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ASSOCIATION OF THE PAI-1 4G/5G POLYMORPHISM WITH BLOOD PRESSURE IN THE QUEBEC FAMILY STUDY: INTERACTIONS WITH ADIPOSITY, PHYSICAL ACTIVITY, AND THE ACE I/D POLYMORPHISM

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By

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ABSTRACT

ASSOCIATION OF THE PAI-1 4G/5G POLYMORPHISM WITH BLOOD PRESSURE IN THE QUEBEC FAMILY STUDY: INTERACTIONS WITH ADIPOSITY, PHYSICAL ACTIVITY, AND THE ACE I/D POLYMORPHISM

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Background: Blood pressure (BP) is associated with increased plasma levels of plasminogen activator inhibitor-1 (PAI-1) and angiotensin converting enzyme (ACE). ACE and its insertion/deletion (I/D) polymorphism have been well studied for their association with BP. However, the role of the PAI-1 4G/5G polymorphism in the association between PAI-1 and BP is less clear. Furthermore, factors such as adiposity, physical activity (PA), and other genes may influence BP. Thus the aims of this study were to examine the association of the 4G/5G polymorphism with BP, and its interactions with adiposity, physical activity, and the ACE I/D polymorphism on BP.

Methods: Eight hundred and fifteen subjects from the Quebec Family Study with valid data for height, weight, body mass index (BMI); percent fat; PA levels; systolic, diastolic and mean arterial BP (SBP, DBP, MAP); and genotype were analyzed.

Results: PAI-1 4G/5G genotype was not associated with any BP variable (SBP, DBP, MAP) or hypertension. No significant interactions were found between PAI-1 genotype and BMI or PAI-1 genotype and PA for any BP variable (SBP, DBP, MAP). Overweight and obese subjects had elevated BP (SBP, DBP, MAP) regardless of genotype, whereas normal weight 4G/4G subjects had the highest mean SBP of all normal weight groups. In the lowest tertile of PA, 4G/4G subjects had the highest mean SBP of all groups. When the PAI-1 and ACE genotypes were combined, a significant gene-gene interaction resulted for DBP only. Individuals with the ACE II genotype had the highest mean DBP values across all PAI-1 genotypes.

Conclusions: Overall, these results suggest the PAI-1 4G/5G genotype is not associated with BP or hypertension in Caucasian adults (males and females). We found limited evidence for an interaction between ACE and PAI-1 genotypes on BP variables, with a significant interaction resulting for DBP only. Furthermore, the interaction of PAI-1 genotype with adiposity and PA measures were not significant for any BP variable (SBP, DBP, MAP). However, individuals with the 4G/4G genotype may have elevated SBP, especially when combined with the phenotypes of overweight/obesity and low PA. The association of PAI-1 and ACE genotypes, along with their interactions with adiposity and PA needs to be further studied in a larger sample of adults.

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KEY TO ABBREVIATIONS

ACE I/D	angiotensin-converting enzyme insertion/deletion
ACSM	American College of Sports Medicine
ANCOVA	analysis of covariance
Ang II	angiotensin II
BMI	body mass index
BP	blood pressure
BRFSS	Behavioral Risk Factor Surveillance System
CARDIA	Coronary Artery Risk Development in young Adults
CDC	Centers for Disease Control and Prevention
CI	confidence interval
CHD	coronary heart disease
CVD	cardiovascular disease
DBP	diastolic blood pressure
DNA	deoxyribonucleic acid
FFM	fat-free mass
FM	fat mass
GxE	gene by environment
GxG	gene by gene
GLM	general linear model
HERITAGE	HEalth, RIsk factors, exercise Training And GEnetics
HTN	hypertension
ICD	International Classification of Disease
LSD	least squares difference
MAP	mean arterial pressure
MET	metabolic equivalent (working metabolic rate/resting metabolic rate)
MVPA	moderate to vigorous physical activity
NHANES	National Health and Nutrition Examination Survey
OR	odds ratio
PAI-1 4G/5G	plasminogen activator inhibitor type 1 four/five guanine bases
PCR	polymerase chain reaction
PRIME	Prospective Epidemiological Study of Myocardial Infarction
QFS	Quebec Family Study
RAS	renin-angiotensin system
RCT	randomized controlled trial
SBP	systolic blood pressure
SD	standard deviation
SE	standard error
tPA	tissue plasminogen activator
U.S.	United States

CHAPTER 1: INTRODUCTION

General background of cardiovascular disease and hypertension

Cardiovascular disease (CVD) refers to the division of diseases that involve the heart and/or blood vessels. In the United States (U.S.), CVD is the leading cause of death accounting for 654,092 deaths in 2004 [1]. The established, or traditional, risk factors for CVD are well-recognized and include modifiable and non-modifiable variables. Modifiable risk factors include elevated blood pressure (BP), obesity, low level of physical activity, and an unhealthy diet (e.g., high sodium, high fat, etc.) [2, 3]. Non-modifiable risk factors include age, sex, race, and genetics [2, 4, 5]. Chronically elevated resting BP, clinically referred to as hypertension (HTN), has been shown to be one of the most potent antecedents of CVD [6]. HTN affects approximately 30% (approximately 50 million) of U.S. adults aged 20 or older [7], and has been associated with the early development of CVD [8]. The clinical manifestation of heart disease and HTN typically occurs in middle- to-older age; however, the pathological processes associated with development of these diseases begin early in life [8]. Furthermore, CVD risk factors including BP track moderately well from childhood to adulthood [9-11]. Thus, prevention of CVD and HTN is an important public health issue.

Associations between obesity and physical activity with BP

The prevention of CVD and HTN could be enhanced through efforts to decrease obesity and physical inactivity. In addition to being risk factors for CVD, obesity and physical inactivity are also related to BP (i.e., risk factors for HTN). In the U.S., the prevalence of obesity is approximately 32% among adults, and this prevalence increases with age [12]. Obesity is an independent risk factor for the development of HTN [13, 14], and the positive relationship between body fatness and BP has been well-established in adults [15-17] of both genders, and in virtually all societies, age groups, and ethnic groups [18]. However, the correlations between indicators of body fatness and BP in adults are low-to-moderate (r=0.10-0.50) [19-25]. Furthermore, BMI has been shown to account for approximately 12-30% of the variation in BP [18, 26]. Conversely, at the upper end of the body fatness spectrum (i.e., obesity), studies have shown a 2- to 6-fold higher risk for HTN in obese compared to non-obese adults [13, 14, 17].

Similar to the relationship between body fatness and BP, the association between physical activity and BP in adults is also well-known. Exercise, or increased habitual physical activity, has been widely established as a cornerstone therapy for the primary prevention, treatment, and control of HTN in adults [27]. The positive effects of habitual physical activity and exercise on resting BP are clear in adults, with the average training-induced reductions in SBP and DBP ranging from 2 to 11 mmHg and 1 to 8 mmHg, respectively [27-31]. The magnitude of training responses is increased in relation to initial BP levels with BP response being more pronounced in individuals with mild to moderate HTN when compared to the response in normotensive individuals [31].

Prospective cohort studies have the ability to examine how behavioral and lifestyle factors influence the risk of developing HTN. Several prospective cohort studies have found a decreased risk of HTN in physically active compared to sedentary individuals [27, 32-37]. For example, the Coronary Artery Risk Development in Young Adults (CARDIA) study found that more physically active men had a 27% reduced risk of HTN compared to non-active men [33]. Another study found that increasing BMI was associated with a higher risk of HTN [37]; however, the protective effect of increased physical activity on risk for HTN remained in both overweight and normal weight subjects [37]. Thus, an inverse relationship between physical activity and BP levels on the incidence of HTN is evident. These findings suggest that obese adults with low levels of physical activity would have the highest average BP levels, whereas normal weight adults with high physical activity levels would have the lowest average BP levels.

Although it is well-established that habitual physical activity reduces BP [27], there is great heterogeneity in the magnitude of the reduction in BP across exercise training studies [27, 30, 38]. Some individuals become hypertensive despite a physically active lifestyle and/or a normal body weight, whereas some individuals who are sedentary and/or overweight may have normal BP. Similarly, although exercise training and weight loss programs may lower BP on average, there are significant inter-individual differences in the BP response [39]. For example, subjects in the HEalth, RIsk factors, exercise Training, And GEnetics (HERITAGE) study experienced an average submaximal exercise SBP and DBP decrease of 7 and 3.5 mmHg, respectively, after a 20-week exercise training program [40]. However individual BP responses in the HERITAGE study ranged from large decreases to no changes, or even a slight increase. This variation in BP response to exercise is an example of normal biologic diversity and most likely originates from interactions with genetic factors [41].

Summary

Overall, the relationships between body fatness, physical activity, and BP are well-established. Higher levels of body fatness are associated with increased BP and risk for HTN, and physical activity is related to BP in a dose-response manner, with the risk of HTN greatly increased in sedentary individuals. However, given the overall low-to-moderate correlations between body fatness and resting BP among adults and the heterogeneity and inter-individual variation of BP response to exercise training and habitual physical activity, other variables, such as genetic factors, should be considered to explain additional phenotypic variance in resting BP.

Genetics of Blood Pressure

In addition to environmental or lifestyle factors, genetic factors also explain some of the phenotypic variance in BP. The estimated heritability of BP is 30% [42] and familial factors (e.g., similar environmental factors such as diet or physical activity levels) influence BP as well [43]. Furthermore, data from twin and family studies have shown a significant genetic component affecting BP responses to exercise training [44-46]. Genome wide scans have identified several chromosomal regions related to BP [47-50], including the q arm of chromosome 7 [48, 50, 51]. These regions provide focal points for examining candidate genes that may influence individual variation in BP. One possible candidate gene for BP is plasminogen activator inhibitor type 1 (PAI-1) [52] which is located on chromosome 7 (7q21.3-q22.3) [53].

PAI-1 and BP

PAI-1 is the primary inhibitor of fibrinolysis. Fibrinolysis refers to the capacity to dissolve fibrin resulting in the removal of small blood clots. PAI-1 is secreted by several cells including the endothelium and adipose tissue [54]. Elevated PAI-1 levels are the primary cause of impaired fibrinolysis and are associated with CVD [55-58], HTN [59-62], and obesity [63-65] in adults. Furthermore, PAI-1 deficiency in mice has been shown to prevent both HTN [66] and obesity [67, 68].

PAI-1 levels are known to be associated with both BP and body fatness. Low-to-moderate correlations (r=0.10-0.40) between PAI-1 and BP in adults have been reported [60, 62, 65, 69-73] [71] with correlations perhaps being stronger in obese individuals [69]. Moreover, individuals with HTN have significantly higher PAI-1 levels than normotensive individuals [61, 71]. The association between PAI-1 level and BP has been shown in men and women and normal weight and obese individuals. PAI-1 levels have also been shown to be associated with longitudinal changes in BP [62, 69, 72].

Several studies have also shown that BMI is positively correlated with PAI-1 levels (r=0.40-0.70) [59, 65, 69, 70, 73-79]. Furthermore, obese individuals have higher levels of PAI-1 compared to non-obese individuals [64, 65]. In the Caerphilly Study, obese men had 50% higher PAI-1 activity levels compared to normal weight men [65].

It is clear that elevated BP and obesity are each associated with high PAI-1 levels in adults. Few studies have shown evidence for both of these relationships (i.e., PAI-1 and BP; PAI-1 and body fatness) simultaneously in the same sample. Results from a study of mildly hypertensive adults (n=96) showed significant correlations of r=0.39 between PAI-1 and BMI and r=0.24 between PAI-1 and SBP ($P \leq 0.05$) [70]. In a study of almost 100 adult men, hypertensive individuals had significantly higher BMI and PAI-1 levels compared to normotensive individuals [71]. A study of children and adolescents found that PAI-1 levels were significantly higher in hypertensive, obese, and both hypertensive and obese subjects when compared to healthy controls [80]. The results from this latter study summarize the trends exhibited in previous studies, and demonstrate the PAI-1 levels that would be expected in adults. Thus, it is likely that hypertensive and obese individuals would have higher PAI-1 levels than normotensive or normal weight individuals, respectively, and individuals who are both hypertensive and obese would have the highest PAI-1 levels of all individuals. As BP is known to be positively associated with adiposity measures and PAI-1 levels independently, future studies should examine the combined influence of adiposity and PAI-1 levels on BP.

Physical activity/exercise and PAI-1

Both acute and chronic physical activity influences PAI-1 levels. More specifically, PAI-1 activity decreases in response to acute exercise as well as in adaptation to chronic exercise [81, 82]. The magnitude of the decrease in PAI-1 activity to acute bouts of exercise is dependent upon the exercise intensity and duration [82]. Furthermore, multiple studies have shown that PAI-1 levels decrease with weight reduction via increased physical activity and caloric restriction [81]. This suggests that elevated adult PAI-1 levels are reversible by lifestyle changes, especially those geared towards weight reduction. Thus, it is possible that a normal weight adult who regularly participates in high amounts of physical activity would have the lowest PAI-1 levels, while obese adults who regularly participate in physical activity having PAI-1 levels similar to normal weight inactive adults. Additionally, obese, sedentary adults probably possess the highest PAI-1 levels.

Although increased physical activity typically leads to reduced PAI-1 levels, there may be large variation in individual PAI-1 response to exercise. For example, among overweight or obese adults who underwent 6 months of endurance training, men showed small decreases in PAI-1 activity with training (−2.6±1.4 IU ml^{-1}) while women showed small increases in PAI-1 activity with training (+0.9±0.9 IU ml^{-1}) [83]. A study of obese children showed changes in PAI-1 level ranging from -69.0 to +54.0 μg/L after 4 months of training [84]. This variation may be representative of genetic differences among individuals. Indeed, results from a randomized controlled trial (RCT) showed that regular moderate physical activity did not decrease PAI-1 levels in the study group as a whole [85]. However, individuals homozygous for the 4G allele of a common PAI-1 polymorphism experienced a 36% reduction in PAI-1 levels [85]. Womack et al. have suggested the possibility of a substantial genetic influence (e.g., polymorphisms) on the fibrinolytic response to exercise, and that the role of polymorphisms be further investigated in future studies involving this response [82].

PAI-1 4G/5G polymorphism

Aside from environmental factors (e.g., obesity and physical activity) influencing PAI-1 levels, gene polymorphisms are also involved in the regulation of plasma levels of PAI-1 [86]. A genetic polymorphism is a difference in DNA sequence among individuals, groups, or populations that gives rise to different forms (e.g., human ABO blood types) [87]. To be considered a polymorphism, the genetic variant must appear in at least 1% of the population. The alternative forms of a gene are called alleles, which occupy a specific chromosomal location (locus). At each autosomal (non-sex chromosome) locus, an individual possesses two alleles (e.g., A or B allele), one inherited from the mother and one from the father, which may be the same or different. Individuals usually have three possible combinations of alleles at a specific site (e.g., AA, AB, or BB), and each combination may result in different phenotypic forms of various traits (e.g., high, normal, or low BP).

