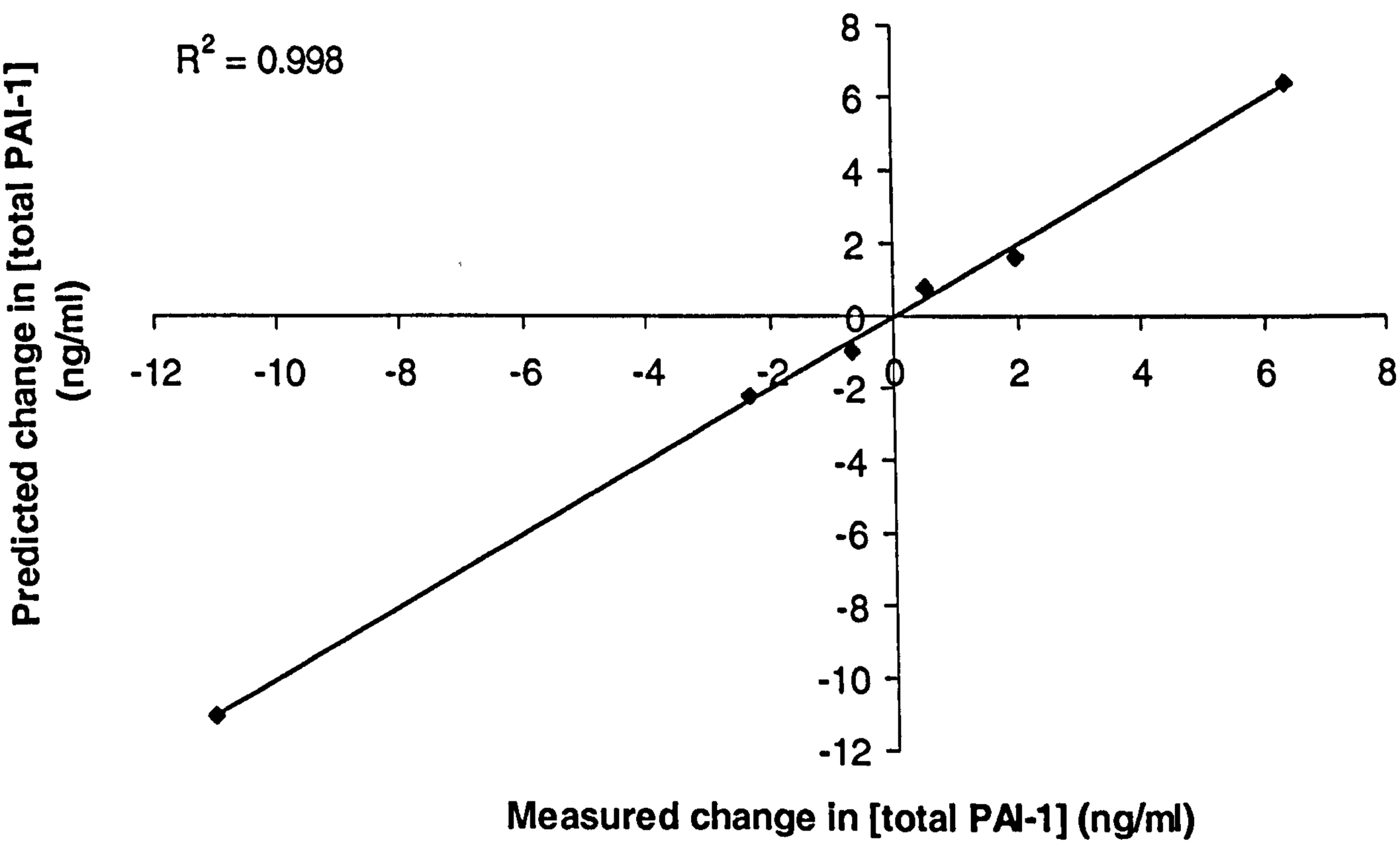

Figure 8.2: The correlation between observed changes in [total PAI-1 Ag] with exercise at 70% VO_2 max and change in [total PAI-1 Ag] as predicted by the multiple regression equation.

With exercise at 50% VO_2 max, the first factor to enter the model (figure 8.3) to predict actual change in total PAI-1 concentration was resting total PAI-1 concentration (coefficient = -0.654) followed by resting total cholesterol (coefficient = -1.597). This model accounted for 99.6% of the variability in total PAI-1 concentration with an acute bout of exercise at 50% VO_2 max (constant = 10.882, $R^2 = 0.996$, P = 0.000).

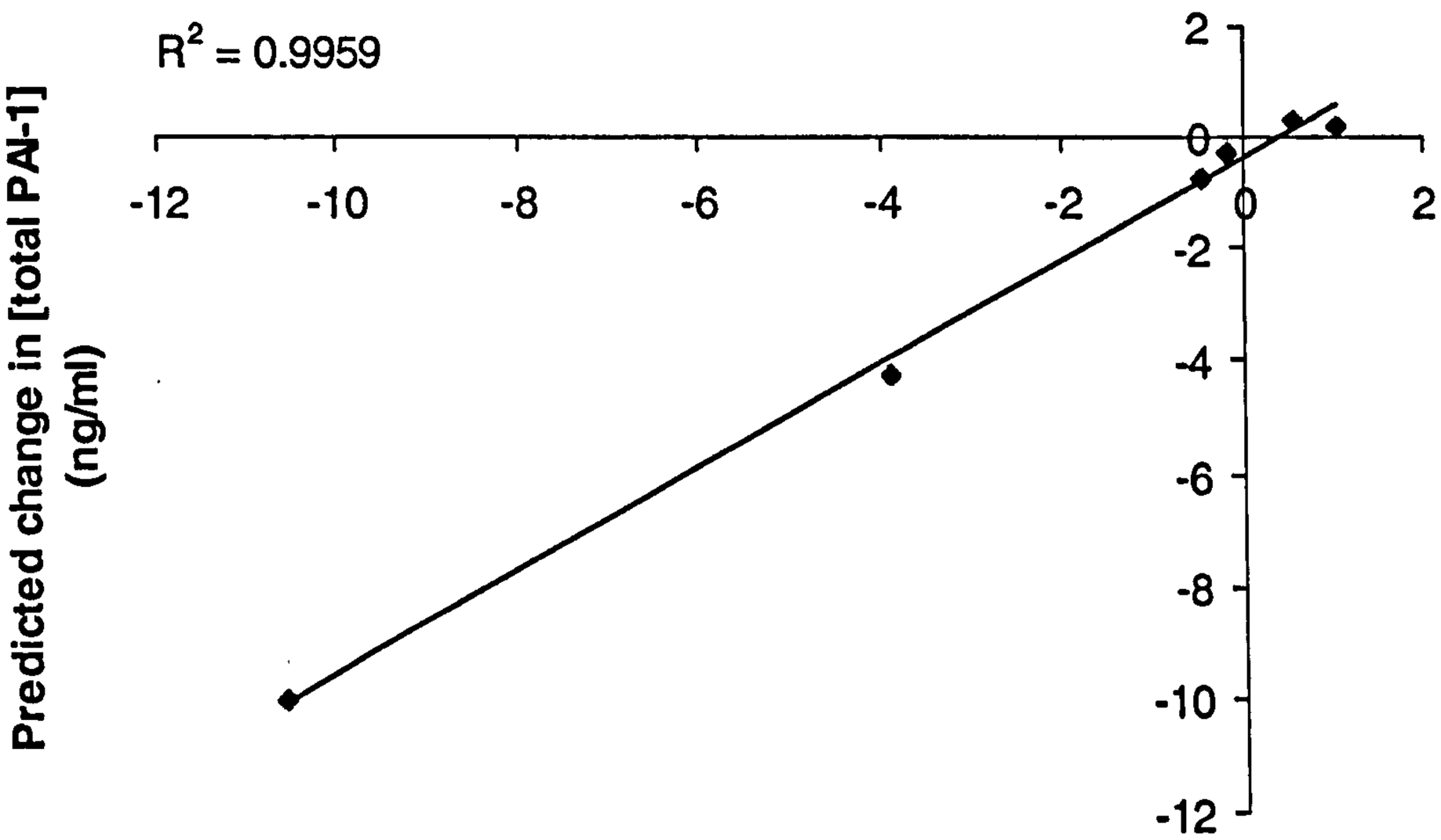

Figure 8.3: Correlation between observed changes in [active PAI-1] with exercise at 50% VO_2 max and the changes predicted by the multiple regression model.

8.3.5 Active PAI-1 Ag

Active PAI-1 concentrations significantly fell from (median) 3.55 ng/ml to 2.20 ng/ml as result of exercise at 70% VO_2 max. During the recovery period following the moderate intensity exercise bout, active PAI-1 remained significantly decreased with respect to pre exercise levels (T+30 Vs PRE P=0.002, T+60 Vs PRE P=0.023, T+120 Vs PRE P=0.012). No significant changes in active PAI-1 concentrations were observed with exercise at 50% VO_2 max or in the post exercise period.

Table 8.4: The effect of 70% and 50% VO_2 max intensity exercise on active plasma PAI-1 concentrations. Data is shown as median and range. $^{++}$= Significantly different from rest time point $P \leq 0.01$. $^{+}$ = Significantly different from rest $P \leq 0.05$. * = Significantly different from 70% trial $P \leq 0.05$.

	70% VO_2 max Trial		50% VO_2 max Trial	
Time Point (min)	Median [active PAI-1] (U/ml)	Range	Median [active PAI-1] (U/ml)	Range
T0	3.55	1.60 to 11.50	3.50	0.70 to 6.6
END	2.20^{+}	0.92 to 6.27	2.84*	0.74 to 3.03
T+30	2.68^{++}	0.97 to 3.96	2.48	0.58 to 5.66
T+60	2.65^{+}	1.09 to 4.61	2.63	2.42 to 3.13
T+120	2.76^{++}	0.47 to 3.65	2.92	1.23 to 5.51

As a result of exercise at 70% VO_2 max, active PAI-1 concentrations were significantly lower compared to after exercise at 50% VO_2 max (END 70% VO_2 max 2.20 (0.92 to 6.27), END 50% VO_2 max 2.84 (0.74 to 3.03) P = 0.043). There were no significant differences between groups at any other time point in the trial.

To attempt to predict percentage and actual (U/ml) changes in active PAI-1 concentration from resting variables, a stepwise multiple regression model was again used. Actual change in PAI-1 concentration with exercise at 70% VO_2 max could be predicted with an accuracy of 74%. Only resting active PAI-1 concentrations entered the model (constant = 0.479, coefficient = -5.34 P = 0.027).