A common, single base-pair polymorphism (four or five guanine bases; 4G or 5G) residing on chromosome 7 has been identified in the promoter region of the human PAI-1 gene, -675 base pairs from the transcription start site [58]. Both alleles (4G and 5G) bind a transcriptional activator, whereas the 5G allele also binds a repressor protein, which decreases binding of the activator [88]. Thus, the 4G/5G polymorphism affects the binding of proteins regulating transcription of the PAI-1 gene. Adults homozygous for the 4G polymorphism (4G/4G) exhibit higher levels of PAI-1 [58, 88-90] and show an increased risk for HTN [52], myocardial infarction [88, 91, 92], obesity [69, 93], and diabetes [94].

This dissertation will focus on the association of the PAI-1 4G/5G genotype with BP and its interactions with adiposity and physical activity. Recently, adults homozygous for the 4G allele (4G/4G genotype) were found to be at increased risk of HTN (OR=1.9, 95% CI: 1.1-3.0) compared to 5G allele carriers. This increased risk was independent of age, BMI, and PAI-1 levels [52]. The authors found that DBP was significantly higher in 4G/4G individuals compared to 5G carriers, whereas there was a trend towards higher SBP in 4G/4G individuals compared to 5G carriers [52]. In another study, the odds for being obese were nearly three-fold higher in individuals with the 4G/4G genotype (OR=2.8, 95% CI: 1.6-4.8) [93]. Furthermore, two studies have shown the 4G/4G genotype to be more frequent in obese compared to non-obese children [95, 96]. Thus, it is evident that the 4G allele is the risk allele of the PAI-1 4G/5G polymorphism. Further studies are needed that examine the role of the PAI-1 4G/5G genotype on adiposity and BP and their interaction.

It is plausible that adults with the 4G/4G genotype would have higher mean resting BP values than adults with the 5G/5G genotype, with the 4G/5G genotype having intermediate BP values. Also, it is possible that a higher proportion of hypertensive and obese adults have the 4G/4G genotype as compared to normotensive and normal fat adults. Thus in combination, taking into consideration obesity status and genotype, it is possible that obese adults with the 4G/4G genotype would have the highest mean resting BP values, while lean 5G/5G adults would possess the lowest mean resting BP values.

Gene-environment and gene-gene interactions

A central concept in multi-factorial phenotypes such as BP is the gene-by-environment (GxE) interaction [97]. GxE interaction implies that in combination, the effect of genotype and environment deviates from the additive or multiplicative effects of the two factors [5]. The interaction between PAI-1 genotype, environmental variables (e.g, obesity and physical activity), and BP is an area that needs further study. GxE interaction in the PAI-1 4G/5G polymorphism has been studied in adults with the results showing that the relationship between PAI-1 levels and cholesterol or triglycerides differed according to the 4G/5G genotype [98]. Several studies have examined the GxE interaction on BP in adults [26, 99-103]. However, few investigators have considered the influence of the PAI-1 4G/5G polymorphism and its interaction with environmental factors on BP in adults. Incorporating key behavioral and physiologic traits in a genetic association study can lead to a better understanding of how the interactions between DNA sequence variants and non-genetic variables are related to BP in adults.

Since BP is a multi-factorial phenotype, interactions other than GxE may help to explain the variance within BP levels. Gene-by-gene (GxG) interactions have been widely accepted as an important contributor to the complexity of mapping complex phenotypes such as BP given that most human phenotypes are polygenic [104]. For example, individuals of similar adiposity have been shown to have a wide variance in BP values, which may be indicative of pleiotropy or GxG interaction [105].

Angiotensin converting enzyme and its relationship to PAI-1

Angiotensin converting enzyme (ACE) is a candidate gene for BP that has pleiotropic and epistatic effects. Pleiotropy is the phenomenon of one gene being responsible for or affecting more than one phenotypic characteristic (e.g., ACE I/D affects both adiposity and BP). Epistasis is a form of interaction between genes located at different loci, in which one combination of such genes has a dominant effect over other combinations (e.g., ACE I/D x PAI-1 4G/5G genotypes affecting BP). ACE is one of the primary enzymes in the renin-angiotensin system (RAS), a complex system that plays a critical role in maintaining BP homeostasis. Increased levels of ACE are associated with increased BP as the actions of ACE result in the activation of a potent vasoconstrictor (Angiotensin II). It is thought that the RAS is involved in PAI-1 production. Several in vivo and in vitro studies have shown Angiotensin II (Ang II), the end-product of the RAS, to induce increased PAI-1 levels [106-110]. Thus, increased levels of ACE lead to increased levels of Ang II and consequently increased levels of PAI-1. Furthermore, ACE inhibition has also been shown to lower PAI-1 levels [109]. Both ACE and PAI-1 have been shown to be expressed in human adipose tissue and adipocytes, and both may be associated with obesity [54, 111, 112]. It has been hypothesized that adipose tissue, as a source of Ang II, may be the link between elevated PAI-1 levels and HTN, especially in obesity [54].

The relationship between ACE, PAI-1, and BP levels warrants further examination. However, it is not always practical or cost-effective to measure ACE and

PAI-1 levels as these levels change in response to many variables (e.g., exercise, food consumption, and smoking) and follow circadian rhythms. Thus, reliable markers that are easily and inexpensively measured are needed to represent ACE and PAI-1 levels. Polymorphisms for these genes may represent practical markers. For example, the 4G/5G polymorphism is associated with PAI-1 levels. Thus, genotyping an individual for the 4G/5G polymorphism may provide a gauge of overall PAI-1 levels without having to control for all of the variables that may affect PAI-1 levels (e.g., circadian rhythms). Furthermore, polymorphisms can be easily and quickly determined simultaneously in many individuals at the same time.

ACE I/D polymorphism

An insertion deletion (I/D) polymorphism of the ACE gene has been shown to account for over half the variance in circulating levels of ACE [113]. Specifically, the DD genotype is associated with the highest ACE levels of the three possible genotypes (II, ID, DD) and displays a positive association with BP and body fatness [48, 114-116]. Similar to the PAI-1 4G/5G genotype, the ACE I/D genotype results in graded ACE levels with II individuals having the lowest, ID intermediate levels, and DD individuals the highest values [113]. The ACE I/D genotype may also be associated with PAI-1 levels. A study in adults found triglycerides, BMI, sex, smoking, and the PAI-1 4G/5G and ACE I/D gene polymorphisms independently predicted plasma PAI-1 levels [76]. In this study, individuals with the ACE DD genotype displayed the highest PAI-1 levels compared to ACE II and ID individuals [76].

The important role ACE plays in the RAS and its association with PAI-1 suggests that the relationship between the PAI-1 4G/5G polymorphism and BP or adiposity may be dependent on the epistatic effects of the ACE I/D polymorphism. This interaction of ACE and PAI-1 genotypes appears biologically plausible and has been studied previously in adults [91, 117]. For example, individuals homozygous for both risk alleles (combined genotype of ACE DD and PAI-1 4G/4G) had an overall odds ratio of 3.10 (95% CI: 1.51-6.36) for early onset of coronary heart disease [117].

Based on the aforementioned results, it is thought that the PAI-1 and ACE genes may play a key role in the pathogenesis of HTN and/or obesity. This notion is further supported by the associations observed between the PAI-1 4G/5G and ACE I/D polymorphisms and BP, HTN, body fatness, and obesity in population studies. Thus, the interaction of the PAI-1 and ACE gene polymorphisms needs further examination, especially within the pathways leading to HTN and obesity. In combination it is possible that ACE DD/ PAI-1 4G/4G individuals would have the highest BP levels and ACE II / PAI-1 5G/5G individuals would have the lowest BP levels.

Summary and Purpose of Dissertation

Increased PAI-1 levels are positively associated with CVD, HTN, and obesity. Furthermore, the 4G/5G genotype of the PAI-1 gene is an important determinant of PAI-1 with the 4G/4G genotype having the highest average levels of all genotype groups. Given that individuals homozygous for the 4G allele are at increased risk of being hypertensive or obese, and that increased physical activity levels have been shown to decrease PAI-1 levels, it is believed that a gene-fatness and gene-physical activity interaction on resting BP involving the PAI-1 4G/5G genotype may be present in adults. Furthermore, a GxG interaction is believed to exist between the PAI-1 4G/5G and ACE

I/D polymorphisms on BP. Thus, the overall purpose of this dissertation is to examine the associations between the PAI-1 4G/5G polymorphism, ACE I/D polymorphism, body fatness, physical activity, and resting BP in adults.

The hypotheses, based on the specific aims, will be examined using data from the Québec Family Study (QFS) (Principal Investigator, Claude Bouchard). Participants in QFS were measured for multiple phenotypes including body fatness (percent fat as estimated by underwater weighing), habitual, free-living physical activity levels (assessed by 3-day activity record), and resting BP. Blood samples were also obtained from consenting participants. Phase 1 of QFS (1978-1982) involved the initial recruitment and data collection from parents and their offspring living within 50 miles of Québec City, while Phases 2 (1992-1997) and 3 (1997-2002) involved re-measurement of participants from the previous Phase(s), as well as recruiting new families having one or more obese member. As a longitudinal family study, QFS has been paramount in providing measures of familial resemblance and heritability for numerous phenotypes and traits, and has produced over 200 publications. This dissertation will utilize cross-sectional data from QFS obtained in Phases 2 and 3 (1992 to 2002), which includes 844 adults aged 18 to 94 years with PAI-1 genotype data.

The potential for a statistical test to reject a null hypothesis (i.e., show significant differences between groups) depends on the power of the study. Adequate statistical power is conventionally thought to be 0.80 or 80%, which represents the likelihood that four times out of five a false null hypothesis will be correctly rejected [118]. Power calculations rely on the significance level effect size (ratio of mean difference between the two groups being compared divided by the pooled standard deviation of the two groups), and the sample size of each of the groups [119]. Typically, researchers can only change the level of significance and sample size in order to improve the power of a study. However, it should be noted that since this is a secondary data analysis, the subsequent power calculations involve a fixed sample size (n = 844).

Specific aims

Specific Aim 1a) To examine the main effect of PAI-1 4G/5G genotype (4G/4G, 4G/5G, 5G/5G) on systolic (SBP), diastolic (DBP), and mean arterial blood pressure (MAP).

BP was measured through auscultation with a mercury sphygmomanometer, with the units in mmHg. It is hypothesized that there will be a significant main effect of PAI-1 genotype on all 3 BP variables. Specifically, it is hypothesized that the PAI-1 4G/4G genotype group will have the highest, 4G/5G intermediate, and 5G/5G the lowest mean values of BP (mmHg) for all 3 variables (SBP, DBP, and MAP). Since SBP and DBP reflect only one beat of the heart (contraction and relaxation pressures respectively), these indices may not represent an individual's true BP profile, as they do not reflect pressure throughout the entire cardiac cycle. MAP is defined as the average arterial BP during a single cardiac cycle. It is calculated as (SBP-DBP/3) + DBP. The reason MAP is important is that it reflects the hemodynamic perfusion pressure of the vital organs, as perfusion can be insufficient even if SBP or DBP is not. A low MAP indicates decreased blood flow through the organs, whereas a high MAP indicates an increased cardiac workload.

The research hypothesis will be tested statistically using a general linear model (GLM) testing for the main effect of PAI-1 genotype on BP for the entire sample.

Dependent variables will be SBP, DBP, and MAP (in mmHg). PAI-1 genotype (4G/4G, 4G/5G, 5G/5G) will be the independent variable, controlling for age (continuous: yrs), sex (M/F), smoking (current, former, never), BP medication (Y/N), BMI (continuous: kg/m^2), and moderate to vigorous physical activity (MVPA) (continuous: activity score). BMI will be used as a covariate instead of percent fat because more subjects had valid data for BMI compared to percent fat. The research hypotheses will be rejected with a P value of ≤ 0.05. Least squares difference (LSD) post-hoc test will be performed to detect specific genotype group differences if a significant main effect for genotype is found in the GLM.

The power to detect genotype differences in BP is predicted to be approximately 80.4% with an effect size of 0.17. This is based on adult literature showing a 2 mmHg higher BP (both SBP and DBP) for 4G/4G individuals compared to 5G carriers [52]. Furthermore the standard deviation (SD) for BP seen in summary statistics of the present sample is ~12 mmHg.

Specific Aim 1b) To examine if there is an association between PAI-1 4G/5G genotype and HTN in this sample of adults.

It is hypothesized that PAI-1 4G/4G individuals will have significantly higher odds of being hypertensive than the other genotypes (4G/5G and 5G/5G). This hypothesis will be tested using a logistic regression with HTN (dichotomous: Y/N) as the dependent variable and genotype (4G/4G, 4G/5G, 5G/5G) as an independent variable, along with age (continuous: yrs), sex (dichotomous: M/F), BMI (continuous: kg/m^2) and MVPA (continuous: activity score) entered as both independent variables and covariates.

For the purposes of statistical analyses, adults were classified as hypertensive with SBP ≥ 140 mmHg or DBP ≥ 90 mmHg, or if they were currently taking medication to control HTN. Thus, the classification of HTN in this dissertation is not based on a clinical diagnosis. A significance level of $P \leq 0.05$ will be used. Odds ratios will be calculated to determine the impact of these variables, and more specifically PAI-1 genotype on HTN.

Predicted required sample size calculation for the regression was performed using an expected power of 0.80; an alpha level of 0.05, using the 6 predictors previously mentioned, and anticipated effect size of 0.17. The effect size was calculated based on a 2 mmHg BP difference between 4G/4G versus 5G carriers with a pooled standard deviation of 12 as described previously. This resulted in a minimum required sample size of 92. This study has a minimum of 190 subjects of each genotype and a total of 844 subjects. Approximately 18% of the present sample was classified as hypertensive (n=123 according to BP measurements obtained from a single visit or the current use of BP medication), which should be sufficient to incorporate all of the genotypes. Thus this sample should have enough statistical power to detect an association between the PAI-1 4G/5G genotype and HTN, if one is present.

Specific Aim 2a) To examine the interactions of PAI-1 4G/5G genotype (4G/4G, 4G/5G, 5G/5G) and adiposity as a continuous variable (BMI and percent body fat) on blood pressure (SBP, DBP, MAP).

BP was measured through auscultation with a mercury sphygmomanometer, with units in mmHg. Body weight was measured to the nearest 0.1 kg using a standard beam scale, and height measured to the nearest millimeter by stadiometer. BMI was calculated as weight (kg)/height (m) ^{2}. Percent body fat was estimated using the Siri formula [120] using body density obtained by hydrostatic weighing.

It is hypothesized that the interaction of PAI-1 4G/5G genotype and adiposity (both BMI and percent fat) will be significant for all 3 BP variables (SBP, DBP, MAP). The research hypothesis will be tested statistically using a GLM with BP (continuous: SBP, DBP, MAP in mmHg) entered as the dependent variable. PAI-1 genotype (4G/4G, 4G/5G, 5G/5G) and PAI-1 genotype x adiposity interaction (genotype x BMI or genotype x percent fat) will be the independent variables, controlling for age (continuous: yrs), sex (M/F), smoking (current, former, never), BP medication (Y/N), and MVPA (continuous: activity score). The research hypotheses will be rejected with a P value of ≤ 0.05.

The power to detect an interaction between the PAI-1 genotype and adiposity is estimated to be 86%. This is based on BP being 2 mmHg higher in PAI-1 4G/4G individuals as described earlier [52], along with studies showing obese adults having higher BP values than normal fat adults. For example a case-control study found BP increases of 15-20 mmHg in obese subjects compared to normal fat controls [69]. As a result of the large sample size in the present study (n=844), using a conservative estimate of a 4 mmHg increase in BP of obese compared to normal fat subjects, or a larger 10 mmHg increase did not change the estimated power. The standard deviation used was 12 mmHg as derived from BP variables in the present sample.