With exercise at 50% VO_2 max (figure 8.4) resting triglyceride concentration was the first factor to enter the model (coefficient = 6.551) followed by VO_2 max (coefficient = 0.154) allowing prediction of active PAI-1 concentration with 96.7% accuracy.

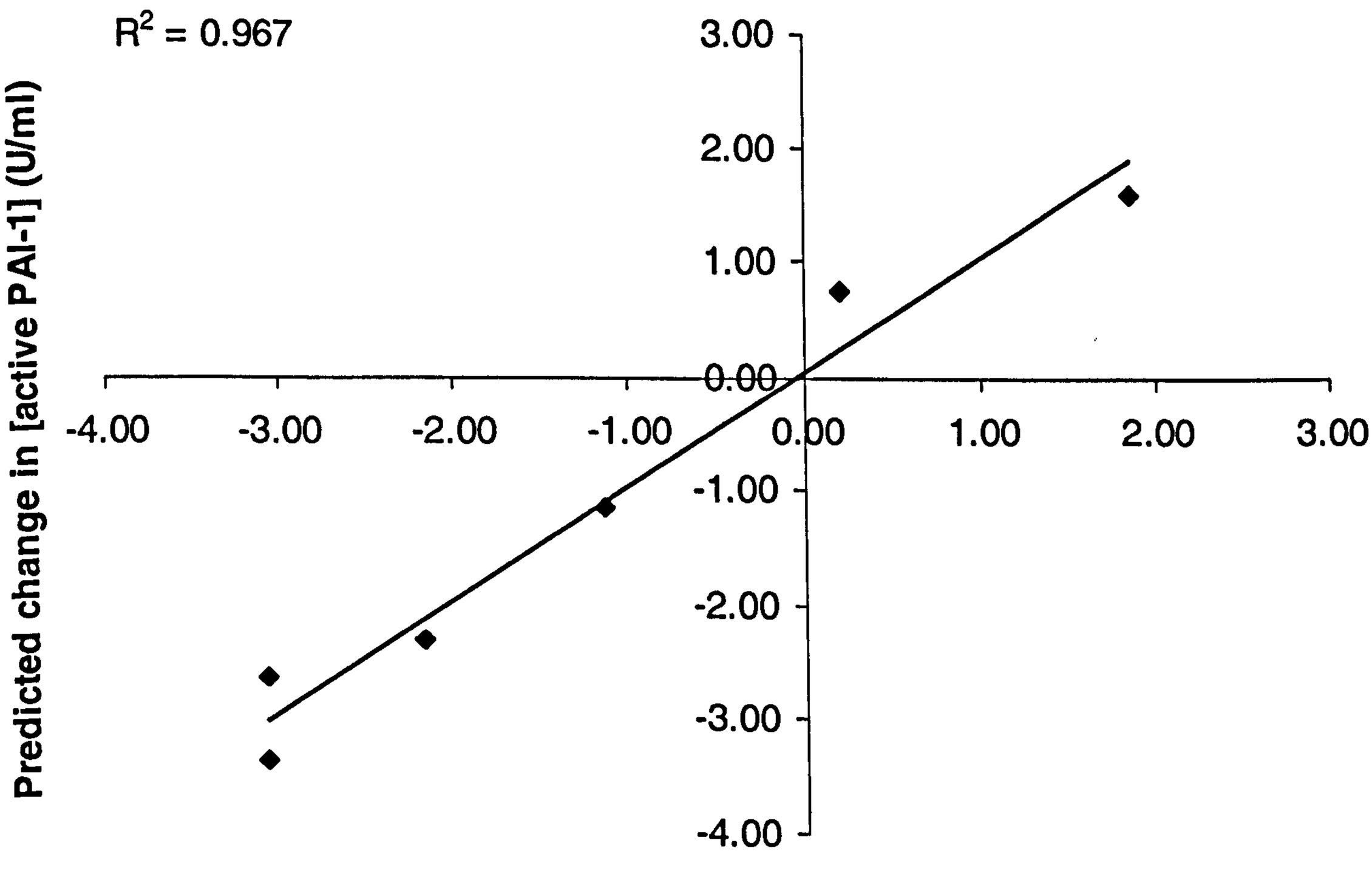

Figure 8.4: Correlation between observed changes in [active PAI-1] with exercise at 50% VO_2 max and changes in [active PAI-1] predicted by the multiple stepwise regression model.

8.4 Discussion

The primary aim of this research study was to determine the effect of exercise intensity on plasma fibrinolytic capacity in overweight and obese pre menopausal females. The secondary aim was to determine if the change in PAI-1 concentrations could be predicted from anthropometric measures and resting blood markers of the IRS

8.4.2 Summary of the Protocol

6 overweight and obese premenopausal women undertook two acute exercise bouts of different intensities on separate days. The moderate intensity exercise bout was performed at 70% VO_2 max and the low intensity exercise bout was performed at 50% VO_2 max. Each exercise bout lasted for a duration equivalent to approximately 300 kcal energy expenditure. Blood samples were taken pre exercise (T0), immediately following the exercise bout (END), 30 minutes post exercise (T+30), 60 minutes post exercise (T+60) and 120 minutes post exercise (T+120). Samples were analysed for concentrations of total PAI-1, active PAI-1, t-PA, insulin, leptin, total cholesterol, triglyceride, HDL cholesterol, LDL cholesterol and vWF.

8.4.3 Resting Data

At rest all t-PA concentrations (Mean $\pm$ S.D., 5.40 $\pm$ 1.21 ng/ml, normal range = 3-10 ng/ml) and total PAI-1 concentrations (Median (range) 8.70 (3.40 to 11.50) ng/ml, normal range = 4 to 43 ng/ml) were within the normal healthy range. A single subject however presented with elevated active PAI-1 concentrations at rest that may be indicative of a reduced fibrinolytic capacity (11.50 U/ml, normal range = 1-7 U/ml). All others presented with active PAI-1 concentrations within the normal healthy range (Median (range) 3.50

(0.7-11.50) despite a waist circumference ≥ 88cm which is indicative of the metabolic disturbances associated with the IRS in premenopausal women (Han et al 1995). The lack of metabolic disturbances in this group despite a waist circumference ≥ 88 cm further underlies the cardioprotective role of oestrogen (Huber 2001a).

8.4.4 Exercise Data

Exercise at 70% VO_2 max significantly increased t-PA concentrations by 101% before returning to base line levels 30 minutes post exercise. This is consistent with numerous research studies at similar exercise intensities in sedentary and active populations (98 % Van den Burg et al 1994, 113 % Szymanski & Pate 1994). The rise in t-PA with sustained submaximal exercise is postulated to occur as a result of a reduction in blood flow to the liver that in turn, may result in reduced hepatic t-PA clearance (Chandler et al 1995). Post exercise, hepatic blood flow is restored and t-PA rapidly decreases to baseline levels (Booth et al 1987). Exercise at 50% VO_2 max did not significantly affect plasma t-PA concentrations and may therefore reflect a less significant alteration in hepatic blood flow. Alternatively, as serum catecholamine concentration is the main determinant of levels of t-PA levels, affecting both t-PA secretion and clearance (Chandler et al 1997). Low intensity exercise (50% VO_2 max) may not have produced sufficiently large alterations in plasma catecholamines to significantly increase plasma t-PA concentrations.

8.4.5 PAI-1 with Exercise

a) Exercise at 70% VO_2 max

Total PAI-1 concentration did not significantly alter with exercise at either 50% or 70% VO_2 max. This is consistent with a number of studies (Fernhall et al 2000, Womack et al 2001, Kawano et al 2000) as well as the studies shown in chapters 5 and 7. There was however a significant 38% reduction in active PAI-1 concentrations with exercise in the 70% VO_2 max group. These results are similar to Womack et al (2001) who reported a 40% reduction in active PAI-1concentrations with no significant change in total PAI-1 following exercise at 65% VO_2 max for 30 minutes in patients with peripheral artery disease. This group and others (DeSouza et a 1997) suggested that the change in active PAI-1 without a significant change in total PAI-1 represented an increase in t-PA/PAI-1 complexes. The assay used in this study to measure total PAI-1 however is specific for active and latent PAI-1 only. A reduction in active PAI-1 therefore with no change in total PAI-1 concentrations represents a concomitant increase in latent PAI-1 concentrations with higher intensity (70% VO2 max) exercise.

Although PAI-1 is synthesised in the active form, it 'spontaneously' converts to the latent form. The largest reservoir of latent PAI-1 is within the alpha granules of platelets (Huber 2001b). The latent PAI-1 is converted to the active conformation upon platelet activation but the exact mechanism by which this occurs is unknown (Huber 2001a). Once secreted from the alpha granule, if available, the active PAI-1 is stabilised by vitronectin to increase its biological half-life (van Meijer & Pannekoek 1995) (figure 8.5).

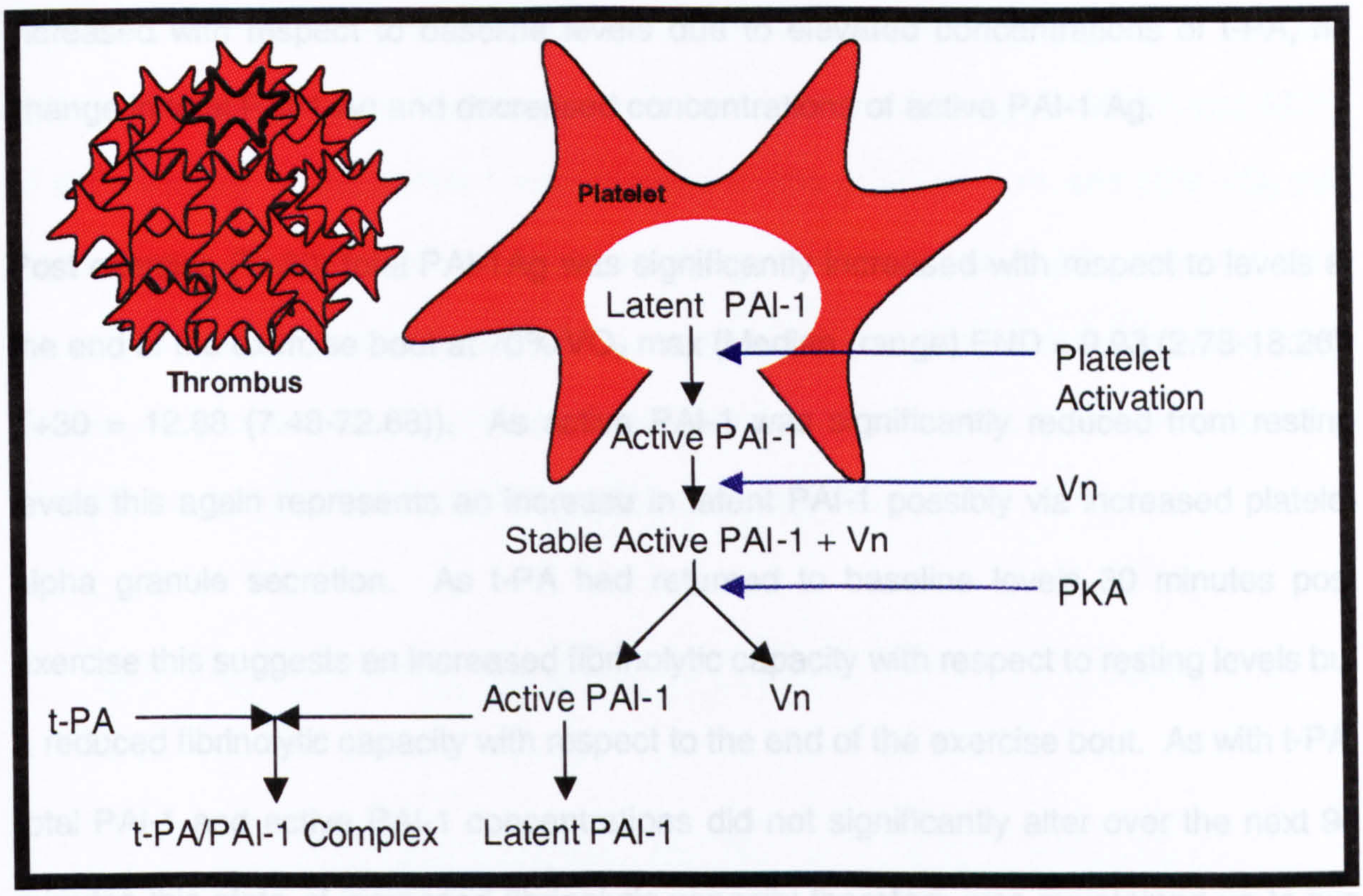

Figure 8.5: A diagrammatic representation of the conversion of latent PAI-1 to active PAI-1 and back to latent PAI-1. Vn = vitronectin, PKA = phosphokinase A

Morgenstern et al (2001) however have reported that phosphokinase A (PKA) is translocated onto the fibrin network of the thrombus from the surface of platelets along with PAI-1 and vitronectin. Phosphorylation of vitronectin by PKA reduces its grip on PAI-1 unleashing it in its free form. The free PAI-1 can then assume its latent (non-inhibitory) conformation allowing more t-PA to remain in the free form to trigger the formation of active plasmin and initiate fibrinolysis (Morgenstern et al 2001).

The decrease in active PAI-1Ag therefore is most likely to be due to increased numbers of t-PA/PAI-1 complexes formed as a result of elevated t-PA concentrations whereas the increase in latent PAI-1 may be due to increased alpha granule release of PAI-1 from activated platelets. A measure of t-PA activity would be useful to clarify this further.

Overall therefore fibrinolytic activity at the end of the exercise bout at 70% VO_2 max is increased with respect to baseline levels due to elevated concentrations of t-PA, no change in total PAI-1 Ag and decreased concentrations of active PAI-1 Ag.

Post exercise (T+30) total PAI-1Ag was significantly increased with respect to levels at the end of the exercise bout at 70% VO_2 max (Median (range) END = 9.93 (2.78-18.26), T+30 = 12.88 (7.48-72.68)). As active PAI-1 was significantly reduced from resting levels this again represents an increase in latent PAI-1 possibly via increased platelet alpha granule secretion. As t-PA had returned to baseline levels 30 minutes post exercise this suggests an increased fibrinolytic capacity with respect to resting levels but a reduced fibrinolytic capacity with respect to the end of the exercise bout. As with t-PA, total PAI-1 and active PAI-1 concentrations did not significantly alter over the next 90 minutes this state of increased fibrinolytic capacity therefore was maintained up to two hours post exercise.

b) Exercise at 50% VO_2 max

Exercise at 50% VO_2 max did not elicit any significant changes in t-PA, total PAI-1 or active PAI-1 either at the end of the exercise bout or post exercise. This is consistent with numerous studies and with the model produced by Streiff & Bell (1994) which states that as exercise approaches 50% VO_2 max the release of t-PA begins although large increases are not seen until exercise intensity reaches 70% VO_2 max. A decrease in PAI-1 is not seen until exercise intensity reaches approximately 70% VO_2 max.

8.4.6 Predicting Changes in PAI-1 with Exercise

The multiple regression models allow prediction of actual change (ng/ml) in total and active PAI-1 concentration from resting variables. The models explain 99.8% and 99.6% of the variability of actual PAI-1 with exercise in this group at 70% and 50% VO_2 max respectively. These models although promising must be viewed with caution as there are only 6 subjects in each group. If they could be validated in a larger population these models will prove useful to clinicians and exercise physiologists in the prescription of exercise programmes in overweight and obese subjects.

Chapter 9

The effect of exercise training on fibrinolytic variables in sedentary, overweight and obese premenopausal volunteers

Chapter 9: The effect of exercise training on fibrinolytic variables in sedentary, overweight and obese premenopausal volunteers

9.1 Introduction

The previous two studies (chapters 8 & 9), demonstrated that acute exercise bouts, in overweight and obese premenopausal females, of moderate (70% VO_2 max) or high (graded VO_2 max test) intensity resulted in significant increases in t-PA concentrations and significant reductions in active PAI-1 concentrations. Exercise at 50% VO_2 max however did not result in significant changes in either active or total PAI-1 concentrations.

Numerous studies have shown an improvement in fibrinolytic capacity following exercise training (Traber 1999, Stratton *et al* 1994, Bowman *et al* 1996). The primary aim of this study therefore is to determine whether these results can be reproduced in overweight and obese premenopausal women. Furthermore, this study aims to determine whether training at 50% or 70% VO_2 max for a duration equivalent to an energy expenditure of 300 Kcals is the most effective in improving fibrinolytic capacity both at rest and following a graded maximal oxygen uptake test.

9.2 Subjects & Methods

19 overweight and obese, sedentary, premenopausal females were randomly assigned to one of three experimental groups, exercise training at 70% VO_2 max for a duration equivalent to the expenditure of 300 Kcals, 3 times per week, for 12 weeks, exercise training at 50% VO_2 max for a duration equivalent to the expenditure of 300 Kcals, 3 times per week, for 12 weeks or a control, no exercise group. Inclusion criteria for the study were BMI >27 kg/m^2 and a waist circumference >88cm. "Sedentary was defined as <20 minutes recreational exercise per week and having a non-manual profession.

9.2.2 Experimental Design

All subjects underwent a physical examination prior to entry into the study. Volunteers arrived at the laboratory at 9.00h (to standardise for the effects of diurnal variation) having fasted over night, abstained from smoking for 8 hours, alcohol for 24 hours and strenuous exercise for 48 hours. No subject was taking prescription or "over the counter" medication for 6 weeks prior to enrolment into the study. All experimental visits were conducted in the early follicular phase (day 7-11) of each subject's menstrual cycle.

On the experimental days, subjects underwent measures of height, weight, percent body fat (by Bioelectrical impedance (BodyStat 1500^{TM})), waist circumference, hip circumference and blood pressure. Blood pressure was determined by the mean of 3 values recorded 10 minutes apart (Omron 711) in the seated position. A 21G cannula was then inserted into a suitable forearm vein (after 3 minutes of venous occlusion at 100 mmHg). Subjects then rested in the seated position for 15 minutes before a 25 ml blood sample (PRE) was taken. Maximal oxygen uptake was determined by means of a maximal fitness test employing a modified Bruce protocol. Expired air was analysed on a breath-by-breath basis using the Metamax 3B online telemetry system for FEO_2, $FECO_2$ volume and temperature allowing calculation of VO_2 (all volumes were corrected to STPD). Heart rate was also recorded by the Metamax via a Polar^{TM} heart rate monitor and perceived exertion was recorded in the last 30 seconds of each stage using the Borg scale. Maximal oxygen uptake considered to have been achieved when subjects fulfilled 3 of the following 4 criteria.

1. A heart rate $\geq$ 220-subject's age
2. VO_2 that remained unchanged over 2 consecutive stages
3. A perceived exertion rating $\geq$ 18 over two consecutive stages
4. RER $\geq$ 1.1

Subjects then performed a 3 minute cool down period (2.7 km/h, 0% gradient) before a second 25ml blood sample (POST) was taken.

Following the initial fitness assessment subjects were randomly assigned into 1 of 3 experimental groups

1. 70% VO_2 max exercise intensity for a duration equivalent to 300kcal
2. 50% VO_2 max exercise intensity for a duration equivalent to 300 kcal
3. control-no exercise.

Both exercise groups then underwent a further exercise session on a separate day to establish the speed and gradient that elicited an intensity of either 50% or 70% VO_2 max. During this visit energy expenditure was assessed by indirect calorimetry and time taken to expend 300kcal calculated by averaging the caloric expenditure over a 10-minute period following 20 minutes of exercise at the given intensity.

9.2.3 Training Period

The two exercise groups attended a local fitness centre 3 times per week for 12 weeks. Each session was fully supervised and consisted of a three minute warm up period (2.7 km/h, 0% gradient) followed by walking exercise at a speed and gradient equivalent to 50% or 70% VO_2 max for a duration equal to an energy expenditure of 300kcal. The speed and gradient used to elicit the given intensity were reassessed every 14 days. Finally, a 3-minute cool-down period consisting of low intensity walking exercise (2.7 km/h, 0% gradient) was performed. Subjects were required to achieve a minimum of 90% compliance to remain in the study. All subjects were asked not modify any dietary practices and the control group were asked not to make

any changes in physical activity levels. This was monitored by means of a 3-day food and activity diary at 0 weeks, 6 weeks and 12 weeks.

Following the 12 weeks training session subjects returned to the laboratory to repeat the resting and exercise measures.