Specific aim 2b) To test the interactions of PAI-1 4G/5G genotype (4G/4G, 4G/5G, 5G/5G) and BMI as a categorical variable (normal weight vs overweight/obese) on blood pressure (SBP, DBP, MAP).

Subjects will be classified based on adult BMI cut points of < 25 kg/m^2 classified as normal weight, ≥ 25 to <30 kg/m^2 overweight, and ≥ 30 kg/m^2 obese. The overweight and obese categories will be combined into one category classified as overweight/obese. This will result in 6 separate PAI-1 genotype/fatness groups: 1) normal weight, 5G/5G; 2) normal weight, 4G/5G; 3) normal weight, 4G/4G; 4) overweight, 5G/5G; 5) overweight, 4G/5G; and 6) overweight, 4G/4G. Subjects homozygous for the high risk allele are found in groups 3 and 6 and for the low risk alleles in groups 1 and 4, with groups 2 and 5 being heterozygous with intermediate risk.

The decision to use cut points for overweight/obesity is based on a working model for the interaction between genetic and environmental factors described by Talmud [5]. This model suggests that the importance of GxE interaction only occurs when an individual with a high-risk genetic profile (e.g., possessing PAI-1 4G/4G genotype) enters a high-risk environment (e.g., obesity or low physical activity), making the effect on risk (i.e., for elevated BP) so great that premature disease states develop (e.g., HTN). Thus by combining the subjects into groups based on genotype and obesity, the differences in BP on both ends of the risk spectrum (i.e., “good” genes with normal fat and “bad” genes with obesity) can be seen. Observing actual BP differences in these combined genotype/fatness groups may be more clinically useful than testing the subjects as a whole. When plotted together, it can be seen whether a GxE interaction exists based on the values of the group means [5].

It is hypothesized that a significant PAI-1 4G/5G genotype x BMI category interaction will result for all 3 BP variables (SBP, DBP, MAP). Specifically it is hypothesized that group 1 (normal weight, 5G/5G) will have the lowest average BP for all 3 variables (SBP, DBP, and MAP), with groups 2, 3 and 4 (normal weight, 4G/5G; normal weight, 4G/4G; and overweight, 5G/5G) having similar average BP values, and groups 5 and 6 (overweight, 4G/5G; and overweight, 4G/4G) having the highest average BP for all 3 variables.

To test this, a GLM will be run with BP variables (SBP, DBP, MAP in mmHg) as the dependent variable. PAI-1 genotype (4G/4G, 4G/5G, 5G/5G) and PAI-1 genotype x fatness interaction will be the independent variables, controlling for overweight/obese (Y/N), age (continuous: yrs), sex (M/F), smoking (current, former, never), BP medication (Y/N), and MVPA (continuous: activity score). The research hypothesis will be rejected with a P value of $\leq$ less than 0.05. LSD post-hoc test will be performed to detect specific genotype/fatness group differences if a significant interaction for PAI-1 genotype x BMI category is found in the GLM model.

The power to detect the interaction of PAI-1 genotype x BMI category is predicted to be 99%. This is based on BP being 2 mmHg higher in PAI-1 4G/4G individuals as described earlier [52], along with studies showing overweight adults having higher BP values than normal weight adults. For example a case-control study found BP increases of 15-20 mmHg in obese subjects compared to normal weight controls [69]. As a result of the large sample size in the present study, using a conservative estimate of a 4 mmHg increase in BP of overweight compared to normal weight subjects, or a larger 10 mmHg increase did not change the estimated power. The standard deviation used was 12 mmHg as derived from BP variables in the present sample.

Specific Aim 3a) To examine the interaction of PAI-1 4G/5G genotype (4G/4G, 4G/5G, 5G/5G) and physical activity as a continuous variable (total physical activity score and MVPA score) on blood pressure measures (SBP, DBP, and MAP in mmHg) in adults.

A 3-day activity diary was used to estimate energy expenditure based on physical activity levels. Two days could fall on any day of the week, but the third day had to be a weekend day. In the activity record the 24 hour day was divided into 96 periods of 15 min. For each 15-min period, energy expenditure was quantified on a scale of 1 to 9. Examples of activities included in each category were explained and illustrated in detail to all participants in materials given to them for personal use. The total daily physical activity level is defined as the sum of all activity scores over 3 days. For the purposes of this study, activities from categories of energy expenditure 6-9, which have energy cost equal to or greater than 4.8 METs will be used to define MVPA. Specifically, the total activity scores from levels 6-9 over the 3-day period will be used as MVPA. The intra-class correlation for test–retest reliability of the 3-day activity diary studied among 61 subjects reached 0.96 [121].

It is hypothesized that there will be a significant PAI-1 genotype x total physical activity and PAI-1 genotype x MVPA interaction for all 3 BP variables. The results are expected to be stronger when using MVPA compared to total physical activity in the analyses. The hypothesis that there will be a significant genotype by physical activity interaction on BP will be tested using a GLM with BP measures (SBP, DBP, MAP in mmHg) as the dependent variable and PAI-1 genotype (4G/4G, 4G/5G, 5G/5G) and PAI-1 genotype x total physical activity interaction or PAI-1 genotype x MVPA interaction will be the independent variables, controlling for age (continuous: yrs), sex (M/F), smoking (current, former, never), BP medication (Y/N), BMI (continuous: kg/m^2), and total physical activity or MVPA (continuous: activity score). The research hypothesis will be rejected with a P value of ≤ 0.05.

The power to detect the interaction of PAI-1 genotype and physical activity on BP is predicted to be 40%. This is based on BP being higher in PAI-1 4G/4G individuals as described earlier [52], along with data in adults showing BP (SBP and DBP) differences between the top and bottom quintiles for physical activity ranging from 4 to 11 mmHg [122]. The standard deviation used was 12 mmHg as found in BP variables of the present sample, which has 844 subjects.

3b) To examine the interactions of PAI-1 4G/5G genotype (4G/4G, 4G/5G, 5G/5G) and physical activity as a categorical variable (tertiles of total physical activity and MVPA) on blood pressure (SBP, DBP, MAP).

Subjects will be grouped into tertiles of total physical activity and MVPA. This will result in 9 separate PAI-1 genotype/fatness groups: 1) low tertile, 4G/4G; 2) low tertile, 4G/5G; 3) low tertile, 5G/5G; 4) middle tertile, 4G/4G; 5) middle tertile, 4G/5G; 6) middle tertile, 5G/5G; 7) high tertile, 4G/4G; 8) high tertile, 4G/5G; 9) high tertile, 5G/5G. The decision to use cut points for physical activity is based on the working model for the interaction between genetic and environmental factors described by Talmud, previously described in specific aim 2b (Figure 1) [5].

It is hypothesized that there will be a significant PAI-1 4G/5G genotype by tertile of physical activity interaction for both total physical activity and MVPA and for all 3 BP variables (SBP, DBP, MAP). Specifically it is hypothesized that group 1 (low tertile, 4G/4G) will have the highest average BP for all 3 variables (SBP, DBP, and MAP), but that group 7 (high tertile, 4G/4G) will have similar BP to groups 8 and 9 (high tertile, 4G/5G; and high tertile, 5G/5G.

To test this, a GLM will be run with BP variables (SBP, DBP, MAP in mmHg) as the dependent variable. PAI-1 genotype (4G/4G, 4G/5G, 5G/5G) and PAI-1 genotype x total physical activity or MVPA tertile interaction will be the independent variables, controlling for age (continuous: yrs), sex (M/F), smoking (current, former, never), BP medication (Y/N), and BMI (continuous: kg/m^2). The research hypothesis will be rejected with a P value of ≤ less than 0.05. LSD post-hoc test will be performed to detect specific genotype/tertile group differences if a significant interaction for PAI-1 genotype x physical activity tertile is found in the GLM model.

The power to detect the interaction of PAI-1 genotype and physical activity on BP is predicted to be 34%. This is based on BP being higher in PAI-1 4G/4G individuals as described earlier [52], along with data in adults showing BP (SBP and DBP) differences between the top and bottom quintiles for physical activity ranging from 4 to 11 mmHg [122]. The standard deviation used was 12 mmHg as found in BP variables of the present sample, which has 844 subjects.

Specific Aim 4) To determine if an ACE I/D by PAI-1 4G/5G genotype interaction exists for BP (SBP, DBP, MAP measured in mmHg)

The interaction of ACE I/D genotype (II, ID, and DD) and PAI-1 4G/5G genotype (4G/4G, 4G/5G, and 5G/5G) will result in 9 possible groups: 1) II and 5G/5G, 2) II and 4G/5G, 3) II and 4G/4G, 4) ID and 5G/5G, 5) ID and 4G/5G, 6) ID and 4G/4G, 7) DD and 5G/5G, 8) DD and 4G/5G and 9) DD and 4G/4G. It is hypothesized that there will be a significant interaction of the ACE and PAI-1 genotypes on all 3 BP variables (SBP, DBP, MAP). Specifically, it is hypothesized that group 1 (II and 5G/5G) will have the lowest mean BP values for all 3 measures (SBP, DBP, and MAP) and group 9 (DD and 4G/4G) the highest mean BP values for all 3 variables, with all other groups having intermediate BP values.

A GLM will be used with BP variable (SBP, DBP, MAP in mmHg) as the dependent variable and PAI-1 genotype (4G/4G, 4G/5G, 5G/5G) and ACE I/D genotype (II, ID, DD) as the independent variables, controlling for age (continuous: yrs), sex (M/F), smoking (current, former, never), BP medication (Y/N), BMI (continuous: kg/m^2), and MVPA (continuous: activity score). The research hypothesis will be rejected with a P value of less than 0.05. LSD post-hoc test will be performed to detect specific combined genotype group differences if a significant interaction of PAI-1 genotype x ACE genotype is found in the GLM.

The power to detect combined genotype group differences is predicted to be 93%. This is based on the previously discussed BP difference of 2 mmHg between PAI-1 4G/4G and 5G carriers. In studies of ACE I/D and BP, a range of effect size from 1 to more than 20 mmHg has been seen between the three genotypes, as ACE is a well known candidate gene for BP [123]. In order to be conservative and for ease of calculations, a difference of 2 mmHg between ACE genotypes was used in the power analysis. A sample size of 844 along with a standard deviation of 12 mmHg was used, as these are actual values from the present sample.

Results from testing these working hypotheses should enhance our understanding of the genetic architecture of PAI-1 and ACE on BP. Genetic architecture has been defined as: a) the number of genes that influence a trait, b) the number of alleles for each gene, c) the frequencies of the alleles in the population, and d) the influence of each gene and its alleles on the trait [124]. It is this last feature of genetic architecture that is of particular interest in this dissertation. To provide appropriate background for this research a literature review on selected topics of CVD, BP, obesity, physical activity, PAI-1, the PAI-1 gene 4G/5G polymorphism, and the ACE I/D polymorphism is provided in Chapter 2. Chapter 3 contains the detailed methodology from the QFS used in this dissertation. Results are presented in Chapter 4, and Chapter 5 contains the discussion of the findings. The final chapter (Chapter 6) summarizes the findings herein and provides recommendations for future research in this area of study.

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CHAPTER 2: LITERATURE REVIEW

INTRODUCTION

The following chapter contains the literature review for the present dissertation topic. As this dissertation involves the examination of several risk factors for CVD, the general epidemiology of CVD is summarized. Next, background information on BP levels in adults is given, including studies of HTN in adults. Studies examining the influence of body fatness and physical activity on BP levels in adults are then reviewed. Lastly, the genetics of BP is introduced, specifically studies examining the association between PAI-1 and its 4G/5G polymorphism or ACE and its I/D polymorphism and BP/HTN are summarized.

GENERAL EPIDEMIOLOGY OF CARDIOVASCULAR DISEASE

Cardiovascular disease and its risk factors. CVD refers to the division of diseases that involve the heart and/or blood vessels (e.g., International Classification of Disease (ICD)-9 390-459, 745-747; ICD-10 I00-I99, Q20-Q28). CVD is the leading cause of death in both low-to-middle and high income countries globally [1]. For example CVD accounted for 1 of every 2.8 deaths (861,826 deaths or 35.2%) in the United States (U.S.) in 2004 [2]. Recently, the American Heart Association estimated that over 79 million American adults (37.1%) are living with one or more forms of CVD, including coronary heart disease, HTN, and stroke [2]. Over 250 variables have been identified as having an association with CVD [3-5]. The established risk factors can be considered as modifiable or non-modifiable. The non-modifiable risk factors include age, sex, race, maturity, and heredity [3-5], while the modifiable risk factors include both biological risk factors such as elevated BP, obesity, and dyslipidemia and lifestyle risk factors such as a low level of physical activity (PA), smoking, and a poor diet (e.g., high sodium, high fat, etc.) [4, 6]. Recently, new emerging biological risk factors have been identified and include plasminogen activator inhibitor type 1 (PAI-1), fibrinogen, C-reactive protein, and endothelial dysfunction [6]. In summary, the widespread prevalence of CVD combined with preventable risk factors, which influence early onset of disease and also track from childhood to adulthood, constitute an important consideration for the prevention of CVD.

BLOOD PRESSURE LEVELS IN ADULTS

Definition of hypertension. HTN, commonly referred to as high blood pressure, is a medical condition in which blood pressure is chronically elevated (ICD-9 401-404; ICD-10 110-115). HTN is a potent antecedent of CVD in adults [7] and the relationship is continuous and independent of other risk factors [8]. In adults, HTN is defined as systolic BP (SBP) ≥ 140 mmHg or diastolic BP (DBP) ≥ 90 mmHg. HTN is oftentimes divided into two stages based on severity: stage 1 HTN- SBP between140-159 mmHg or DBP between 90-99 mmHg; or stage 2 HTN- SBP ≥160 mmHg or DBP ≥ 100. Pre-HTN is defined as SBP between 120 and 139 mmHg or DBP between 80 and 89 mmHg on multiple readings. Pre-HTN is not a disease category, but a designation used to identify individuals at high risk of developing HTN [8]. Furthermore, although HTN is directly related to CVD, risk for CVD increases with increasing BP levels starting near the pre-HTN range. For instance, for adults aged 40 to 70 years, each increment of 20 mm Hg in SBP or 10 mm Hg in DBP doubles the risk of CVD across the BP range of 115/75 to 185/115 mm Hg [9]. Similar to adult HTN, childhood HTN has been associated with the early development of CVD [10]. The longer a person lives with high BP the greater their likelihood of adverse effects, potentially leading to an earlier and/or an intensified cardiovascular event.

Prevalence of hypertension in adults. In 2000, it was estimated that the prevalence of HTN was 26% of the adult population globally, and this prevalence would increase by 24% in developed countries by the year 2025 [11]. In North America, the age- and sex-adjusted prevalence of HTN was estimated to be 28% [12]. Nearly 1 in 3 U.S. adults had HTN in 1999-2000, a 30% increase compared to 1988-1994 [13]. In Canada, the prevalence of HTN more than doubled (increase of 60%) over a 10-year period from 1995 to 2005 (adjusted HTN prevalence of 245 per 1000 adults) [14]. The increased prevalence of HTN has been associated with increased obesity levels and an aging, growing population [13].