9.2.4 Laboratory Methods

Blood samples were distributed between sealed evacuated test tubes containing no additives, sodium citrate or EDTA. The EDTA samples were analysed immediately for haemoglobin concentration (GDS systems) and haematocrit (micro centrifugation Hawksley International). Sodium citrate samples were centrifuged at 3000rpm (IEC Centra-2) for 15 minutes and the resultant plasma was pipetted into eppendorf tubes and immediately frozen at -80°C. The plasma samples were analysed by ELISA for t-PA Ag (Biopool International procedure 101005), Total PAI-1 Ag (Biopool International procedure 101005), active PAI-1 Ag (Technoclone procedure 16075) and vWF (Immunozym procedure 5450200). No additives samples were allowed to clot for 30 minutes before centrifugation at 3000rpm (IEC Centra-2) for 15 minutes and the resultant serum was pipetted into eppendorf tubes and stored at -20°C. The serum samples were analysed for insulin by MEI (Imx systems), leptin by ELISA (R&D systems procedure DLP00), Total cholesterol (Beckman Synchron CX systems procedure 467825), triglyceride (Beckman Synchron CX systems 445850) and HDL cholesterol (Sigma diagnostics procedure 345L). LDL concentration was determined using the Friedman equation.

9.2.5 Calculations and Statistical Analysis

Plasma volume changes were calculated according to the method of Dill & Costill (1974). All data shown is corrected to the plasma volume of the PRE sample of the

initial max test. Data was assessed for normality of distribution using the Shapiro-Wilks test. Comparisons between PRE and POST samples were determined using paired T tests and comparisons between groups were analysed using one way analysis of variance (ANOVA) followed by Tukey's post hoc analysis for normally distributed data and Mann Whitney tests for skewed data. The relationships between measures were determined using Spearman's rho correlation coefficients and multiple stepwise regression models. Two tailed significance levels are reported and differences were considered significant when $P \leq 0.05$.

9.3 Results

9.3.1 Pre Training Data

The pre training resting anthropometric measures and fibrinolytic variables are shown in table 9.1. BMI was significantly lower in the 70% training group compared to the 50% training group (P=0.005). There were no significant differences in any other anthropometric or fibrinolytic variables between groups at rest.

Table 9.1: Anthropometric, blood pressure, fitness and fibrinolytic variables in the 3 research groups. Data is shown as mean ± standard deviation for normally distributed data or median (range).for skewed data ** = Significantly lower than 50% training group P <0.01.

Marker	Group 1 (70% VO_2 max) N = 7	Group 2 (50% VO_2 max) N = 7	Group 3 (No Exercise) N = 5
Body Mass (kg)	83.05 (72.70 to 92.00)	86.55 (72.70 to 100.30)	93.65 (74.00 to 110.00)
BMI (kg/m^2)	29.78 (28.38 to 33.39)**	34.47 (28.07 to 38.10)	32.39 (27.50 to 35.51)
Body Fat (%)	40.95 ± 2.17	44.53 ± 4.43	40.05 ± 6.18
Fat Mass (kg)	33.41 (28.28 to 38.36)	40.03 (26.68 to 49.84)	39.43 (22.20 to 51.92)
Waist Circumference (cm)	89.5 (88.0 to 97.0)	96.0 (88.0 to 108.0)	93.5 (89.0 to 106.0)
Waist to Hip Ratio	0.79 (0.74 to 0.95)	0.82 (0.77 to 0.96)	0.86 (0.79 to 0.94)
VO_2 max (mls/kg/min)	20.95 (12.40 to 25.90)	17.26 (10.66 to 24.17)	20.11 (11.58 to 23.51)
Systolic Pressure (mmHg)	131 (120 to 138)	132 (125 to 140)	135 (120 to 160)
Diastolic Pressure (mmHg)	86 (60 to 96)	91 (78 to 98)	84 (80 to 96)
t-PA (ng/ml)	5.7 (4.2 to 8.9)	10.3 (3.2 to 11.2)	9.1 (5.6 to 13.9)
Active PAI-1 (U/ml)	3.85 (0.50 to 9.80)	6.05 (2.50 to 14.60)	4.45 (1.51 to 12.50)
Total PAI-1 (ng/ml)	8.35 (5.10 to 13.00)	10.05 (7.00 to 29.00)	11.75 (8.70 to 17.30)

9.3.2 Post Training Resting Data

a) 70% VO_2 max Training Group

After the three month training period (table 9.2) the group exercising at 70% VO_2 max showed significant reductions in body mass (P=0.026), BMI (P=0.049), body fat percentage (P=0.000), fat mass (0.000), waist circumference (P=0.000) and waist to hip ratio (P=0.005).

Table 9.2: Changes in anthropometric, blood pressure, fitness and fibrinolytic variables after 3 month exercise training period. Normally distributed data is shown as mean ± SD, Skewed data is shown as median (range.) * = significantly different from baseline P≤0.05, **= significantly different from baseline P≤ 0.01. += Significantly different from 70% group P≤0.05, ++= significantly different from 70% group P≤0.01, $= significantly different from 50% group P ≤ 0.05, $$=significantly different from 50% group P≤0.01.

Marker	Group 1 70% VO_2 max N=7	Group 2 50% VO_2 max N=7	Group 3 No Exercise N=5
Body Mass (kg)	-2.30 (-8.60 - 1.40)*$	0.35 (-3.80 to 3.00)+	0.25 (-0.50 to 2.60)+
BMI (kg/m^2)	-0.92 (-3.09 to 0.97)*$	0.20 (-1.42 to –1.12)+	-0.04 (-0.29 to 0.80)+
Body Fat (%)	-3.60 ± 2.62**	-3.15 ± 2.22**	0.22 ± 0.92++$$
Fat Mass (kg)	-4.25 ± 3.08**	-2.68 ± 2.91**	0.47 ± 1.43++$
Waist Circumference (cm)	-8.5 ± 3.6**$	-4.50 ± 3.75**+	2.00 ± 3.16++
Waist to Hip Ratio	-0.03 (-0.10 to 0.00)**	-0.02 (-0.07 to 0.02)*	0.01 (-0.01 to 0.03)+
VO_2 max (mls/kg/min)	6.67 (2.37 to13.39)**	4.54 (1.90 to 9.14)**	0.34 (-1.19 to 3.47)+$
Systolic Pressure (mmHg)	-8 (-21 to -2)**	-7 (-9 to –2)**	-5 (-13 to 8)+
Diastolic Pressure (mmHg)	-5 (-16 to -4)**	-7 (-17 to –3)**	-1 (-7 to 8)+
t-PA (ng/ml)	6.20 (6.60 to 9.01)	7.39 (2.51 to 12.18)	3.62 (0.24 to 13.77)+
Active PAI-1 (U/ml)	-0.55 (-3.90 to 9.70)**	1.15 (-8.20 to 16.30)	4.90 (0.30 to 10.11)+
Total PAI-1 (ng/ml)	-5.60 (-10.50 to -3.10)*	-3.25 (-11.70 to 19.00)	-3.15 (-4.90 to 10.80)+

This group also showed significantly lowered systolic blood pressure (P=0.002), diastolic blood pressure (P=0.002) resting active PAI-1 concentrations (P=0.002) and resting total PAI-1 concentrations (P=0.012) as well as significantly higher VO_2 max (P=0.000) and resting t-PA concentrations (P=0.046) post training.

b) 50% VO_2 max Training Group

The group exercising at 50% VO_2 max showed significant reductions in body fat percentage (P=0.000), fat mass (P=0.000), waist to hip ratio (P=0.036), VO_2 max (P=0.002), systolic pressure (P=0.002) and diastolic pressure (P=0.002). No significant changes in the fibrinolytic profile were recorded in this group.

c) Control Group

As expected, no significant changes occurred in any of the resting anthropometric or fibrinolytic variables in the non-exercising control group.

All the measured factors were significantly improved in the 70% group when compared to controls but in the 50% group only body fat percentage (P=0.012), fat mass (P=0.017) waist circumference (P=0.003) and VO_2 max (P=0.028) were significantly improved when compared to the control group.

9.3.4 Post Training Fibrinolytic Response to Exercise

a) t-PA

As a result of the graded maximal oxygen uptake test, as with the pre training test, (table 9.3) t-PA rose significantly in all groups (group 1 P=0.002, group 2 P=0.002, group 3, P=0.028). The percentage changes in t-PA with maximal exercise were not significantly different however between pre and post training in any of the groups. As with the resting value, t-PA was significantly elevated post exercise in group 1 with respect to the pre training value (P=0.002) but was not significantly altered in either group 2 or 3.

Table 9.3: Changes in t-PA concentration with maximal exercise pre and post training. Data is shown as median (range)* = significantly different from resting value P≤0.05, ** = significantly different from resting value P≤0.01. $ = Significantly elevated with respect to pre training value P≤ 0.05, $$=significantly elevated with respect to pre training value P≤0.01.

Time Point	Group 1 70% VO_2 max N=7	Group 2 50% VO_2 max N=7	Group 3 No Exercise N=5
Pre Training			
Resting t-PA (ng/ml)	5.70 (4.20 to 8.90)	10.25 (3.20 to 11.20)	9.10 (5.60 to 13.90)
Exercise t-PA (ng/ml)	11.65 (7.10 to 13.40)**	10.85 (5.70 to 30.70)**	14.75 (2.00 to 22.60)**
Change in t-PA with exercise (%)	76.63 (42.11 to 141.51)	58.44 (-12.38 to 174.11)	41.58 (-64.29 to 198.28)
Post Training			
Resting t-PA (ng/ml)	6.50 (4.10 to 9.10)$	7.65 (3.10 to 12.80)	7.90 (5.90 to 14.00)*
Exercise t-PA (ng/ml)	11.35 (10.00 to 15.80)**$$	17.75 (5.60 to 34.20)**	10.55 (3.50 to 18.90)*
Change in t-PA with exercise (%)	110.44 (52.36 to 263.41)	95.42 (53.13 to 362.16)	43.77 (-46.97 to 72.86)

b) Total PAI-1

As a result of the post training graded maximal oxygen uptake test, as with the pre training test, total PAI-1 concentrations (table 9.4) did not significantly alter from resting values in any of the groups. Total PAI-1 was significantly lowered in group 1 both at rest and following the exercise test (P=0.012 in both cases) when compared to pre training values. There were no significant variations in either resting or exercise PAI-1 in either of the other two groups.

Table 9.4: Changes in total PAI-1 concentration with maximal exercise pre and post training. Data is shown as median (range)* = significantly different from resting value P≤0.05, ** = Significantly different from resting value P≤0.01. $ = Significantly different from pre training value P≤ 0.05, ++ = Significantly different from 70% group P≤ 0.01.