Risk factors influencing blood pressure levels. There are many well known modifiable and non-modifiable risk factors for BP including age, race/ethnicity, obesity, PA levels, family history (genetics), smoking, and diet. BP is known to rise with age, with the magnitude of the increases different for each individual. The Framingham Heart Study found that there was a linear rise in SBP from the ages of 30 through 84 years, with a concurrent increase in DBP and mean arterial pressure (MAP) [15]. However, after the age of 50 to 60 years, DBP declined and MAP reached an asymptote. The authors suggest the likely cause of the decline in DBP co-occurring with the continual increase in SBP seen with aging, is due to increased large artery stiffness [15]. Furthermore, the decline in DBP seems to be the result of not the cause of the disease process, as these patterns were evident in both normotensive and hypertensive subjects. Race and ethnicity is also known to be associated with BP levels and prevalence of HTN. Non-

Hispanic blacks (36.2%) and Mexican Americans (25.4%) had higher prevalence rates of HTN compared to non-Hispanic whites (23.4%), in the U.S [13]. This dissertation will specifically focus on the risk factors of body fatness, PA, and genetics which are explained in more detail in later sections.

Tracking of BP. Tracking refers to the tendency for an individual to remain in a respective rank over time in relation to their age-sex group [16, 17]. Statistically, tracking is expressed as either a correlation coefficient between a measurement of a characteristic between two points in time, or the percentage of subjects that maintain a relative rank over time. Typically as the time interval between observations increases, the tracking coefficients decrease (e.g., tracking coefficient of 0.60 for observations separated by 2 years and 0.20 for those separated by 20 years). Many of the CVD risk factors, including BP [18-20], fatness [21, 22], PA [23-25], and cholesterol levels [19, 26], have been shown to track moderately well from childhood to adulthood.

The tracking coefficients for BP levels from childhood to adulthood ranges from 0.20-0.60 [27]. Both SBP and DBP track consistently between various studies, with tracking coefficients being larger and more consistent for SBP. This may be a result of SBP being more easily measured and having a smaller measurement error than DBP [16]. One of the first studies to show tracking of BP in children and adolescents (ages 5-18) was the Muscatine Study, which showed tracking coefficients of 0.41 and 0.30 for SBP and 0.27 and 0.18 for DBP in adolescents for observations separated by 2 and 6 years, respectively [17]. In Muscatine, the risk for SBP above the 90th percentile in young adults (age 20-30 years) was 2.4-fold higher among those who had ever exceeded age- and sex-specific SBP 90th percentile values as children or adolescents [28]. The Fels

Longitudinal Study has examined the long term tracking of BP following subjects from birth to the age of 40 years old [29]. Tracking coefficients of 0.24 for SBP and 0.20 for DBP were found for observations separated by 20 years. These results translate into a moderate risk (Relative Risk = 1.9-2.6) for adult HTN among adolescents with high normal BP.

A recent systematic review and meta-regression found the average tracking coefficients to be 0.38 for SBP and 0.28 for DBP [30]. BP tracking was found to increase with increasing baseline age and decrease with increased length of follow up. Specifically, tracking coefficients adjusted for baseline age were 0.43, 0.38, 0.31, and 0.23 for SBP and 0.31, 0.30, 0.21, and 0.15 for DBP for follow-up lengths of <5 years, 5-9 years, 10-14 years, and ≥ 15 years respectively [30]. Furthermore, this review found that BP tracking did not vary drastically based on number of BP measurements or by race. Thus, the overall evidence suggests childhood or early adulthood BP may predict adult HTN [16, 28, 29, 31].

In summary, the prevalence of HTN has been increasing in adults worldwide. The risk of CVD disease increases with increasing levels of BP beginning near pre-hypertensive levels, making regular monitoring of BP levels important. Furthermore, since BP levels increase with age and track from childhood to adulthood, it is necessary to address and minimize the risk of increased BP early in life. Many risk factors for elevated BP have been identified including modifiable risk factors such as obesity and physical activity levels. As the increased prevalence of HTN parallels the obesity epidemic, the prevention of HTN and obesity remains a priority.

BODY FATNESS AND BLOOD PRESSURE IN ADULTS

Obesity. Obesity refers to a state of excess body fat or a state above normal adiposity at which health problems are likely to prevail [6]. Increases in the prevalence of obesity have been observed in many countries throughout the world, including the U.S. [32]. In the U.S., the prevalence of obesity is approximately 32% among adults, and this prevalence increases with age up to the age of 60 [33]. Specifically, 29% of adults aged 20-39 years were obese compared to 37% of adults aged 40-59 years, however prevalence drops to 31% in those 60 and older [33]. This drop could be the result of early mortality in obese individuals, thus they may not live long enough to be included in the study after the age of 60.

The prevalence of obesity significantly increased in the U.S. from 1999-2000 to 2003-2004 in men only (28% to 31%). Similar to HTN rates, the prevalence of obesity is higher in non-Hispanic blacks (45%) and Mexican Americans (37%) than non-Hispanic whites (30%) [33]. The association between obesity and CVD is well-established [34, 35]. Obesity is also associated with the development of cardiovascular risk factors including HTN, type 2 diabetes, dyslipidemia, stroke, and the metabolic syndrome [36]. Furthermore, obese children are at an increased risk of CVD in adulthood [37]. Thus, as previously described, the increasing prevalence of obesity is most likely a major contributor to the increased prevalence of CVD and HTN seen worldwide.

Association between body size, fatness, and blood pressure in adults. Positive associations exist among body weight, height, and high BP. A direct relation between weight and BP has been documented as early as five years of age [38]. A 1 standard deviation (SD) increase in weight has been associated with a 0.3 SD increase in systolic pressure and a 0.08 SD increase in DBP in children and young adults [39]. Height was also independently related to BP at all ages during childhood and adolescence [40]. A 1 SD increase in height has been associated with a 0.03 SD reduction in both systolic and diastolic BP [39]. Thus, for any given weight a taller individual had lower BP. Furthermore, BMI, an indicator of body size that divides weight by height squared (e.g., kg/m^2), has been shown to account for approximately 12-30% of the variation in BP [41, 42].

A positive relationship between body fatness and BP is well recognized in adults [43-45]. In general, cross-sectional studies show low-to-moderate positive correlations (r=0.10-0.50) between various indicators of body fatness (e.g., BMI, waist circumference, skinfolds, percent body fat) and BP in adults [46-52]. In a study of 430 healthy adult men and women, SBP and DBP was found to correlate the strongest with weight (r=0.35-0.51), followed by fat free mass (r=0.34-0.51), BMI (r=0.34-0.48), fat mass (r=0.32-0.41), and percent body fat (r=0.20-0.26) [52]. In this study, all of the correlations were higher in men than women. This study should however be regarded with caution. The results suggest that indices of body fatness can be used in place of measuring or estimating percent body fat directly, since of all the significant correlations percent body fat had the lowest correlations with SBP and DBP. But percent body fat was estimated from a triceps skinfold measure in this study. Furthermore it is generally agreed that central rather than peripheral obesity is more closely related to CVD. Thus, waist circumference or waist-to-hip ratio may have been an important marker to include in this study. However this study does indeed show evidence that several markers of body fatness may be useful if time and/or resources does not permit the measurement of every indicator.

An increased risk for HTN also occurs with increasing levels of BMI. A longitudinal study of adults found that increasing levels of BMI were associated with higher risk of HTN [53]. Multivariate-adjusted hazards ratios of HTN based at different levels of BMI (<25, 25 to 29.9, and ≥30 kg/m^2) were 1.00, 1.18, and 1.66 for men, and 1.00, 1.24, and 1.32 for women, respectively [53]. The relationship between obesity and BP has been observed across all societies, ages, races, and in both sexes [42]. In particular, obese adults have a 2- to 6-fold higher risk for HTN compared to non-obese adults [45, 54, 55]. The positive association between body size, body fatness, and BP is evident. As obesity is associated with increased BP and risk for HTN, it would be expected that adult BP levels will continue to rise in conjunction with the increasing levels of obesity. However this phenomenon is not limited to just obese adults, as BP has been shown to increase across increasing BMI values. Thus controlling weight and body fatness may help prevent elevated BP and/or HTN in adults.

PHYSICAL ACTIVITY AND BLOOD PRESSURE IN ADULTS

General background of physical activity. PA is defined as any force exerted by the skeletal muscles that results in energy expenditure above the resting level [56, 57]. PA has been listed as the number one leading health indicator by Healthy People 2010 [58]. Thus, the measurement of PA is an essential element in the PA sciences.

There are many methods available to measure PA in adults. In general, there is a trade-off between practicality and accuracy among PA measurement tools [59]. Preferably, a PA measurement tool should reliably and validly assess all 4 dimensions of

PA: frequency, intensity, duration, and type. However, no such measurement tool currently exists [59]. Consequently, a large variety of methods have been used to measure PA, including self-report methods such as questionnaire, activity logs, and diaries as well as objective measures of PA such as doubly labeled water, heart-rate monitoring, pedometers, and accelerometers [59].

Physical activity levels in adults. The Centers for Disease Control and Prevention (CDC) and American College of Sports Medicine (ACSM) PA guidelines recommend adults accumulate at least 30 minutes of moderate intensity PA on most days, or 20 minutes of vigorous PA on 3 or more days of the week [60]. Estimates of PA levels in adults are typically derived from large, population based studies such as the Behavioral Risk Factor Surveillance System (BRFSS) or the National Health and Nutrition Examination Survey (NHANES). The BRFSS is a state-based system of telephone surveys about health risk behaviors including PA. Since 2001, BRFSS has used six survey questions about PA in three domains (household work, transportation, and discretionary/leisure time) to quantify frequency, duration, and intensity. These questions are asked in all states once every 2 years. Respondents are asked to provide information on overall frequency and duration of time spent in bouts of 10 minutes or more of PA of moderate intensity (e.g., brisk walking or gardening) and vigorous intensity (e.g., heavy yard work, running, or aerobics) during a usual week. Moderate-intensity activity is described to respondents as any activity "that causes small increases in breathing and heart rate," and vigorous-intensity activity is described as any activity "that causes large increases in breathing or heart rate." Respondents are classified as active based on meeting the CDC/ACSM guidelines [61].

In 2007, BRFSS estimated that 49.5% of U.S. adults did participate in MVPA regularly, and 50.5% did not [62]. These PA levels are actually an increase from the 2001 BRFSS results, which found 53.9% did not meet the adult PA guidelines, whereas 46.1% did. Regardless of the year there are distinct sex differences in PA levels, for example in 2007 only 47.5% of females met the guidelines versus 51.5% of males [62]. In 2001, 56.8% of females reported not meeting the guidelines compared to only 50.3% of males [61]. The BRFSS data also shows a step-wise decrease in regular MVPA with age. For example, in 2007 60.9% of adults aged 18-24 met the guidelines, compared to 51.9% of those aged 35-44, 47.5% of those aged 55-64, and 39.3% of those aged 65 years or older [62].

Thus, recent analysis of BRFSS data shows evidence of an overall trend towards increased PA and decreased physical inactivity among U.S. adults [62]. PA self-reports have been deemed reliable and valid for use in research [63]. Others believe self-report measures of PA to have limitations for people of all ages [64]. Subjective measures of PA may tend to underestimate MVPA, as this type of activity is more sporadic and therefore less memorable and quantifiable [65]. Furthermore, like any subjective measure, the BRFSS data are subject to recall and reporting bias. Few studies have assessed the prevalence of PA in relatively large numbers of adults using objective measures (e.g., accelerometry).

Recently, NHANES 2003-2004 employed an objective measure of PA, by implementing the use of accelerometry in a large sample of U.S. adults. The results follow similar trends to those found in the BRFSS in that PA levels were higher in males compared to females and that PA declines considerably with age [66]. However, NHANES accelerometry based PA data found drastically different results for the overall prevalence of PA, with less than 5% of adults meeting the recommend PA guidelines [66]. These differences may be attributed to multiple factors. The high estimates found from self-reported PA levels could result from misclassification of sedentary-to-light activity as moderate, or from overestimates of activity duration. NHANES accelerometry data bases MVPA on single cut points for all adults from accelerometer counts derived from walking, which may exclude activities involving upper body movement or the additional energy cost of load carrying, that truly were of moderate intensity. However, walking is the most prevalent form of leisure time PA in the U.S. [67], and walking is a large part of occupational and transportation activity which is included in accelerometry data. These cut points may also not be appropriate for all ages, since PA is known to decline with age as well. Also, the NHANES results are based on data obtained from one or more valid day of accelerometry data. Close to 30% of the subjects had only 1 to 3 days of valid accelerometry data, and the 20-39 year old age range was particularly less compliant [66]. Thus if these subjects participated in PA on the other 4-6 days of the week, it would have been missed, which could result in the decrease in prevalence of PA seen in NHANES. Accelerometry data also does not include activities such as biking or swimming, which may also affect the results.

Further analysis of NHANES accelerometry data classified adults into 5 classes based on their weekly MVPA patterns. The results show that the 2 least active classes averaged less than 25 min of MVPA per day and represented 79% of the total study population [68]. Only 1% of the study population was classified into the most active group which averaged 134 min of MVPA per day [68]. An inactive class emerged that averaged only 5 minutes of MVPA per day and represented almost 34% of the total sample population [68]. Thus it can be seen that as a whole, a very large proportion of U.S. adults obtain low levels of MVPA.

Overall the results show men are more physically active than women and that PA significantly declines with age [61, 66]. These trends were also apparent in the proportions of adults meeting current PA guidelines, as more men met the guidelines than women and the overall proportion of adults meeting PA guidelines greatly declined with age [61, 66]. Furthermore, it can be seen that prevalence of adherence to PA guidelines is higher in studies implementing self-reported measures of PA compared to studies implementing accelerometry as an objective measure of PA. Regardless of the method used to assess PA, there are still a substantial number of adults not meeting the recommended amount of weekly PA, and a large proportion that are sedentary.

Association between physical activity and blood pressure. Of the independent risk factors for CVD, physical inactivity is the most prevalent modifiable risk factor, followed by smoking, elevated cholesterol, and HTN [69]. The landmark Harvard Alumni Study found that men with weekly energy expenditures greater than 2000 kcal/week had a 39% decrease in CVD and 24% reduction in cardiovascular mortality [70, 71]. The estimated population attributable risk for CVD mortality associated with physical inactivity was 16%, compared to 9% for HTN [70]. Subsequent studies have also found a decreased risk of mortality in those regularly participating in PA [72, 73], and this reduced risk occurs even in individuals participating in PA levels below the recommended amount [74]. Further studies have replicated these results [75], including a meta-analysis showing that CVD risk decreases linearly with increasing percentile of PA

[76]. Thus it is clearly evident that engaging in PA, whether below the recommended guidelines or above, results in a reduction of health risks including mortality.

Exercise, or increased habitual PA, has also been widely established as a cornerstone therapy for the primary prevention, treatment, and control of HTN in adults [77]. Several studies, including the Harvard Alumni Study [78, 79], have shown higher levels of PA associated with a reduced incidence of HTN [77]. In the CARDIA study, over 15 years of follow up showed that individuals who were more physically active compared to less active experienced a reduced risk of HTN (hazard rate ratio=0.83) after adjustment for covariates [80].