Time Point	Group 1 70% VO_2 max N=7	Group 2 50% VO_2 max N=7	Group 3 No Exercise N=5
Pre Training			
Resting total PAI-1 (ng/ml)	8.55 (5.10 to 13.00)	10.05 (7.00 to 29.00)	11.75 (8.70 to 15.20)
Exercise total PAI-1 (ng/ml)	8.95 (4.90 to 11.80)	9.70 (6.50 to 21.70)	9.50 (7.00 to 37.90)
Change in total PAI-1 with exercise (%)	19.54 (-60.80 to 63.38)	-11.19 (-60.34 to 210.00)	-13.45 (-53.95 to 203.20)
Post Training			
Resting total PAI-1 (ng/ml)	2.75 (2.00 to 8.20)$	8.15 (3.80 to 26.00)	13.65 (8.80 to 26.00)
Exercise total PAI-1 (ng/ml)	5.10 (2.70 to 7.90)$	12.85 (3.40 to 37.90)++	13.75 (6.70 to 37.90)++
Change in total PAI-1 with exercise (%)	56.39 (-43.84 to 169.23)	59.29 (-40.38 to 119.08)	-26.91 (-35.42 to 201.10)

Post training exercise total PAI-1 was significantly lower in group 1 with respect to both group 2 (P=0.002) and group 3 (P=0.046). There were no significant differences between groups at any other time point. Finally, There was no significant

difference in percentage change in total PAI-1 with maximal exercise in any of the groups.

c) Active PAI-1

Finally, active PAI-1 (table 9.5) was significantly decreased as a result of the post training maximal oxygen uptake test in group1 (P=0.02), unlike the pre training test in all groups and the post training test in groups 2 and 3. Active PAI-1 concentrations were also significantly lower both at rest (P=0.002) and after exercise (P=0.002) compared to pre training levels in group 1. There were no significant changes in resting or exercise active PAI-1 concentrations in group 2 and at rest in group 3. Following the exercise bout active PAI-1 was significantly elevated in group 3 compared to pre training levels.

Table 9.5: Changes in active PAI-1 concentration with maximal exercise pre and post training. Data is shown as median (range)* = Significantly different from resting value P≤0.05, **= significantly different from resting value P ≤0.01. $ = Significantly different from pre training value P≤ 0.05 + = Significantly different from group1 P≤ 0.05.

Time Point	Group 1 70% VO_2 max N=7	Group 2 50% VO_2 max N=7	Control No exercise N=5
Pre Training			
Rest active PAI-1 (U/ml)	3.85 (0.50 to 9.80)	6.05 (2.50 to 14.60)	4.45 (1.51 to 12.50)
Exercise active PAI-1 (U/ml)	4.15 (0.50 to 15.40)	5.45 (2.13 to 7.70)	5.85 (0.92 to 17.00)
Change in active PAI-1 with exercise (%)	20.88 (-68.00 to 192.00)	28.10 (-58.24 to 10.00)	13.88 (-39.25 to 100.00)
Post Training			
Resting active PAI-1 (U/ml)	2.60 (1.10 to 17.50)$	7.95 (1.00 to 21.40)	11.20 (3.80 to 17.30)+
Exercise active PAI-1 (U/ml)	1.00 (0.50 to 13.80)**$	5.25 (0.50 to 13.50) +	10.10 (1.30 to 14.80)*+
Change in active PAI-1 with exercise (%)	-70.46 (-86.36 to 0.00)	-31.48 (-73.68 to 150.00)	-26.23 (-27.27 to 197.37)

Active PAI-1 concentrations were significantly lower at rest in group 1 when compared to group 3 (P=0.028) and exercise active PAI-1 concentrations were significantly lower in group 1 when compared to groups 2 (P=0.026) and 3 (P=0.028). As with t-PA and total PAI-1, there was no significant difference in the percentage change in active PAI-1 concentrations with maximal exercise before and after the training period.

To determine the possible effect of other markers of the Insulin Resistance syndrome on the fibrinolytic variables multiple Spearman's rho correlations were performed.

9.3.5 Correlations with Resting Total PAI-1

Total PAI-1 concentrations (table 9.6) were significantly correlated with resting systolic blood pressure pre and post training both at rest (pre training R=0.481, P=0.001, post training R=0.428, P=0.018) and after the graded maximal oxygen uptake test (pre training R=0.398 P=0.029, post training R=0.428 P=0.018). Similarly, HDL cholesterol was significantly negatively correlated at all measured time points (pre training rest, R= -0.375 P=0.051, exercise (R=-0.402 P=0.028, post training R=-0.374 P=0.042 exercise R=-0.471 P=0.009).

Table 9.6: Correlations between total PAI-1concentrations at rest and post exercise, pre and post training. Significant correlations are shown in red. * = Significant correlation $P \leq 0.05$, ** = significant correlation $P \leq 0.01$.

Marker	Pre Training Resting total PAI-1 (ng/ml)		Post Training Resting total PAI-1 (ng/ml)		Pre Training Exercise total PAI-1 (ng/ml)		Post Training Exercise total PAI-1 (ng/ml)	
	R	P	R	P	R	P	R	P
BMI (kg/m^2)	0.249	0.185	0.465**	0.010	0.162	0.392	0.465**	0.010
Body Fat (%)	0.123	0.516	0.232	0.217	0.395*	0.031	0.232	0.217
Fat Mass (Kg)	0.168	0.374	0.414*	0.023	0.325	0.079	0.414*	0.023
Waist Circumference (cm)	0.115	0.546	0.375*	0.041	0.204	0.280	0.375*	0.041
Waist to Hip Ratio	-0.346	0.061	0.219	0.244	0.457*	0.011	0.219	0.244
VO_2 max (mls/kg/min)	-0.661**	0.000	-0.247	0.188	0.090	0.636	-0.247	0.188
Systolic Pressure (mmHg)	0.481**	0.001	0.428*	0.018	0.398*	0.029	0.428*	0.018
Diastolic Pressure (mmHg)	0.101	0.594	0.219	0.245	0.437*	0.016	0.219	0.245
Body Mass (kg)	0.152	0.424	0.376*	0.041	0.361*	0.050	0.376*	0.041
Insulin (pmol/L)	-0.118	0.585	0.682**	0.000	0.168	0.376	0.645**	0.000
Total Cholesterol (mmol/L)	0.197	0.297	0.303	0.103	0.155	0.413	0.177	0.350
Triglycerides (mmol/L)	0.163	0.390	0.296	0.112	0.078	0.683	0.432*	0.017
HDL Cholesterol (mmol/L)	-0.372*	0.051	-0.374*	0.042	-0.402*	0.028	-0.471**	0.009
LDL Cholesterol (mmol/L)	0.192	0.310	0.405*	0.026	0.301	0.105	0.149	0.430
Leptin (pmol/L)	0.132	0.488	-0.007	0.970	-0.138	0.466	-0063	0.741
vWF (U/ml)	-0.125	0.512	-0.128	0.499	0.614**	0.000	-0.099	0.604
t-PA (ng/ml)	0.596**	0.001	0.466**	0.009	-0.097	0.609	0.167	0.377
Active PAI-1 (U/ml)	-0.212	0.261	0.584**	0.001	0.207	0.273	0.491**	0.006

Resting total PAI-1 concentrations were significantly correlated to resting t-PA concentrations (pre training R=0.596 P=0.001, post training R=0.466 P=0.009) and exercising total PAI-1 concentrations were significantly correlated to body mass (pre training R=0.361 P=0.050, post training R=0.376 P=0.041).

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9.3.6 Correlations with Active PAI-1

Finally, as with total PAI-1, active PAI-1 concentrations (table 9.7) were significantly correlated with resting systolic blood pressure both pre and post training at rest and after the acute bout of maximal exercise (pre training rest R=0.467 P=0.009, exercise R=0.511 P=0.044, post training rest R=0.512, P=0.004, exercise R=0.481 P=0.007).

Table 9.7: Correlations between active PAI-1 concentrations at rest and post exercise, pre and post training. Significant correlations are shown in red * = Significant correlation $P \leq 0.05$, ** = significant correlation $P \leq 0.01$.

Marker	Pre Training Resting active PAI-1 (U/ml)		Post Training Resting active PAI-1 (U/ml)		Pre Training Exercise active PAI-1 (U/ml)		Post Training Exercise active PAI-1 (U/ml)	
	R	P	R	P	R	P	R	P
BMI (kg/m^2)	0.018	0.924	-0.026	0.891	-0.027	0.885	-0.030	0.873
Body Fat (%)	0.087	0.649	0.031	0.869	-0.035	0.854	0.101	0.596
Fat Mass (Kg)	0.019	0.921	0.083	0.663	-0.090	0.636	0.063	0.743
Waist Circumference (cm)	-0.208	0.270	0.186	0.324	-0.174	0.358	0.180	0.341
Waist to Hip Ratio	-0.066	0.270	0.542**	0.002	-0.209	0.267	0.421*	0.021
VO_2 max (mls/kg/min)	0.076	0.729	-0.108	0.571	0.324	0.080	-0.190	0.315
Systolic Pressure (mmHg)	0.467**	0.009	0.512**	0.004	0.511*	0.044	0.481**	0.007
Diastolic Pressure (mmHg)	0.189	0.690	-0.242	0.197	0.327	0.078	-0.118	0.535
Body Mass (kg)	-0.032	0.317	0.070	0.714	-0.194	0.303	-0.048	0.800
Insulin (pmol/L)	0.045	0.812	0.437*	0.016	0.161	0.397	0.302	0.105
Total Cholesterol (mmol/L)	0.034	0.860	-0.147	0.439	-0.121	0.525	-0.080	0.674
Triglycerides (mmol/L)	-0.123	0.517	-0.059	0.756	-0.153	0.419	0.037	0.846
HDL Cholesterol (mmol/L)	-0.176	0.351	-0.364*	0.048	0.166	0.381	-0.312	0.094
LDL Cholesterol (mmol/L)	0.125	0.511	-0.060	0.754	-0.094	0.621	-0.088	0.645
Leptin (pmol/L)	0.361*	0.050	0.093	0.627	0.018	0.925	-0.087	0.647
vWF (U/ml)	0.008	0.967	-0.252	0.178	-0.190	0.314	0.191	0.311
t-PA (ng/ml)	0.302	0.104	0.514**	0.004	0.189	0.316	0.175	0.355
Active PAI-1 (U/ml)	-0.212	0.261	0.584**	0.001	-0.170	0.369	0.491**	0.006

No other factors were significantly correlated with either resting or post exercise active PAI-1 concentrations both pre and post training.

9.4 Discussion

9.4.1 Summary of the study

19 sedentary, overweight and obese premenopausal women underwent a maximal oxygen uptake test before being randomly assigned to one of three groups. Group 1 exercised at 70% VO_2 max for a duration equivalent to 300 kcal energy expenditure three times per week for 12 weeks, group 2 exercised at an intensity of 50% VO_2 max for a duration equivalent to 300kcals energy expenditure three times per week for 12 weeks and group 3 had no exercise intervention for 12 weeks. Measurements were recorded at rest and after a graded treadmill maximal exercise test before and after the exercise intervention.

9.4.2 Pre Training Data

Pre training, there were no significant differences in anthropometric measures, fitness levels, fibrinolytic variables or other markers of the Insulin Resistance syndrome between the three training groups with the exception of BMI which was significantly lower in group 1 compared to group 2. This difference was not considered meaningful as BMI is a crude predictor of the metabolic abnormalities associated with the IRS and there were no significant differences in body fat percentage, fat mass, waist circumference or waist to hip ratio which are factors that can influence fibrinolytic variables either directly or via other markers of the IRS.

9.4.3 The Effect of Exercise Training on Fibrinolysis

a) Resting t-PA

Following the three month exercise training programme, significant increases in resting t-PA were observed in the group trained at 70% VO_2 max compared to both pre training levels and control group values. There were no significant increases in t-PA in the group trained at 50% VO_2 max compared to either pre training levels or controls. Fibrinolytic activity in the blood is regulated by the release of t-PA from the endothelial cells, the rapid inhibition of t-PA by PAI-1 and the rate of clearance of t-PA by the liver (Stratton *et al* 1991). In most individuals approximately 90% of circulating t-PA is in complex with PAI-1 (Chandler *et al* 1997). Following endurance exercise training however, Stratton *et al* (1991) demonstrated that the fraction of t-PA circulating in the free form is increased.

The increase in t-PA concentration recorded in this study following 70% VO_2 max exercise training did not occur with a concomitant significant increase in vWF. The lack of a significant change in vWF, (which is concurrently secreted with t-PA by the endothelial cells) suggests that the elevated t-PA concentrations post training are not due to an increase in secretion. An alteration in the metabolism of t-PA with exercise training therefore, via a reduced hepatic clearance of free t-PA or t-PA/PAI-1 complexes may be the source of the elevated t-PA concentrations. More work, including the measurement of t-PA activity needs to be conducted on the kinetics of t-PA clearance to elucidate the mechanism responsible for elevated t-PA at rest following exercise training.

The majority of studies, both longitudinal (DePaz *et al* 1992) and cross sectional (Szymanski & Pate 1994, Kvernmo & Osterund 1997) have shown either unchanged (DePaz *et al* 1992)or significantly reduced t-PA Ag at rest (Szymanski & Pate 1994, Stratton *et al* 1994) in regularly physically active subjects. These studies have

however, reported significant increases in t-PA activity and therefore a greater proportion of t-PA in the active form (Stratton *et al* 1991). A measure of t-PA activity in this study would confirm whether this is the case in overweight and obese premenopausal women. The studies that have reported a reduced plasma t-PA Ag with exercise training have employed protocols that included either high intensity exercise (80-85% VO_2 max, Stratton *et al* 1991) or long training periods (athletes Vs controls, Szymanski & Pate 1994, >6 months Stratton *et al* 1991). It may be that exercise training results in an initial increase in t-PA Ag followed by a long term reduction when active PAI-1 decreases. Another possibility for this rise in t-PA Ag is the effect of the process' of venous occlusion and cannulation. There may be a greater t-PA response to venous occlusion and cannulation post training and although there was 15 minutes between cannulation and withdrawal of the first sample both pre and post training t-PA Ag may still have been elevated.

b) T-PA Following a Maximal Exercise Test

As with the pre training results, t-PA Ag increased significantly with the graded maximal oxygen uptake test in all three groups but only the group training at 70% VO_2 max displayed increased t-PA concentrations post exercise compared to pre training values. There have been several reports of elevated t-PA Ag and activity post exercise following exercise training $\geq$ 70% VO_2 max (Szymanski & Pate 1994, Speiser *et al* 1988). As with the resting values this rise in t-PA was not accompanied by a similar rise in vWF indicating that rise in t-PA is not solely due to elevated endothelial store release. This again suggests that a modification in t-PA clearance occurs, improving the fibrinolytic profile both at rest and during maximal exercise. Another possibility is that increased physical fitness allows subjects to increase the time spent at intensities greater than 50% VO_2 max during the graded oxygen uptake test and therefore increase the release of t-PA.

The lack of change in resting and post exercise t-PA concentrations in the group trained at 50% VO_2 max compared to controls and pre training values suggests that there is a training intensity threshold for these alterations to occur. Although the intensity threshold for t-PA release is 50% VO_2 max, the greatest increases in t-PA concentration occur with exercise intensities greater than 70% VO_2 max (Streiff & Bell 1994) suggesting that regular sustained large increases in t-PA concentration as a result of increased intensity exercise training may be required for training adaptations to occur. More work needs to be conducted on the effect of exercise training intensity on fibrinolytic variables, in an attempt to elucidate the mechanism by which elevations in t-PA occur.

c) Resting PAI-1

Both total PAI-1 and active PAI-1 were significantly reduced at rest in the 70% group (group 1) compared to controls and pre training levels. As with t-PA, neither total nor active PAI-1 was significantly changed in the 50% group (group 2) or controls compared to pre training values. This finding is consistent with many other longitudinal (Stratton *et al* 1991) and cross sectional studies (Speiser *et al* 1988). Stratton *et al* (1991) reported a 58% decrease in PAI-1 activity in older male volunteers following a 6 month training programme at 80-85% VO_2 max and Speiser *et al* (1988) reported significantly lower PAI-1 Ag and activity levels in young athletes compared to sedentary age-matched controls. Kvernmo & Osterund (1997) reported no difference in PAI-1 Ag or activity between athletes and controls. In this study however, the control group were physically fit individuals (mean VO_2 max = 52 mls/kg/min) suggesting that there is a maximal training effect.

These findings suggest that exercise training at an intensity equal or greater than 70% VO_2 max decreases the stimulus for PAI-1 synthesis and secretion. As PAI-1 is released from multiple sites, it is impossible from the measures used in this study to

determine if this reduction is purely due to decreased PAI-1 secretion or is compounded by increased PAI-1 clearance from the circulation. The decrease in active PAI-1 may be a consequence of increasing t-PA concentrations. The magnitude of the rise in t-PA compared to the decrease in PAI-1 further suggests that the rise in t-PA observed in this group is also accompanied by an increase in the free active fraction of t-PA suggesting that, if measured, an increase in t-PA activity similar to that described in non-obese populations (Stratton *et al* 1991, DePaz *et al* 1997) would be observed.

d) PAI-1 Following a Maximal Exercise Test

As with t-PA, the lack of change in both resting and exercise concentrations of total and active PAI-1 50% VO_2 max training group compared to controls and pre training values suggests that there is a training intensity threshold for these modifications to occur. The threshold for decreases in PAI-1 concentration with acute exercise is 70% VO_2 max (Streiff & Bell 1994). As group 2 trained at an intensity below this threshold, significant alterations in either total or active PAI-1 did not occur.

There was a significant decrease in active PAI-1 concentrations as a result of the post training maximal exercise bout in group 1(median (range) 2.60 (1.10 to 17.50) REST Vs 1.00 (0.50 to 13.80) END P=0.001). Furthermore, both total and active PAI-1 concentrations were significantly decreased with respect to pre training values in this group. There were no significant differences in either total or active PAI-1 with acute exercise or exercise training in either the group trained at 50% VO_2 max or controls.

As with t-PA the decreases in active PAI-1 concentrations observed in the 70% group with maximal exercise may purely be due to an increased duration of exercise at an intensity greater than 70% VO_2 max during the post training oxygen uptake test.

The significant lowering of both total and active PAI-1 factors at rest however, indicates that this may not be the only reason. Exercise training at an intensity ≥ 70% VO_2 max is known to reduce platelet activity and aggregation (Rauramaa & Vaisanen 1999) both at rest and during exercise. Decreased secretion of PAI-1 from the alpha granules of activated platelets may account for the reductions in active and total PAI-1compared to pre training values observed in the 70% group. More work is needed to determine the effect of exercise training on fibrinolytic factors in obese populations both at rest and during exercise.

Overall we can conclude that exercise training at 70% VO_2 max three times per week for 12 weeks results in favourable changes in the fibrinolytic capacity of overweight and obese premenopausal women, as shown by elevated t-PA and reduced total and active PAI-1. Exercise training at 50% VO_2 max neither improves nor reduces fibrinolytic capacity.

9.4.4 Correlations with PAI-1

When evaluating the correlations between total and active PAI-1 and other markers of the IRS it is important to remember the fluctuating nature of PAI-1 (Fig 9.1). Active PAI-1 'spontaneously' converts to the latent conformation due to a lack of cystiene residues (Huber 2001a). This latent conformation can be reactivated as a result of platelet activation as well as denaturing agents such as SDS, guanidium chloride and urea (Debrock & Declerk 1997). The third conformational change results in the formation of complexed PAI-1 via the pseudosubstrate actions of active PAI-1 on t-PA (van Meijer & Pannekoek 1995). These reactions occur rapidly and may therefore mask any potential effects of other markers of the IRS.

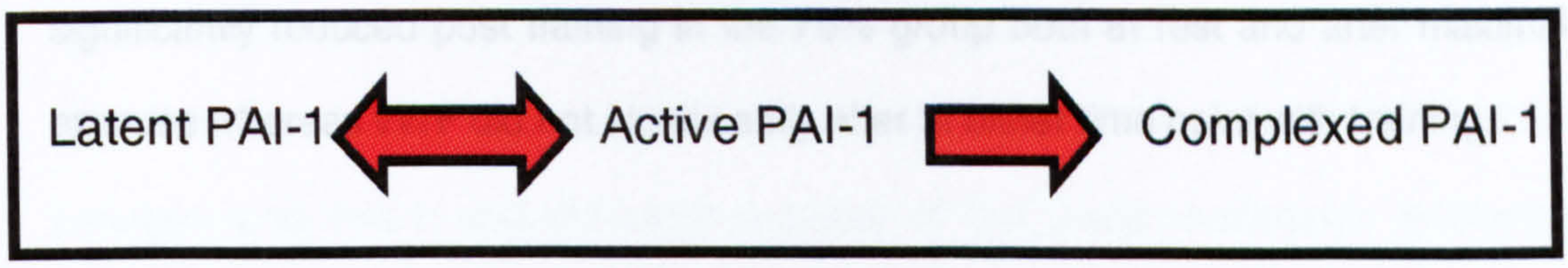

Figure 9.1: Schematic representation of the major conformations of PAI-1

Another consideration is that the main actions of PAI-1 occur locally and may not therefore be reflected at the cannulation site. These factors must be remembered when interpreting the statistical relationships between PAI-1 and other markers of the IRS.