Furthermore, chronic endurance exercise training reduces resting BP in normotensive and hypertensive individuals. The evidence shows that chronic endurance exercise can decrease resting BP by 3-7 mmHg for both SBP and DBP [77]. The magnitude of training responses vary widely by study, but typically increase as a function of initial BP levels, being more pronounced in patients with mild to moderate HTN than in normotensive subjects. A meta-analysis of 54 RCTs showed significant decreases of 3.84 mmHg for SBP and 2.58 mmHg for DBP with aerobic exercise training and reductions were found in all BP and weight groups [81]. Another meta-analysis of 35 training studies focusing solely on normotensive adults, found overall post-training reductions in resting SBP of 4.4 mmHg and 3.2 mmHg for DBP [82].

Many studies have found a decreased risk of HTN in physically active compared to sedentary individuals as well [53, 77, 79, 80, 83-85]. The CARDIA study found that men participating in higher levels of PA had a 27% reduced risk of HTN compared to sedentary men [80]. A longitudinal study of adults found that regular PA reduced the risk of HTN in both men and women, and in both normal weight and overweight adults, as well as after controlling for baseline BP levels [53]. Overall, the level of evidence for exercise training and reduction of HTN falls into evidence category A which means that the results from endpoints of well-designed RCTs provide a consistent pattern of findings from a substantial number of studies [77].

It is also important to note that although resting BP may not decrease in all normotensives after training, this does not mean that these individuals did not improve their overall BP profile as assessed by ambulatory BP. In adults, the exercise training induced weighted net reduction in ambulatory BP was 3mmHg for both SBP and DBP [77]. Aerobic training has also been shown to decrease exercise BP at a fixed workload. The weighted net training reduction in exercise SBP has been shown to be 7 mmHg [77]. Also, in the HERITAGE family study it was shown that a 20 week endurance training program in healthy adults led to small changes in resting BP, but led to substantial and clinically important reductions in exercise BP (decrease of 7 and 3.5 mmHg in SBP and DBP, respectively) [86].

Acute bouts of endurance exercise have also been shown to induce a phenomenon known as post-exercise hypotension, which can last for several hours after exercise [87]. Post-exercise hypotension is particularly significant for hypertensive individuals as it lowers the BP to normal levels during a major portion of the daytime, when BP is typically at its highest level [77]. This effect over the course of many years may reduce the risk of later onset of HTN.

There are several possible reasons for the small and inconsistent results found in studies involving PA and BP in adults including the aforementioned type of BP measured (i.e., resting, ambulatory, exercise), as well as subject issues (i.e., inclusion of hypertensive subjects), methods used to measure PA, the possible role of diet, and genetic factors. In studies examining the effects of PA/exercise on BP, a majority of the subjects are normotensive. Furthermore there is difficulty in getting a true sedentary control group in RCTs. It has been concluded that when evaluating the role of PA in reducing the risk of HTN, it is important to recognize that benefits may come from an active lifestyle independent of a formal exercise training program [88].

The method through which PA is measured may influence its relationship to BP. The previously mentioned studies all involved subjective measures of PA, mostly self-report questionnaires. Few studies exist that examine the association between BP and PA, while employing objective PA measures. In adults, PA was assessed by heart rate monitoring with individual calibration of the relationship between heart rate and energy expenditure in the Isle of Ely Study [89]. This study showed significant differences across quintiles of PA, and PA level exhibited significant but low correlations to systolic ($r = -0.13$ men, -0.19 women) and diastolic ($r = -0.17$ both sexes) BP [89].

Furthermore, although beyond the scope of this dissertation, it is apparent that diet can affect BP levels. Thus, diet is oftentimes controlled for in studies involving predictors of BP, such as exercise. Other studies have compared the combined influence of diet and exercise to diet alone on BP [90-93]. A study by Becque et al randomized 63 obese children into a dietary intervention or dietary intervention plus exercise for 20 weeks [90]. SBP decreased by 16 mmHg in children apart of the diet and exercise group, while children in the diet only group experienced a significant decrease of 10 mmHg, and SBP in the children of the control group was unchanged [90]. Both intervention groups of children lost similar amounts of weight (~2 kg), suggesting that aerobic PA reduces BP above and beyond diet and weight loss alone [90]. A RCT in non-obese adults found that caloric restriction and exercise both individually resulted in substantial improvements in CVD risk factors, and specifically no difference between the caloric restriction or exercise groups in terms of changes in BP after one-year [94]. Thus the individual effects of diet and exercise on BP may be comparable in adults.

Genetics of BP response and PA levels. Individual BP responses in the HERITAGE study ranged from large decreases to no changes, or even to slight increases. Thus, the HERITAGE study has shown that some people may exhibit more pronounced BP responses to endurance training than others, despite identical training programs and similar initial BP levels [95]. This kind of variation is an example of normal biologic diversity and most likely originates from interactions with genetic factors [95]. Furthermore, it has been suggested that genetic factors account for 17% of the reduction in resting SBP following exercise training among adults after controlling for baseline levels and age [96]. Genetic factors may not only influence individual BP response to exercise, but also levels of PA. The first phase of QFS found that genetic factors accounted for 29% of the variance of habitual PA [97]. This is in agreement with the heritability found in QFS for different types of PA phenotypes, such as 19% for total PA and 25% for an indicator of physical inactivity [98]. Evidence from the Framingham Family Study revealed that active fathers were 3.5 times more likely to have active offspring and active mothers 2.0 times more likely to have active offspring than inactive fathers or mothers, respectively. Furthermore, offspring from parents that were both active, were 6 times more likely to be active than children of two inactive parents. These results suggest that genetics along with other factors transmitted across generations, predispose offspring to be active or inactive.

In summary, PA levels decline with increasing age and males are more active than females. Few adults are meeting the current PA recommendations of ≥ 30 min of MVPA on most days. In adults, PA has been shown to decrease resting and exercise BP both acutely and longitudinally. In studies examining the association between PA and BP in adults it is important to consider changes in exercise as well as resting BP, to include hypertensive as well as a control group of adults, and to use accurate measures of PA. The role of diet on BP has been shown to be important, but PA has been shown to be independently associated with reduced BP. Lastly, sex differences in PA and BP levels along with the wide range of BP responses to identical training, highlights the importance of considering other factors that may influence BP such as genetics. Furthermore, genetic factors have been shown to influence both BP and PA levels in adults.

GENETICS OF BLOOD PRESSURE

Genetic aspects of blood pressure. Given the low-to-moderate correlations between PA, obesity, and BP among adults it seems important to consider that other factors may explain the remaining phenotypic variance in resting BP. A possible explanation for the unexplained variance in BP of adults could be the result of genetic variability between individuals. It is well-known that familial factors influence BP as children from families with HTN tend to have higher BP than children from normotensive families [99]. The estimated heritability of BP is 30% [100] and recent genome wide scans have identified numerous chromosomal regions and possible candidate genes which encode components of physiologic pathways pertaining to BP regulation [101-104].

DNA sequence variations in these candidate genes are then compared between hypertensive patients and normotensive controls to examine if an association exists. However, association studies have produced mixed results [105], which is more than likely due to the fact that BP is a complex, polygenic trait and each candidate gene contributes a small portion to the overall variance in BP. The most well-studied candidate genes for BP include angiotensinogen and ACE [95]; however, factors associated with thrombosis and fibrinolysis (e.g., PAI-1) are currently being studied more extensively [106, 107]. Although some chromosomal regions are specific to BP, other regions involve other phenotypes such as obesity, suggesting pleiotropy.

Plasminogen activator inhibitor type-1 gene. Until recently, PAI-1 had not been directly examined as a candidate gene for BP [108], although its association with BP has been reported numerous times [109]. Furthermore, PAI-1 is located on chromosome 7q (7q21.3-q22.3) [110], a region previously identified as having a relationship with BP [102, 104, 111]. PAI-1 is the primary inhibitor of fibrinolysis. Fibrinolysis is defined as the capacity to lyse inappropriate or excessive clots and the fibrinolytic system degrades fibrin clots in blood vessels through the actions of its main enzyme, plasmin [112]. The inactive plasma enzyme plasminogen serves as the precursor to active plasmin. Tissue plasminogen activator (tPA) is the prominent activator of fibrinolysis, through rapid conversion of plasminogen to plasmin, which then binds to fibrin and degrades the clot (Figure 1). PAI-1 binds to tPA forming an inactive complex, consequently inhibiting fibrinolysis (Figure 2). Therefore, fibrinolytic potential is largely determined by the balance between tPA and PAI-1 levels.

Elevated levels of plasma PAI-1 have been shown to be the primary cause of impaired fibrinolysis and are associated with CVD [113-117], HTN [109, 118-121], obesity [122-124], metabolic syndrome [125, 126], and stroke [127]. For example, over 10,500 men were followed in the Prospective Epidemiological Study of Myocardial Infarction (PRIME) and the OR for CVD associated with a 1 standard deviation (SD) rise in PAI-1 level was 1.38 (95% confidence interval (CI): 1.27-1.49) [117].

Relationship of PAI-1 to body fatness and BP. A heritability study in families showed that 35% of the variance in PAI-1 levels was explained by age, sex, BP (both SBP and DBP), and BMI [128]. BMI accounts for approximately 12-16% of the BP variance in young healthy men [41, 129]. Previous studies have shown that BMI also positively correlates to PAI-1 levels [117, 118, 124, 129-136], with obese individuals having a higher concentration of PAI-1 than non-obese individuals. For instance, the PRIME cohort study has shown the correlation between BMI and PAI-1 levels to be 0.39 [117]. In a study of over 1000 men and women, PAI-1 levels were found to be positively correlated with BMI (r = 0.40) [131]. A smaller study found the correlation between PAI-1 levels and BMI to be even higher, at r= 0.66 [133]. In the Framingham Offspring Study, increasing quintiles of BMI were associated with increasing levels of PAI-1 in both men and women [136]. In adults, the Caerphilly Study showed PAI-1 activity increased with increasing BMI, with obese men having a 50% increase in PAI-1 activity compared to normal weight men after adjusting for lifestyle variables [124]. In fact, a study found that not only did the obese patients have significantly elevated PAI-1 levels compared to lean controls, but obese patients' PAI-1 levels reached pathological values comparable to a hypofibrinolytic state [135].

Also, PAI-1 deficiency in mice has been shown to prevent obesity and insulin resistance even when fed a high-energy diet [137, 138]. Similar results were shown in genetically obese (ob/ob) mice lacking the PAI-1 gene (-/-) [139]. The PAI-1 $^{-/-}$ ob/ob mice weighed significantly less and had improved hyperinsulinemia as compared to wild-type PAI-1 $^{ob/ob}$ mice [139]. These results suggest that PAI-1 levels may play a role in the development of obesity. PAI-1 deficiency in mice has also been shown to prevent HTN [140]. Higher PAI-1 levels may reflect endothelial dysfunction [141], an important component of HTN, by accelerating perivascular and medial fibrosis [142]. Suppression of PAI-1 in animal models with HTN has been shown to protect against these adverse vascular changes [140]. A review in humans showed patients with HTN have significantly elevated PAI-1 levels as compared to normotensive controls [109].

Furthermore studies have shown a positive relationship between PAI-1 and BP in adults [129, 135, 143]. A stepwise increase in PAI-1 activity was demonstrated in normotensives through borderline hypertensives and untreated hypertensives to the highest values found in the treated with antihypertensive drugs group [119]. The Framingham Offspring Study showed PAI-1 levels increased as a function of increasing SBP and DBP, and this correlation persisted after adjustment for age, BMI, smoking habits, alcohol intake, diabetes mellitus, and serum levels of total cholesterol and triglycerides [120]. PAI-1 levels were also shown to be significantly and positively associated with longitudinal changes (mean 3 yrs) in SBP in the Framingham Offspring Study [142].

Epidemiological studies have supported these clinical findings with low to moderate correlations between PAI-1 and BP. In general, low-to-moderate positive correlations (r=0.10-0.40) between PAI-1 and BP in adults have been reported [109, 119, 120, 124, 129, 134, 135, 142, 143]. PAI-1 activity showed significant correlations of 0.12 with SBP and 0.16 with DBP in the Caerphilly Study [124]. In a case-control study PAI-1 levels correlated with SBP in obese subjects only (r=0.38), suggesting that the relationship may be stronger at the higher end of the body fatness spectrum [135]. In a study of adult men, PAI-1 activity showed significant correlations of 0.27 for both SBP and DBP [143]. The PAI-1/BP relationship is stronger at the high ends of the BP spectrum as well. Individuals with HTN have significantly higher PAI-1 levels than normotensive individuals [109, 143].

A positive relationship exists between obesity and BP and PAI-1 levels in adults. However, few studies have shown evidence for both of these relationships (i.e., PAI-1 and BP; PAI-1 and body fatness) in the same cohort. A study of mildly hypertensive adults found individual positive correlations of 0.39 between PAI-1 and BMI and 0.24 between PAI-1 and SBP, but did not analyze the data using all 3 variables simultaneously [129]. A study of men found that hypertensive individuals had significantly higher BMI and PAI-1 levels compared to normotensive individuals, but these data were used for descriptive purposes only and were not analyzed any further [143]. In a study of emerging atherosclerotic risk factors in children and adolescents, it was found that PAI-1 levels were significantly higher in hypertensive and obese children compared to healthy controls, with children that were both hypertensive and obese exhibiting the highest PAI-

1 levels of any group [144]. Further studies are needed to examine the combined effects of adiposity and PAI1 levels on BP.

Association between physical activity and PAI-1 levels. Fibrinolytic responses to acute bouts of exercise and adaptations to chronic aerobic exercise have been studied extensively. Two notable reviews reached the same overall conclusions with regards to PAI-1, in that PAI-1 activity decreases in response to acute exercise as well as in adaptation to chronic exercise [145, 146]. For example, PA was inversely associated with PAI-1 activity in adults of the PRIME study (r= -0.06) [117]. The magnitude of the decrease in PAI-1 activity to acute bouts of exercise is dependent upon the intensity and duration of exercise, as well as possibly related to training status and sex of the individual [146]. Changes in fibrinolytic activity seem to occur in a threshold pattern, with exponential changes in activity occurring once threshold intensity is reached [147-149]. Duration may also be of importance as large changes in fibrinolytic activity including decreases in PAI-1 have been observed in long duration exercise, with studies including lengths of 1 hour up to the duration of a marathon run [146]. However a study performed in our laboratory showed that higher intensity, shorter duration (20 min) exercise resulted in greater fibrinolytic response (e.g., greater PAI-1 decrease) than moderate intensity, equicaloric exercise of longer duration [147].

Chronic adaptations to exercise are exhibited by habitually active individuals having the lowest resting PAI-1 levels when compared to inactive individuals [145]. The authors explain that this effect has been repeatedly demonstrated using various exercise intensities and durations [145]. However, El-Sayed found that high-intensity training significantly decreased PAI-1 activity, but low-intensity training did not [150].

Furthermore there may be an effect of aging, as training elicited significant changes in older (60-82 year olds) but not younger (24-30 year olds) adults [151]. Training status may also affect PAI-1 response to exercise as resting levels of PAI-1 have been shown to correlate with maximum oxygen consumption [152, 153]. Overall there does not seem to be differences in fibrinolytic response to exercise in trained versus untrained or healthy versus patients with CVD per se (i.e, both groups experience decreases in PAI-1 with exercise) [146]. However resting levels of PAI-1 are higher in sedentary individuals and patients with CVD, causing a possible blunted response to exercise as there is more PAI-1 present to inhibit fibrinolysis regardless of the transient exercise induced decreases.

Sex differences also exist in PAI-1 response to exercise. A study involving 6 months of endurance training found exercise training decreased PAI-1 activity more in men than women [154]. Decreases in PAI-1 levels (-31%) resulting from weight reduction, healthy diet, and increased PA were found in the intervention group only from a diabetes prevention study of overweight and obese adults [155]. Weight reduction was the most important factor explaining the decrease in PAI-1 in the intervention group [155]. These decreases in PAI-1 were found to persist through a 3-year follow-up of a sub-group of 97 subjects from the same study [155]. Additionally, changes in PAI-1 over the 3-year follow-up period were significantly associated with the number of lifestyle changes made during the first year including fats in diet, weight change, and exercise [155]. These results suggest that elevated PAI-1 levels are mostly reversible by lifestyle changes, especially those geared towards weight reduction (i.e., PA and diet) in overweight/obese adults.

Studies of fibrinolytic response to exercise have also been performed in children. A clinical study of 102 obese children and 105 age and sex matched controls was performed to examine the effects of a 3-month period of treatment to reduce weight on PAI-1 levels [156]. Treatment for reducing weight included nutritional counseling and increased daily PA, including aerobic exercise one hour per day for 3 days per week. A significant decrease in PAI-1 levels was found in obese children as compared to the control group [156]. However, the decrease in PAI-1 levels was only found in the obese children who had a reduced BMI (experienced weight loss) compared to obese children who did not experience weight loss [156]. Furthermore, the largest decreases in PAI-1 levels were observed in the obese children with the highest baseline PAI-1 levels. Thus weight loss resulting from increased PA and nutritional counseling improves elevated PAI-1 levels in obese children, including the highest risk obese children (e.g., obesity and elevated PAI-1 levels).

In another study of obese children (n=43), PAI-1 levels were measured before and after 4 months of physical training offered 5 days/week (mean attendance = 4 days/week) [157]. The training did not result in significant changes in PAI-1 (-1.9 μg/L ± 31.2). However, there was a wide range of individual variation in the response to training within these obese children (range of -69.0 to 54.0 μg/L for changes in PAI-1) [157]. This variation may be representative of genetic differences between individuals. In their review, Womack et al suggest the possibility of a substantial genetic influence (e.g., polymorphisms) on the fibrinolytic response to exercise, which according to the authors represents an obvious area of future research and future studies should further evaluate the role of polymorphisms on this response [146].

Genetic component of PAI-1 levels. There is a significant genetic component of resting PAI-1 concentration. The Family Heart Study, a study of 512 families from 4 U.S. communities, showed age and gender adjusted familial correlations for PAI-1 levels ranging from 0.09 (mother-son) to 0.29 (sisters), with an average of 0.16 among all first-degree relatives [158]. A study of Spanish families suggests that approximately 30% of the variance in circulating PAI-1 is related to genetic influence [159]. A European study found that PAI-1 levels had a heritability of 24% after taking into account age, sex, and associated polymorphisms [128]. Twin studies have produced heritability estimates ranging from 42% [160] to 71% [161]. In the Family Heart Study, age, anthropometric variables (height, weight, waist-to-hip ratio), and lifestyle variables (including smoking status, alcohol intake, and PA levels) together explained 31% (men) to 42% (women) of the variation in PAI-1 antigen levels [158]. Furthermore, individual-specific environmental factors were found to explain 36% of the variance in PAI-1 levels [160]. Thus it is hypothesized that both environmental factors and gene polymorphisms are involved in the regulation of plasma levels of PAI-1 [160].

PAI-1 4G/5G polymorphism. A common, single guanosine insertion/deletion polymorphism (four or five guanine bases; 4G/5G) has been identified in the promoter region of the human PAI-1 gene, -675 base pairs from the transcription start site [162]. Both alleles (4G and 5G) bind a transcriptional activator, whereas the 5G allele also binds a repressor protein, which decreases binding of the activator due to interference caused by steric hindrance [163]. Thus the 4G/5G polymorphism affects transcription of the PAI-1 gene, and has been shown to explain 56.4% of the calculated heritability of plasma PAI-1 levels [164]. Possible genotypes and general population distribution of each in

Caucasians are 4G/4G (26%), 4G/5G (50%), and 5G/5G (24%) [165], which is in agreement with Mendelian distribution/Hardy-Weinberg equilibrium. The allele frequencies for 4G and 5G respectively, are approximately 0.53 and 0.47 [162]. PAI-1 levels increase in a step-wise manner with regards to the 4G/5G polymorphism with the 5G/5G genotype having the lowest levels, 4G/5G intermediate levels, and 4G/4G the highest levels [162].

Adults homozygous for the 4G polymorphism (4G/4G) exhibit higher levels of PAI-1 [162, 166, 167] and are at increased risk of coronary heart disease (CHD) [168, 169] including myocardial infarction [163, 170, 171], HTN [108], obesity [135, 172], and diabetes [173]. A meta-analysis of 9 studies (1,521 cases and 2,120 controls) found an overall OR for CHD comparing 4G/4G to 5G/5G individuals of 1.30 (95% CI: 1.07-1.58) [168]. The results of the meta-analysis, stratified by subject risk level, showed a significant difference in the effect of the 4G/4G genotype on low- and high-risk populations, with 4G/4G carriers from high risk-populations having twice the risk of CHD [168]. A more recent meta-analysis of 37 studies (11,763 cases and 13,905 controls) of the PAI-1 4G/5G polymorphism found a per-allele relative risk of the 4G allele for coronary disease of 1.06 (95% CI: 1.02-1.10) [169]. Since possessing even one 5G allele seems to be beneficial or one 4G detrimental, researchers typically group subjects as 5G carriers or 4G carriers.

A study of normotensive and hypertensive individuals found the 4G/4G genotype to be associated with increased PAI-1 activity in both groups, with hypertensive individuals having higher PAI-1 activity [174]. In obese patients, PAI-1 levels were significantly correlated to SBP in the 4G/5G and 5G/5G genotype groups (r = 0.45 and

0.49 respectively) and DBP in in the 4G/4G and 4G/5G groups (r = 0.42 and 0.39 respectively) [135]. Most recently the PAI-1 4G/5G polymorphism was found as a risk marker for HTN in Spanish adults [108]. The results from this study show that 4G/4G homozygous individuals are at increased risk of HTN (OR=1.9, 95% CI: 1.1-3.0) compared to 5G allele carriers independent of PAI-1 levels, age, BMI, and low-density lipoprotein cholesterol levels [108]. Furthermore, DBP was significantly higher in 4G/4G individuals compared to 5G carriers (80 vs 78 mmHG respectively), whereas SBP was higher in 4G/4G individuals compared to 5G carriers (127 vs 125 mmHg) but the difference was not statistically significant [108].

The differences in PAI-1 levels between obese patients and lean controls were found to be significant in both 4G/4G and 4G/5G genotype groups [135]. Furthermore BMI was significantly correlated to PAI-1 levels in the 4G/4G and 4G/5G groups (r = 0.52 and 0.38 respectively) [135]. When lean and obese subjects were compared in a study of adults, the odds for being obese as compared to lean was threefold higher in 4G/4G homozygous individuals (OR=2.8, 95% CI: 1.6-4.8) [172]. When grouped by possession of the 4G allele, the odds of being obese as compared to lean was twofold higher in 4G carriers (OR=1.9, 95% CI: 1.3-2.9) compared to 5G/5G individuals [172]. In studies of children, the 4G/4G genotype was found to be more frequent in obese compared to non-obese children [175, 176].

The PAI-1 4G/5G genotype may also influence changes in PAI-1 levels resulting from exercise. A RCT found that regular moderate PA did not decrease overall mean PAI-1 levels in either the exercise or reference groups. But individuals in the exercise group homozygous for the 4G allele experienced a significant 36% reduction in PAI-1 levels [177]. Thus adults with the PAI-1 4G/4G genotype are at increased risk for both HTN and obesity, but may benefit more from increased levels of PA.

In summary, BP is a heritable (~30%) phenotype known to be influenced by familial and environmental factors. Through its role as inhibitor of fibrinolysis, PAI-1 is an emerging candidate gene for HTN and known candidate gene for obesity, as increasing levels of PAI-1 are associated with increases in BP and body fat. Furthermore, PAI-1 deficiency in mice has been shown to prevent both HTN and obesity. Increased levels of PA have been shown to decrease PAI-1 levels, but this response has a wide range of variability and differs by sex suggesting genetic variation in PAI-1 response to PA, which has been shown in one RCT. PAI-1 levels have a large genetic component to them, as a single-base pair polymorphism (4G/5G) has been shown to explain over half the heritability of PAI-1 levels. As such, the PAI-1 4G/4G genotype has been associated with higher levels of PAI-1 and increased risk of HTN and obesity. However few studies have examined the association of the PAI-1 4G/5G genotype on BP levels and how body fatness and PA may influence this association.

GENE x ENVIRONMENT AND GENE x GENE INTERACTIONS

A central concept in multifactoral phenotypes such as BP is the gene-environment (GxE) interaction [178]. GxE interaction implies that in combination the effect of genotype and environment deviates from the additive or multiplicative effects of the two factors [5]. Some studies have examined the GxE interaction in relation to BP levels in adults [41, 179-183]. For example, a recent study indicated that PA or fitness modified the association between DNA sequence variation in the endothelin-1 gene and BP [184].

The DNA sequence variation in endothelin-1 was associated with HTN in low fit subjects, whereas the risk did not differ for high fit subjects [184]. GxE interaction has been detected involving the PAI-1 4G/5G polymorphism, as a study of adults showed the correlations between PAI-1 levels and cholesterol or triglycerides differed according to the 4G/5G genotype [185]. However, no study examines GxE interactions involving the PAI-1 4G/5G polymorphism and BP.

Unfortunately, GxE interactions may not explain all of the variance within BP, as BP is a very complex phenotype. Another interaction that may affect BP is that between genes. Epistasis or gene-gene (GxG) interaction has been widely accepted as an important contributor to the complexity of mapping complex phenotypes such as BP [186]. According to Ma et al, genetic studies that ignore epistasis or GxG interactions are likely to reveal only part of the genetic architecture [187]. Any one candidate gene may only explain a small portion of the variance in complex phenotypes such as BP [188]. However, pleiotropy or GxG interaction can confound the identification of the genetic component of a phenotype, meaning two candidate genes may help explain more of the phenotypic variance. For example, individuals of similar adiposity have been shown to have a wide variance in BP values, which may be indicative of pleiotropy or GxG interaction [189]. A case-control study found that genetic interactions between multiple loci rather than variants of a single gene underlie the genetic basis of HTN seen in their subjects [190]. The authors hypothesize that such interactions may account for the inconsistent findings in previous studies because, unlike their study, prior studies almost always examined single-locus effects and did not consider the effects of variation at other potentially interacting loci. They conclude that some of the confusion from previous studies is probably due to undetected GxG interactions [190].

ACE is one such candidate gene that has pleiotropic and epistatic effects. ACE is an important regulatory enzyme of the renin-angiotensin system (RAS), which is a complex system that plays a critical role in maintaining BP homeostasis. ACE converts inactive angiotensin I into active angiotensin II (Ang II) (vasoconstrictor) and inactivates bradykinin and kallidin (vasodilators). Thus, activation of the RAS results in a vasopressor response mainly through the actions of the ACE enzyme. A polymorphism of the ACE gene consisting of the insertion or deletion (I/D) of a 287-bp fragment in intron 16 has been shown to account for over half the variance in circulating levels of ACE [191]. Specifically, the D/D genotype has been shown to be associated with enhanced conversion of angiotensin I to Ang II, resulting in the highest ACE levels of the three possible ACE genotypes (II, ID, DD) [191]. The DD genotype has also been indicated to have a positive association with BP and body fatness [102, 192-194].

There is a growing body of evidence that suggests ACE through its association with Ang II, modulates fibrinolysis by affecting PAI-1 levels. Several in vivo and in vitro studies demonstrated that Ang II increases resting plasma PAI-1 activity and PAI-1 mRNA [195-199]. The relationship between Ang II and PAI-1 levels may also be dependent on PAI-1 genotype. A study found that the addition of Ang II stimulated increases in PAI-1 expression in endothelial cells, but this increase was only statistically significant for 4G/4G individuals, who showed a 2-fold increase in PAI-1 activity [200]. Furthermore, pharmacologic inhibition of ACE has been shown to lower PAI-1 levels [197, 201-203]. Similar to the PAI-1 4G/5G genotype, the ACE I/D genotype results in graded ACE levels with II having the lowest ACE levels, ID intermediate, and DD the highest [191]. The ACE I/D genotype is also associated with PAI-1 levels, with the ACE DD genotype having higher PAI-1 levels compared to ACE II and ID individuals [131, 195]. Thus in combination with the PAI-1 4G/5G genotype, individuals may be at an even increased risk for HTN if they are homozygous for both of the high risk alleles (PAI-1 4G/4G and ACE DD).

It is known that genetics and candidate genes may influence individual BP levels. However in combination with environmental factors, genetics or GxE interactions may result in differential effects on BP than when analyzed alone. GxE interactions have been examined using the PAI-1 4G/5G genotype and with BP as the dependent variable, but never simultaneously. Furthermore, GxG interactions may explain even more of the variance in BP since any one candidate gene may by itself have small effects on BP. The ACE gene is a gene known interact with other genes, and is associated with BP. A polymorphism of the ACE gene (I/D) is related to increasing ACE levels, as well as increasing BP and body fatness. Thus in combination with the PAI-1 4G/5G genotype, the ACE I/D polymorphism may help to explain which adults are at increased risk for elevated BP and obesity.

SUMMARY

In summary, CVD is the leading cause of death in the U.S. and there is a strong association between BP and obesity with CVD. In general, BP increases with age, and is moderately influenced by PA and body fatness. However, genetics also play an important role in determining individual BP levels. PAI-1 is an emerging candidate gene for BP, as higher levels of PAI-1 have been associated with increased BP. In addition, a 4G/5G polymorphism in the PAI-1 gene accounts for a majority of PAI-1 levels, with 4G homozygous individuals exhibiting higher plasma PAI-1 levels, and thus are at increased risk for HTN. Research questions such as if PA and body fatness modify the association between PAI-1 4G/5G polymorphism and BP phenotypes in adults remain unanswered. Incorporating key behavioral and physiologic traits in a genetic association study will hopefully lead to a better understanding of how the interactions between DNA sequence variants and non-genetic variables affect multi-factorial phenotypes such as BP.