9.4.5 Correlations with Total PAI-1

Total PAI-1 concentrations were significantly correlated with resting systolic blood pressure pre and post training both at rest (pre training R=0.481, P=0.001, post training R=0.428, P=0.018) and after the graded maximal oxygen uptake test (pre training R=0.398 P=0.029, post training R=0.428 P=0.018). This relationship suggests a role for the RAAS in the regulation of PAI-1 as at rest and after the post training exercise bout PAI-1 did not correlate with vWF indicting that this relationship may not solely be a result of shear stress induced endothelial damage and

subsequent platelet and endothelial store PAI-1 release. There was a correlation after the pre training maximal exercise test between total PAI-1 and vWF suggesting that increased endothelial damage as a result of the increased shear stresses of exercise may result in elevations in PAI-1. With exercise training however, this association disappeared suggesting that exercise training alters the PAI-1 response to endothelial damage. Exercise training at both 50% and 70% VO_2 max resulted in significant reductions in systolic and diastolic blood pressures thus reducing the theoretical shear stress on the endothelial wall. The reductions in blood pressure (<10 mmHg) however were not clinically significant and could be accounted for by the variability in both systolic and diastolic blood pressure at rest. Also PAI-1 Ag was significantly reduced post training in the 70% group both at rest and after maximal exercise whereas vWF did not significantly alter at either time point with training.

Similarly, HDL cholesterol was significantly negatively correlated with total PAI-1 at all measured time points (pre training rest, R=-0.375 P=0.051, exercise (R=-0.402 P=0.028, post training R=-0.374 P=0.042 exercise R=-0.471 P=0.009). Negative correlations between HDL and PAI-1 have been observed in numerous epidemiological studies (Vague 1993, Vague *et al* 1995) and this has been attributed to the close metabolic relationship between HDL cholesterol and triglyceride as HDL concentrations are dependent on triglyceride uptake into adipose tissue (Frayn 1995) and there is a large body of evidence (Tremoli *et al* 1993, Nilsson *et al* 1999) to suggest that triglyceride stimulates PAI-1 expression and secretion from a number of different cell types. There was no significant correlation between triglyceride and total PAI-1 in this study however suggesting that this may not always be the case. There is limited research that suggests that glycated HDL cholesterol that occurs with insulin resistance and type II diabetes increases PAI-1 secretion from vascular endothelial cells independently of triglyceride (Ren & Shen 2000). The women in this study did not show the elevated concentrations of insulin associated with resistance

(median (range) insulin pmol/L 6.41 (2.70 to 11.70)) and this group were premenopausal and overweight to mildly obese suggesting that they were unlikely to be experiencing pancreatic beta cell failure. This suggests that an alternative mechanism may be responsible for the relationship between PAI-1 and HDL cholesterol, possibly hepatic blood flow and therefore hepatic uptake of the two molecules.

The assay used for the quantification of total PAI-1 concentrations recognises only non-complexed PAI-1 (latent and active conformations). This allows large variation between total PAI-1 concentration including the complexed form and total PAI-1 as measured by the assay. The variation in the percentage of total PAI-1 in the complexed form may account for the lack of correlations previously described between total PAI-1 and the other markers of the insulin resistance syndrome measured.

9.4.6 Correlations with Active PAI-1

Active PAI-1 concentrations were significantly correlated with resting systolic blood pressure both pre and post training at rest and after the acute bout of maximal exercise (pre training rest R=0.467 P=0.009, exercise R=0.511 P=0.044, post training rest R=0.512, P=0.004, exercise R=0.481 P=0.007). No other factors were significantly correlated with either resting or post exercise active PAI-1 concentrations both pre and post training.

9.4.7 Overall Conclusions

Overall, we have shown that exercise training at an intensity of 70% VO_2 max for a duration equivalent to an energy expenditure of 300 kcal three times per week results in significant improvements in the resting fibrinolytic profile of overweight and obese previously sedentary women as shown by elevated t-PA concentrations and reduced

total and active PAI-1 concentrations. Exercise training at 70% VO_2 max also results in a significant improvement in the fibrinolytic response to maximal exercise, with further increases in t-PA and reductions in active PAI-1 elicited. This is in contrast to the group training at 50% VO_2 max for the same period also for a duration equivalent to 300 kcal energy expenditure in whom no significant fibrinolytic changes were observed. We can therefore deduce that there may an exercise training intensity threshold for fibrinolytic changes to occur.

It has also been shown that the favourable changes in the fibrinolytic profile observed in the 70% VO_2 max group occurred independently of weight loss, loss of body fat, reductions in waist circumference and lowering of the waist hip ratio. Systolic blood pressure was determined to be the main contributing factor to both total and active PAI-1 concentrations possible acting through the RAAS as well as endothelial damage related mechanisms.

The mechanisms and kinetics of PAI-1 production and hepatic clearance are poorly understood. More work is needed to determine the precise effects of exercise training on the regulation of both fibrinolysis *in vivo* and the RAAS.

Chapter 10

Discussion

10.1 Aims of the Research

The aims of this series of studies were firstly, to determine the effect of acute exercise bouts and exercise training on fibrinolytic capacity in overweight and obese volunteers and secondly, to examine the relationships between PAI-1 concentrations and other markers of the IRS to highlight the potential for a role for these factors in the short term regulation of PAI-1.

10.2 Fibrinolytic Response to Acute Exercise in Overweight & Obese Subjects

10.2.1 Tissue Plasminogen Activator (t-PA)

A summary of the results is shown in table 10.1. T-PA increased with acute exercise in all cases, with the exception of 30 minutes at 70% VO_2 max in obese males and 50% VO_2 max for duration equivalent to 300 kcal in overweight and obese premenopausal females. This is consistent with the model of Streiff & Bell (1994) for non obese populations. They suggested that although increases in t-PA are seen with exercise at intensities greater than 50% VO_2 max, large changes are not seen until exercise reaches an 70% VO_2 max. The lack of significant t-PA change in the obese males may be due to the fact that the exercise intensity for this group was based on predicted rather than actual VO_2 max and therefore may have been below the 70% VO_2 max threshold.

T-PA concentrations decreased significantly (70% VO_2 max in overweight and obese premenopausal females) or showed non significant trends towards decreasing (non obese males & obese males 30 minutes post exercise. This is again consistent with the majority of research studies in non obese populations (Van Den Burg *et al* 1994, Szymanski & Pate 1994) and the model proposed by Streiff & Bell (1994). Sustained submaximal exercise results in reduced hepatic blood flow and

*Table 10.1: Summary of the effect of acute exercise on t-PA, total PAI-1 and active PAI-1. BMI is shown as mean ± SEM or median (range). Percentage changes are calculated from rest to immediately post exercise, and from immediately post exercise to recovery period. *=Mean value, all other changes are calculated as median values. N.S.D = no significant difference, - = no sample taken at this point.*

Subject Group	BMI Kg/m^2	Protocol	Effects of an Acute Exercise Bout			Recovery Effects of Acute Exercise		
			T-PA	Total PAI-1	Active PAI-1	T-PA	Total PAI-1	Active PAI-1
Obese Males	31.6 ± 0.88	30 min walking at 70% predicted VO_2 max. 30 min recovery	N.S.D	N.S.D	↓37.7%	N.S.D	↑ 29.9%	N.S.D
Non Obese Males	25.0 ± 0.94	30 min walking at 70% predicted VO_2 max. 30 min recovery	↑ 44.5%	N.S.D	↓23.9%	N.S.D	N.S.D	N.S.D
Overweight & Obese Premenopausal Females	31.1 (27.5 to 38.1)	Graded maximal exercise test to exhaustion No recovery period	↑ 61.9%*	N.S.D	N.S.D	-	-	-
Overweight & Obese Premenopausal Females	30.3 ± 1.29	70% VO_2 max for a duration equivalent to 300 kcal 120 min recovery period	↑ 101.0%*	N.S.D	N.S.D	↓ 56.7%*	30 min = ↑ 161.0% Remained unchanged for following 90 min	N.S.D
Overweight & Obese Premenopausal Females	30.3 ± 1.29	50% VO_2 max for a duration equivalent to 300 kcal 120 min recovery period	N.S.D	N.S.D	N.S.D	N.S.D	N.S.D	N.S.D
Overweight & Obese Premenopausal Females, trained at 70% VO_2 max	29.7 (28.4 to 33.4)	Graded maximal exercise test to exhaustion No recovery period	↑ 110.4%	N.S.D	↓ 70.5%	-	-	-
Overweight & Obese Premenopausal Females, trained at 50% VO_2 max	34.47 (28.1 to 38.1)	Graded maximal exercise test to exhaustion No recovery period	↑ 95.4%	N.S.D	N.S.D	-	-	-

therefore a decreased rate of hepatic clearance of t-PA from the circulation (Chandler *et al* 1995). T-PA rises gradually during the exercise period in parallel to the reduced hepatic blood flow as well as secretion from endothelial stores. When exercise ceases, hepatic blood flow and therefore t-PA concentrations rapidly return to baseline levels (Booth *et al* 1987*).*

A training response was also shown. Regular exercise training at both low (50% VO_2 max) and moderate (70% VO_2) intensities in obese premenopausal females significantly increased the t-PA response to a single graded exercise test to exhaustion. This is consistent with the results of De Paz *et al* 1992 (cross sectional data) who showed that athletes were hyperfibrinolytic compared to controls due to an increased release of t-PA Ag following a maximal exercise test and Ferguson & Guest (1994) who observed that the magnitude of increase in t-PA as a result of acute exercise is dependent on both current and former physical fitness.