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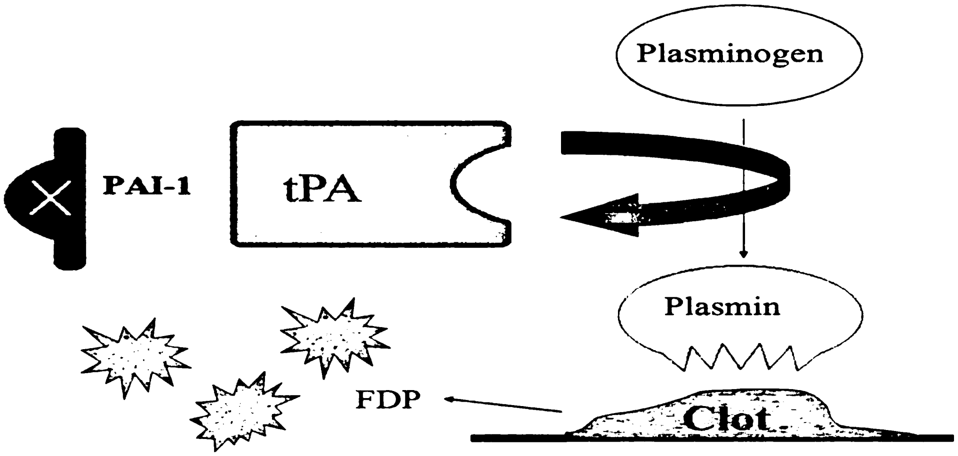

Figure 1: The process of fibrinolysis. Tissue plasminogen activator (tPA) rapidly stimulates the conversion of plasminogen to plasmin, which breaks down small blood clots.

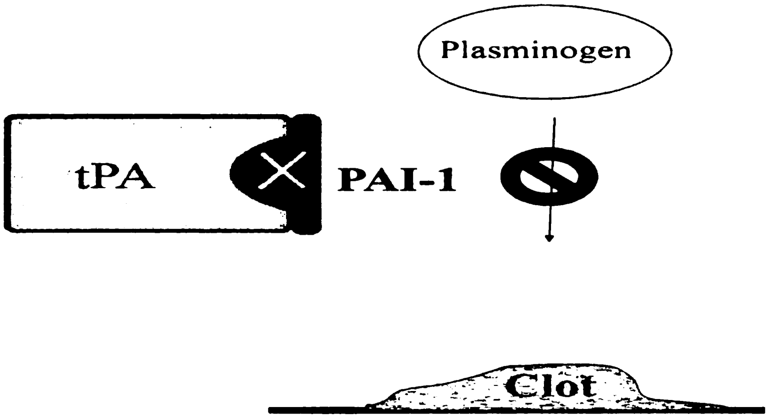

Figure 2: Fibrinolysis is inhibited by plasminogen activator inhibitor-1 (PAI-1), which binds to tPA forming an inactive complex. Thus the blood clot is not dissolved.

CHAPTER 3: METHODS

Study design and Subjects

Subjects included in this dissertation were participants in the Québec Family Study (QFS) which has been described previously in detail [1]. In brief, families of French descent were recruited, mainly through the media and referrals from other participants and colleagues, to participate in a study aimed at examining the genetics of health-related physical fitness. The study was divided into three phases: Phase 1 (1978-1982) involved the initial recruitment and data collection of parents and their offspring living in nearby Québec City (within ~50 miles); Phase 2 (1992-1997) involved re-examination of participants from Phase 1, as well as recruiting new families having one or more obese member; and Phase 3 (1997-2002) involved 5-yr follow up measures of participants from Phase 2, along with the recruitment of new families with at least one obese member. Families were tested for several morphological, physiological, and behavioral variables during their one day visit to the laboratory. During the laboratory visit, blood samples were obtained via venipuncture, and permanent lymphoblastoid cell lines were established for DNA extraction. Age, sex, smoking status, and use of BP medications were determined via questionnaire.

Subjects available for the present analysis were adults (≥17.5 years) with valid data from phases 2 and 3. The sample includes subjects from phase 1 that were followed-up in phase 2. If subjects had valid data from both phases 2 and 3, the data from phase 2 was used. Phenotypes and DNA were available for a total of 894 subjects. A total of 871 individuals (377 men and 494 women) with valid data for age, BMI, BP, and genotype were available for the present study. Informed written consent was obtained from all subjects and the study was approved by the Medical Ethics Committee of Laval University.

Anthropometry

Body weights of all subjects were measured to the nearest 0.1 kg using a standard beam scale, and standing height was measured to the nearest millimeter with a stadiometer. BMI was calculated as weight (kg)/height (m) ^{2}. Overweight was classified as 25 kg/m^2$\leq$ BMI <30 kg/m^2 and obesity as a BMI $\geq$30 kg/m^2. Body density was determined by hydrostatic weighing, and percent body fat was estimated from body density using the Siri equation [2].

Measurement of blood pressure

BP was measured in a 2-hour fasted state early in the morning with a mercury sphygmomanometer and stethoscope. A first reading was taken after a 10-minute rest, followed by additional readings at 2-minute intervals. The mean of 2 consecutive measurements that were <10 mmHg apart on both measures was used for statistical analysis (<1% of subjects required >2 readings to meet the criteria). SBP was determined as the point when the Korotkoff sounds became audible (Kortokoff I) and DBP was determined when the Korotkoff sounds ceased (Korotkoff V). The MAP was calculated as: (SBP-DBP/3) + DBP. A test-retest study on 61 subjects yielded intra-class reliability coefficients of 0.93 and 0.91 for SBP and DBP, respectively [3]. Subjects were classified as hypertensive as defined by a SBP $\geq$ 140 mmHg or DBP $\geq$ 90 mmHg in accordance with the seventh report of the Joint National Committee on Detection, Evaluation and

Treatment of High Blood Pressure [4]; or if they were currently taking anti-hypertensive medication.

Physical activity assessment

A 3-day activity diary was used to estimate total daily energy expenditure and moderate-to-vigorous physical activity (MVPA) [5]. The completed diary included two days that could be recorded on any day of the week, but the third day had to be a weekend day (Saturday or Sunday). In the activity diary, the entire 24-hour day was divided into 96, 15-min periods. For each 15-min period, a code from 1 to 9 was entered. The energy expenditure varies for each code from 1 to 9. Examples of activities included in each category were explained and illustrated in detail to all participants in material given to them for personal use [5]. The total daily physical activity level is defined as the sum of all activity scores (1-9) over 3 days. For the purposes of this study, activities from categories 6-9, which have energy cost equal to or greater than 4.8 METs were used to define MVPA Specifically, the total activity score from levels 6-9 over the 3-day period were used as MVPA. A sample showing a completed activity record for one day along with additional details concerning the activity record can be found in the original paper describing these methods [5]. The intra-class correlation for test–retest reliability of the 3-day activity diary reached 0.96 for mean energy expenditure among 61 subjects re-tested 6 to 10 days after an initial visit [5]. The intra-class correlation was lower (0.71) for the mean frequency of activities in categories 6 through 9. This may be due to a greater fluctuation in these types of activities over the 3 day period, thus making them more difficult to recall, and more inherently variable from day-to-day. A study comparing the 3 day diary from QFS to a temporally matched objective measure of PA found the mean intra-individual correlation between estimated total energy expenditure from an accelerometer and the 3 day diary was 0.74 [6].

PAI-1 4G/5G Genotyping

Genomic DNA was prepared from permanent lymphoblastoid cells by proteinase K and the QIAGEN Blood & Cell Culture DNA Maxi Kit. Polymerase chain reaction (PCR) was performed in a total volume of 6 μL. Twenty ng of genomic DNA were added to a mixture containing a final concentration of 30 μM each dNTP (AMersham Pharmacia Biotech, Inc.); 0.3 U 1xbuffer [10x: Tris-HCl, KCl, $(NH_4)_2SO_4$, and 15 mM $MgCl_2$, pH=8.7]; and 50 nM each for flanking primers (Table 1). After a 5-minute denaturation step at 95°C, 38 cycles were performed as follows: 10 cycles of denaturation at 95°C for 20 seconds, annealing at 55°C for 1 minute; 28 cycles of denaturation at 95°C for 20 seconds and annealing at 52°C for 1 minute. In the same wells, the PCR mixture dNTPs were digested with shrimp alkaline phosphatase (USB), 0.2 U (final volume 11 μL) for 15 minutes at 37°C followed by 20 minutes at 80°C. A minisequencing assay (25) was performed in a final volume of 16 μL (same wells) containing the following: dGTP/ddNTP mix, 1.56 μM each nucleotide, 3.125 nM IRDye tag primer (LICOR) (Table 1), 0.3 U Thermosequenase (Amersham), 0.6xBuffer (10x: 260 mM Tris-HCl, 65 mM $MgCl_2$, pH=9.5). Following a 2-minute denaturation step at 95°C, 30 PCR cycles were performed as follows: denaturation at 95°C for 10 seconds, annealing at 57°C for 30 seconds, extension at 72°C for 5 seconds. Detection was performed on a LICOR automated sequencer (model 4200).

ACE I/D genotyping

The ACE I/D polymorphism was typed with a PCR-based method using three primers, two of which were from outside the insertion sequence (ACE1&3) and one from within the insertion sequence (ACE 2) (Table 1). The final reaction mixture of 15 μl contained 100 ng of genomic DNA, 3.0 mM MgCl2, 200 μM each 2'-deoxynucleoside 5'-triphosphate, 300 nM primers flanking the insertion sequence,140 nM nested primer, 4.7% DMSO, and 1.0 U of Taq polymerase (Pharmacia Biotech, Baie d'Urfe′, PQ, Canada). The PCR protocol (model 9600 thermal cycler, Perkin Elmer,Norwalk, CT) consisted of one cycle at 94°C for 3min, 55°C for 1 min, and 72°C for 1 min, followed by 35 cycles at 94°C for 30 s, 55°C for 30 s, 72°C for 45 s, and finally one cycle at 72°C for 10 min. The PCR products were separated on 3.5% agarose gel and visualized under ultraviolet light after ethidium bromide staining.

Statistical analysis

Data were checked for normality and descriptive statistics were calculated for all variables. Independent t-tests were used to examine differences in continuous variables by sex. Hardy-Weinberg equilibrium was tested by comparing observed genotype frequencies to expected frequencies using a chi-squared test. The main effects of PAI-1 4G/5G genotype (specific aim 1a) and its interactions with adiposity (aims 2a and 2b), physical activity (aims 3a and 3b), and the ACE I/D genotype (aim 4) on BP were tested using the GLM procedure in the SAS Version 9.1 software package. The independent variable was PAI-1 genotype. Covariates included age, sex, smoking, BP medication, MVPA, and BMI or percent fat as all of these variables are known to independently influence BP levels in adults. However, since the main effect of MVPA was not significant in the models, it was excluded in models not testing for the interactions of physical activity. The interaction terms of PAI-1 genotype x BMI (continuous or categorical), PAI-1 genotype x physical activity (continuous or categorical), and PAI-1 genotype x ACE genotype were also added as independent variables within the specific models testing for their effects. The association of PAI-1 genotype with HTN (specific aim 1b) was tested using a multivariate logistic regression model. Data were checked for normality (skewness and kurtosis within ± 1), and BMI was log transformed since it was not normally distributed. Reported adjusted least square means are for untransformed BMI values, but P values were based on transformed BMI values when applicable. Statistical power and p values for alpha-level were discussed in Chapter 1, within the descriptions for each specific aim.

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Table 1: PCR and mini-sequencing primers and PCR product size for genotyping of the PAI-1 4G/5G and ACE I/D polymorphisms.

PAI-1	Forward primer	Reverse primer	Mini-sequencing primer	Amplicon
4G/5G	CGTTGAGGACCACTGCTC	TTGGTCTTTCCCTCACCC	GAGTCTGGACACGTGGGG	418bp

ACE	Flanking primer (ACE1)	Flanking primer (ACE3)	Nested primer (ACE2)	Amplicons
I/D	CATCCTTTCTCCCATTTCTC	ATTTCAGAGCTGGAATAAAATT	TGGGATTACAGGCGTGATACAG	65bp(I)/84bp(D)

CHAPTER 4: RESULTS

GENERAL CHARACTERISTICS

Subject characteristics are presented in Table 2. On average, subjects were 43 years old, 28% were classified as overweight and an additional 27% were obese (55% combined overweight or obese, BMI $\geq$25 kg/m^2). Fifteen percent were classified as hypertensive. Women were significantly fatter and engaged in less MVPA and total PA than men, while men had significantly higher mean DBP and MAP (Table 2).

BMI and percent fat were strongly correlated ($r=0.79$, $P<0.001$) and both showed low-to-moderate correlations with BP measures ($r=0.28-0.34$, $P<0.001$). Total PA and MVPA were significantly correlated ($r=0.65$, $P<0.001$) and both were significantly and inversely correlated with BMI ($r= -0.11$ and -0.10 respectively, $P\leq0.005$) and percent fat ($r= -0.16$ and -0.25 respectively, $P<0.001$), but not with BP measures ($r= -0.01$ to -0.05, $P>0.05$).

Allele and genotype frequencies for the PAI-1 -675 and ACE I/D polymorphisms are shown in Table 3. Half of the subjects were heterozygous (4G/5G) for the PAI-1 polymorphism, while the two homozygous genotypes each approximated one-quarter (27% 4G/4G and 23% 5G/5G). The overall frequency of the 4G allele was slightly higher than the frequency of the 5G allele (0.52 vs 0.48, respectively). In terms of the ACE I/D genotype, the frequency of the D allele was 0.59 with 48% of the subjects being heterozygous (ID) and 35% possessing the DD polymorphism (Table 3). Both the PAI-1 4G/5G and ACE I/D polymorphisms were in Hardy-Weinberg equilibrium.

ASSOCIATION OF PAI-1 GENOTYPE WITH BP AND HTN (Specific Aim 1)

In the overall model for BP, there were statistical main effects for age, sex, smoking, BP medication, BMI, and percent fat ($P<0.001$), whereas total PA, MVPA, and alcohol did not show significant main effects on BP ($P>0.05$). The physical characteristics of the subjects grouped by PAI-1 genotype are shown in Table 4 and represent the adjusted means (Standard Error (SE)). There were no significant main effects of PAI-1 genotype on any of the phenotypes, including the main outcome of resting BP ($P>0.05$). This finding rejects the research hypotheses for specific aim 1a. Furthermore, the prevalence and odds of HTN did not differ by PAI-1 genotype (Tables 4 and 5) which rejects the research hypothesis of specific aim 1b. Only age and BMI significantly influenced the odds of HTN in the logistic regression model which included sex, age, smoking, BMI, MVPA, and PAI-1 4G/5G genotype (Table 5). When BMI was included as a categorical variable (i.e., normal weight, overweight, obese) there was no change in the association with PAI-1 genotype, but obese subjects (OR=2.65, 1.40-5.01 95% CI) and combined overweight and obese subjects (OR=2.28, 1.31-3.94 95% CI) were more likely to be classified as hypertensive than normal weight subjects.

PAI-1 GENOTYPE X ADIPOSITY INTERACTIONS ON BP (Specific Aim 2)

Although there were no main effects of PAI-1 genotype on BP, it may be through interactions with other risk factors (e.g., adiposity and physical activity) that PAI-1 genotype has an effect on BP. Nominal significant PAI-1 genotype x BMI interactions resulted for SBP ($P=0.04$) and MAP ($P=0.04$), but not for DBP ($P=0.14$). However, none of the interactions remained significant after multiple testing was accounted for via

Bonferroni adjustment (P=0.24). Since six different models were run to test the effects of PAI-1 genotype, a p-value of ≤0.008 would be required for statistical significance taking into account the Bonferroni correction. The lack of interactions was further shown by comparing the partial correlations between BMI and BP by genotype. Although slightly lower in the PAI-1 5G homozygotes, there were no significant differences in the partial correlation coefficients between BMI and BP by PAI-1 genotype (Table 6).