10.2.2 Plasminogen Activator Inhibitor type 1 (PAI-1)

No significant changes in total PAI-1 concentrations (Table 10.1) were detected immediately post exercise following the acute exercise bouts. Exercise bouts at 70% VO_2 max for periods equal or greater than 30 minutes in obese males and overweight and obese premenopausal females did however lead to significant increases in total PAI-1 concentration during the recovery period. These rises were seen within the first 30 minutes of the recovery period and remained unchanged for up to 2 hours post exercise.

These results were somewhat surprising, as other groups have observed significant reductions in PAI-1 immediately following exercise and during post exercise recovery from intensities as low as 50% VO_2 max. The increase in PAI-1 Ag post exercise may

be an effect specific to obese populations. The groups studying non obese populations however did not state the time between cannulation and withdrawal of baseline samples. As has been demonstrated in the study in obese Vs non obese sedentary male volunteers, the process of venous occlusion and cannulation results in significant increases in PAI-1 concentrations. It may be that the decrease in PAI-1 seen with exercise by other groups was merely a return to baseline values following the cannulation process.

The results from this research fit well with the model proposed by Streiff & Bell (1994) in that changes in PAI-1 concentration are not seen until exercise intensity reaches 70% VO_2 max although it is not possible to identify a more precise threshold as the critical intensity may lie between 50% and 70% VO_2 max. Based on such evidence we would therefore have expected to see a reduction in PAI-1 concentrations in the overweight and obese premenopausal women following exercise at 70% VO_2 max for a duration equivalent to 300 kcal (mean duration 49.17 min $\pm$ 6.18 min). The number of subjects recruited for this study (i.e. the statistical power) however was based on the fibrinolytic response to exercise of non obese sedentary subjects as obese populations showed more variability in their response the results of the study were subject to type II error.

The rise in total PAI-1 during the recovery from exercise at 70% VO_2 max (sedentary males & overweight and obese sedentary premenopausal females) has not previously been recorded in the literature and may be a phenomenon unique to obese sedentary populations.

Regular exercise training at 50% VO_2 max did not significantly change total PAI-1 concentrations at rest or following a maximal exercise bout. Exercise training at 70% VO_2 max however significantly decreased the total PAI-1 concentration at rest and

following the VO_2 max test although there was no significant change in total PAI-1 concentration from rest to exercise post training. Exercise training at intensities ≥ 70% VO_2 max is known to reduce platelet activity and aggregation both at rest and during exercise (Rauramaa & Vaisanen 1999), which may account for the reduced concentrations of both total and active PAI-1 following exercise training at the higher intensity.

10.2.3 Active Plasminogen Activator Inhibitor type 1 (PAI-1)

As total PAI-1 remained unchanged immediately following acute exercise, active PAI-1 concentrations were measured to gain an insight into overall fibrinolytic capacity. Active PAI-1 was significantly decreased following a single exercise bout at 70% VO_2 max in both obese and non obese sedentary males. As total PAI-1 Ag remained unchanged this may suggest an increase in the number of t-PA/PAI-1 complexes. No other sedentary population in these studies displayed significant decreases in active PAI-1 immediately following acute exercise. Again, low subject numbers and a possible large type II error may be responsible for the lack of significant change.

With regular exercise training at a moderate intensity (70% VO_2 max) although not at a low intensity (50% VO_2 max), overweight and obese premenopausal women showed significant reductions in active PAI-1 concentrations both at rest and following maximal exercise to exhaustion. This may be due to an increased t-PA response to the test following training at 70% VO_2 max increasing the proportion of PAI-1 in the complexed formation with t-PA or, this may be a training effect on active PAI-1 directly resulting in a decreased PAI-1 secretion. Following the training at 70% VO_2 max however the volunteers were physically fitter (as evidenced by increased VO_2 max) and therefore able to exercise at intensities greater than 70% VO_2 max for a longer period of time that may have provided sufficient exercise duration to trigger the PAI-1 response.

10.2.4 Overall Fibrinolytic Capacity

All the individual studies indicated that there was either no change or an improvement in fibrinolytic capacity compared to rest, following acute exercise in overweight and obese populations and therefore it can be suggested that response of obese populations to acute exercise is similar to that of non obese populations. The elevations in total PAI-1 post exercise were compounded by decreasing t-PA concentrations in the groups exercising at 70% VO_2 max suggesting that there may be a reduced fibrinolytic capacity post exercise. A measure of t-PA activity would be needed to confirm or refute this.

There were no significant changes in any of the fibrinolytic variables following exercise at 50% VO_2 max for duration equivalent to the expenditure of 300 kcal. Graded maximal oxygen uptake tests to exhaustion and exercise at 70% VO_2 max for a duration equivalent to 300 kcal energy expenditure (in overweight and obese premenopausal women) as well as a 30 minute exercise bout at 70% VO_2 max (in non obese men) were sufficient to improve fibrinolytic potential by significantly increasing t-PA concentrations. A 30 minute exercise bout at 70% VO_2 max in males was also sufficient to further improve fibrinolytic potential by significantly reducing active PAI-1 concentrations and this was maintained 30 minutes into recovery period. Although t-PA concentrations had returned to baseline at this point fibrinolytic potential was still increased with respect to baseline levels.

It is important to note however from the single study comparing obese and non obese males, that although the obese subjects exhibited a similar fibrinolytic response to acute exercise when compared to the non obese subjects, the obese were still hypofibrinolytic with respect to non obese at all times throughout the trial.

Furthermore, obese populations are known to exhibit elevated concentrations of fibrinogen at rest, possibly due to impaired fibrinolysis suggesting that an increased fibrinolytic capacity may therefore be desirable to reduce thrombotic risk (Ferguson *et al* 1987). Exercise training at 70% VO_2 max (in overweight and obese premenopausal women) was shown to increase fibrinolytic potential both at rest and after a maximal exercise bout. Further research is required to establish if exercise training is sufficient to fully reverse the hypofibrinolytic state observed in obesity.

10.3 Correlations with PAI-1

There was little consistency across the studies with respect to the correlations between PAI-1 concentrations and other markers of the IRS particularly post exercise. This may be due to differences in subject demographics and study protocols as well as variability between subjects.

At rest, anthropometric measures such as body mass, BMI, body fat percentage, waist circumference and waist to hip ratio were however consistently the main predictors of plasma PAI-1 concentrations. These results support numerous research studies reporting that at least one anthropometric variable is the main determinant of plasma PAI-1 concentrations (Jannad-Delanne et al 1998, Vague et al 1989). These results provide further evidence for the postulated role of adipose tissue, particularly visceral adipose tissue in the regulation of PAI-1 expression and secretion (Samad & Loskutoff 1997).

Blood pressure also showed significant correlations with both total and active PAI-1 at rest. Unexpectedly this relationship was not dependent on endothelial damage (measured as vWF) but was dependent on anthropometric measures. This suggested a possible role for the RAAS, a system that not only influences blood pressure via vasoconstriction and blood volume but also stimulates the dose

dependent release of PAI-1 via angiotensin IV (Kerins e al 1995). This system is known to up regulate in obesity via increased concentrations of tissue specific ACE and increased production of plasma angiotensin and plasma renin (Egan et al 2001). *In vivo* studies have shown that infusions of angiotensin II increase PAI-1 Ag selectively in both normotensive and hypertensive individuals (Brown et al 1998).

Blood markers of the IRS showed inconsistent relationships with PAI-1 across the trials highlighting the complex nature of the IRS and the possible limitations of the statistical analyses. Interestingly, HDL cholesterol maintained a significant relationship with PAI-1 concentrations despite controlling for triglyceride. Although the explanation of stimulation of PAI-1 by glycated HDL cholesterol is viable (Ren & Shen 2000) it may not apply to all the populations studied in particular overweight and obese premenopausal females who did not show significant signs of insulin resistance. Changes in hepatic blood flow with exercise would alter the hepatic uptake and clearance of these two factors from the plasma and this may be a more likely explanation for the continuing relationship.

Finally, the use of stepwise multiple regression models to predict the PAI-1 response to exercise from routinely measured markers of the IRS proved a promising approach. The prediction equations accounted for approximately 90% of the variability in PAI-1 with submaximal exercise and 60% of the variability with maximal exercise. The results however should be viewed with caution as the subject numbers in each case were exceptionally low. Further studies therefore incorporating a large population with varying degrees of obesity and resultant co morbidities are required to validate this particular approach.

10.4 Limitations

The major limitation of these studies is the low subject numbers. Although all experiments were powered on published data, there are no published studies on the effect of acute exercise on fibrinolytic variables in obese populations. Numbers therefore were based on non obese populations that may demonstrate less variability than the obese. The low subject numbers can also be attributed to low subject compliance. On average 29 were contacted and 14 patients were screened for every patient that completed the studies.

Secondly, the lack of measure of t-PA activity only allowed speculation as to the change in fibrinolytic capacity with exercise. Measures of t-PA activity as well as measures of fibrin, fibrinogen and their breakdown products would allow a more accurate picture of fibrinolysis to be obtained.

The use of the correlation studies to gain a possible insight into the role of markers of the IRS in the short term regulation of PAI-1 only accounted for immediate dose response reactions of a particular marker on PAI-1 release and, the dual effect of an unmeasured variable on PAI-1 and another marker of the IRS. This approach did not account for delayed dose response reactions involving up regulation of PAI-1 expression and synthesis or delayed responses involving long signalling pathways.

10.5 Further Research

Further research is required into the effect of exercise in both an acute and chronic sense on fibrinolysis in obese populations. Studies employing a number of specific exercise intensities and durations are required to gain a more accurate picture on the effect of acute exercise on fibrinolysis. Training studies are required to clarify if regular exercise training is sufficient to abolish the hypofibrinolytic state observed at

rest and post exercise in obesity in males, premenopausal and post menopausal females.

Studies examining the role of platelet activity (through alpha granule released factors such as platelet factor 4 and β thromboglobulin) cytokines (such ad TNFα and IL-6), endothelial release of PAI-1 as well as hepatic tissue and adipose tissue expression and secretion of PAI-1 are required to fully understand the regulation of PAI-1 secretion, expression and clearance both at rest and during exercise. Furthermore, the role of hepatic blood flow in the secretion and clearance of both t-PA and PAI-1 requires further investigation to determine the exact mechanism by which fibrinolytic capacity changes with exercise. The possible role for RAAS as the link between obesity induced hypertension and elevated PAI-1 concentrations looks promising but further studies will be required to examine this relationship fully. Finally, research needs to be carried out to examine the effect of acute exercise on coagulation pathways so that an overall view of haemostatic balance in the obese during exercise can be obtained.

Chapter 11

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