The results of the interaction of PAI-1 genotype and BMI as a categorical variable (normal weight: BMI < 25 kg/m^2 vs overweight/obese: BMI ≥25 kg/m^2) on BP are shown in Figure 3. The BMI cut point for overweight (i.e., BMI ≥25 kg/m^2) is nearly the same as the median split in this sample (median BMI=25.4 kg/m^2). Although the mean BP values for the overweight/obese groups were higher than the mean BP values of the normal weight groups regardless of PAI-1 genotype (i.e., main effect for weight status), results of the GLM indicate no significant PAI-1 genotype x weight status group interactions. However, the mean SBP of the PAI-1 4G/4G group was the highest within the normal weight groups.

Since a limitation of BMI is that it does not differentiate between fat mass and fat free mass, the interaction of PAI-1 genotype with percent body fat on BP was also examined. When percent fat replaced BMI in the continuous model, the interaction between PAI-1 genotype and percent fat on BP was not significant (SBP: P=0.74, DBP: P=0.96, MAP: P=0.86). Since BMI and percent fat were strongly correlated (r=0.79) and approximately 159 subjects did not have valid percent fat estimates, future analyses and discussion includes BMI only. Overall, these results reject the research hypotheses for specific aim 2.

PAI-1 GENOTYPE X PHYSICAL ACTIVITY INTERACTION ON BP (Specific Aim 3)

There were no significant interactions between PAI-1 genotype and total PA as a continuous variable for any BP measure (SBP: P=0.38, DBP: P=0.83, MAP: P=0.57). Likewise, there were no significant PAI-1 genotype x tertile of total PA interactions (Figure 4). A trend towards significance was observed for the interaction of PAI-1 genotype x tertile of total PA on SBP (P=0.06). Within the lowest tertile of total PA, subjects with the 4G/4G genotype had the highest SBP (126 ± 2.3 mm Hg) while heterozygotes (4G/5G) had the lowest SBP (118 ± 1.8 mm Hg). However, heterozygotes (4G/5G) in the middle and high tertiles of total PA had the highest SBP values (both 122 mm Hg), although they were similar to other genotype group values (120-122 mmHg). No significant PAI-1 genotype x MVPA interactions were found for BP (SBP: P=0.38, DBP: P=0.26, MAP: P=0.25). There were also no significant PAI-1 genotype x tertile of MVPA interactions (SBP: P=0.56, DBP: P=0.75, MAP: P=0.65). Thus, these results reject the research hypotheses for Specific aim 3.

PAI-1 GENOTYPE X ACE GENOTYPE INTERACTION ON BP (Specific Aim 4)

Results for the gene-gene interaction (PAI-1 4G/5G x ACE I/D) are shown in Figure 5. The only significant PAI-1 4G/4G x ACE I/D genotype interaction resulted for DBP ($P<0.05$). Individuals with combined PAI-1 5G/5G and ACE II or DD genotypes had the highest overall mean DBP values, whereas the combined PAI-1 5G/5G and ACE ID DBP value was the lowest. For those with the PAI-1 5G/5G genotype, DBP was 7% and 5% higher in those also homozygous for the ACE I and D alleles, respectively, compared to 5G/5G subjects heterozygous for the ACE genotype (ID). Across PAI-1 genotypes, the ACE II DBP value was always the highest, whereas ACE ID was the lowest for 4G/4G and 5G/5G and ACE DD the lowest for 4G/5G. However, there was no clear pattern based on combined PAI-1 4G/5G and ACE I/D genotype.

Table 2: Physical characteristics of the sample. Values are mean (SD) for males and females, with the total sample also showing the range of values.

	Total (n=894)			Males (n=382)	Females (n=512)	P
Variables	**N**	**Mean**	**Range**	**Mean**	**Mean**	**value**
Age (yrs)	894	42.6 (16.8)	17.6-93.5	42.5 (16.4)	42.7 (17.1)	0.85
BMI (kg/m^2)	887	27.7 (7.7)	16.8-64.9	27.4 (6.4)	27.9 (8.5)	0.37
Percent Fat (%)	718	28.2 (10.8)	2.9-59.8	23.1 (9.1)	32.4 (10.3)	< 0.001
Total PA score	754	680 (109)	459-1294	704 (125)	661 (91)	< 0.001
MVPA score	744	71 (113)	0-765	101 (141)	48 (78)	< 0.001
SBP (mmHg)	883	120 (19.8)	83-223	121 (17.8)	120 (21.2)	0.39
DBP (mmHg)	883	73 (10.3)	48-115	74 (10.5)	72 (10.1)	0.0006
MAP (mmHg)	883	89 (12.4)	62-148	90 (11.9)	88 (12.8)	0.01

P value listed is for sex difference in mean values for each variable.
According to adult BMI cut points 28% of the sample was classified as overweight and 27% as obese. According to single BP measures BP medication use, 15% of the subjects were classified as hypertensive for statistical purposes.

Table 3: Allele and genotype frequencies for the PAI-1 -675 and ACE I/D polymorphisms.

Polymorphism	**N**	**Frequency (SE)**	**HWE (p-value)**
PAI1 -675	851		0.971
4G/4G	234	0.27 (0.008)	
4G/5G	425	0.50 (0.008)	
5G/5G	192	0.23 (0.008)	
4G	659	0.52 (0.01)	
5G	617	0.48 (0.01)	
ACE I/D	871		0.986
I/I	144	0.17 (0.008)	
I/D	420	0.48 (0.008)	
D/D	307	0.35 (0.008)	
I	564	0.41 (0.01)	
D	727	0.59 (0.01)	

Table 4: Physical characteristics of the sample by genotype. Values are adjusted mean (SE).

	PAI-1 4G/5G genotype			
Phenotypes	**4G/4G**	**4G/5G**	**5G/5G**	**P**
N (max/min)	222/197	407/347	186/164	
BMI (kg/m^2)	27.7 (0.5)	27.4 (0.4)	28.1 (0.6)	0.65
Percent Fat (%)	28.1 (0.7)	27.3 (0.5)	28.0 (0.7)	0.52
Total PA score	680 (8)	684 (6)	681 (9)	0.95
MVPA score	79 (8)	68 (6)	68 (9)	0.46
SBP (mmHg)	123 (1.3)	123 (1.2)	122 (1.4)	0.56
DBP (mmHg)	73 (0.8)	73 (0.7)	73 (0.9)	0.83
MAP (mmHg)	90 (0.9)	89 (0.8)	89 (0.9)	0.88
HTN n (% of HTN)	38 (33%)	48 (41%)	30 (26%)	0.13

All variables were adjusted for age and sex. BP measures were also adjusted for smoking, BP medication, and BMI. P values represent main effects of genotype on each phenotype. Max N value reflects subjects with valid data for smoking, BP medication, BMI, BP, and PAI-1 genotype; whereas min N value reflects subjects with valid data for BMI, PA score, BP, and PAI-1 genotype.

Table 5: Odds ratios and 95% confidence intervals for hypertension by selected hypertension risk factors.

Independent variable	**Odds Ratio**	**95% CI**	**P value**
Age (yrs)	1.07	1.04-1.10	< 0.0001
Sex (M vs F)	1.01	0.60-1.72	0.94
Smoking (never vs current)	1.63	0.65-4.07	0.72
4G/4G vs 5G/5G	0.94	0.48-1.84	0.49
4G/5G vs 5G/5G	0.61	0.32-1.16	0.07
MVPA (score)	1.00	0.99-1.09	0.28
BMI (kg/m^2)	1.05	1.02-1.09	0.0004
***Overwt vs normal weight**	1.10	0.56-2.18	0.18
Obese vs normal weight	2.65	1.40-5.01	0.0006
Overwt/obese vs normal wt	2.27	1.31-3.94	0.003

*Analysis with BMI as a categorical variable was run in a separate model.

Table 6: Partial correlations[1] between BMI and blood pressure by PAI-1 genotype.

Genotype	4G/4G	4G/5G	5G/5G
SBP	0.27	0.30	0.21
DBP	0.31	0.30	0.21
MAP	0.32	0.33	0.24

[1] Controlling for age, sex, smoking, and BP medication.

No significant differences in correlation coefficients between groups (P>0.05).

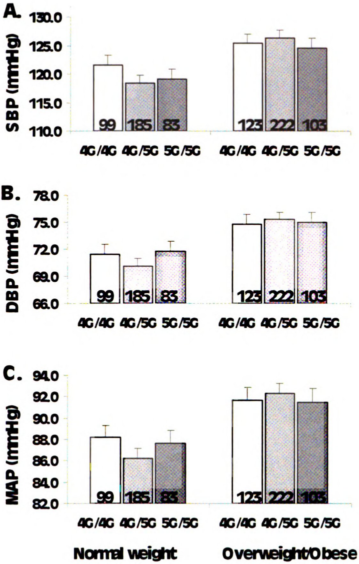

Figure 3: Systolic blood pressure (A), diastolic blood pressure (B), and mean arterial pressure (C) for each genotype of PAI-1 4G/5G by weight status. Significance level of the gene x weight status interaction on: SBP P=0.21, DBP P=0.31, and MAP P=0.20. Number of subjects is indicated inside each histogram bar. Subjects categorized using adult overweight cut points for BMI (≥25 kg/m^2). Values are adjusted for age, sex, smoking, and BP medication.

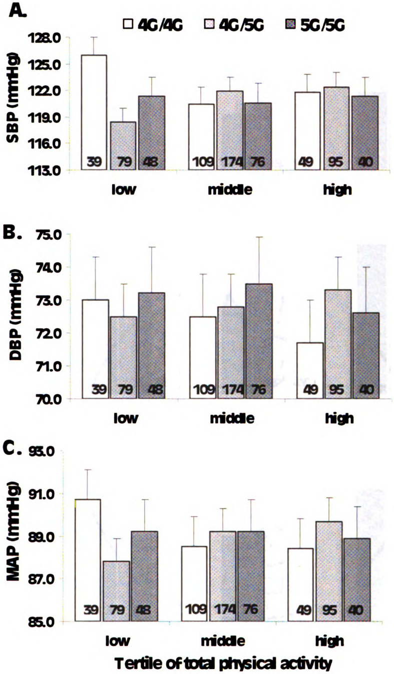

Figure 4: Systolic blood pressure (A), diastolic blood pressure (B), and mean arterial pressure (C) differences for each genotype of PAI-1 4G/5G by tertiles of total physical activity. Significance level of the gene x tertile interaction on: SBP P=0.06; DBP P=0.89; and MAP P=0.47. Number of subjects is indicated inside each histogram bar. Values are adjusted for age, sex, smoking, BP medication, and BMI.

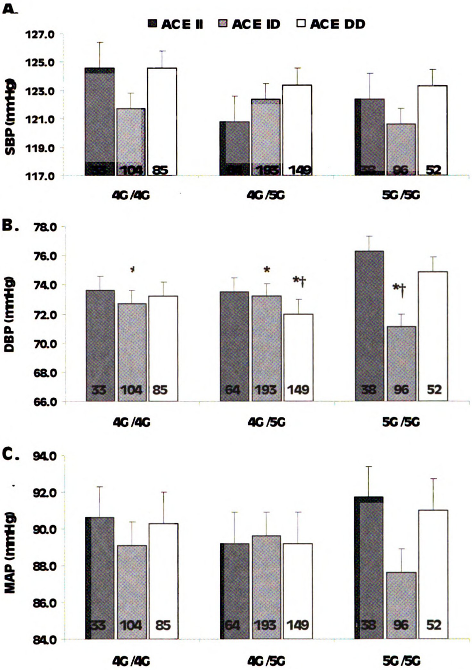

Figure 5: Systolic blood pressure (A), diastolic blood pressure (B), and mean arterial pressure (C) differences for combined PAI-1 4G/5G and ACE I/D genotype. Significance level of the gene x gene interaction on: SBP P=0.71, DBP P=0.03, and MAP P=0.20. Number of subjects is indicated inside each histogram bar. Values are adjusted for age, sex, smoking, BP medication, and BMI.

* P<0.05 for difference compared to DBP of PAI-1 5G/5G, ACE II group.

† P<0.05 for difference compared to DBP of PAI-1 5G/5G, ACE DD group.

CHAPTER 5: DISCUSSION

PAI-1 GENOTYPE AND BP AND HYPERTENSION (Specific Aim 1)

The associations between PAI-1 and variables related to fibrinolysis, thrombosis, and CVD have been studied extensively [1], but its role in HTN is uncertain. A study of knockout mice found that PAI-1 influenced the development of HTN [2]. Although SBP was similar at baseline, PAI-1 $^{-/-}$ mice had significantly lower SBP compared to control mice (112 mmHg vs 141 mmHg, respectively) after long-term exposure to nitric oxide synthase inhibition which is known to induce HTN [2]. Furthermore, a positive association between PAI-1 and BP levels in adult humans has been shown in several cross-sectional and longitudinal studies with hypertensive individuals having the highest PAI-1 levels [3-10]. Although the 4G/4G genotype has been associated with the highest PAI-1 levels, the role of the 4G/5G polymorphism in PAI-1/BP associations has been examined less extensively.

The present study found no difference in BP or HTN between PAI-1 4G/5G genotype (Tables 4 and 5). Although the data are somewhat limited, these results are consistent with three of four previous studies that have examined this association [11-13]. In a study of over 1000 Caucasian Italian adults there were no differences in the prevalence of self-reported HTN by PAI-1 4G/5G genotype [11]. In another study of male smokers (n=208), the prevalence of HTN did not differ by 4G/5G genotype [13]. Aside from having a small sample size of only men, this study was limited by including only smokers which is known to influence both fibrinolysis (e.g., PAI-1 levels) and BP. Furthermore, HTN was defined by BP values ≥160/95 mmHg. In another small sample (n=122) of middle-aged adults (84% men), no differences in HTN were shown by PAI-1 4G/5G genotype [12]. However, PAI-1 genotype was an independent predictor of PAI-1 antigen in hypertensive individuals after adjustment for covariates [12]. Thus, it is possible that the risk for HTN by PAI-1 4G/5G genotype is modulated by PAI-1 levels. Unfortunately, PAI-1 levels were not available in this sample. In contrast to the aforementioned studies showing no association between PAI-1 genotype and HTN, it was recently reported that 4G homozygotes had a higher likelihood of being hypertensive than individuals carrying at least one 5G allele (OR=1.6, 95% CI: 1.1-2.3), and the association was independent of circulating PAI-1 levels, age, BMI, low-density lipoprotein cholesterol, and hyperglycaemia (adjusted OR=1.9, 95% CI: 1.1-3.0) [14]. Furthermore, DBP was significantly higher in 4G homozygotes compared to carriers of the 5G allele (79.6 vs 77.9 mmHg respectively) [14]. Currently, the latter study is the only study to report a positive association between PAI-1 4G/5G genotype and resting BP and HTN.

The discrepancy in results between the present study (and the three others reporting no association) and the latter study reported herein may be due to differences in study design and subject population. Although sample sizes between both studies are similar, the latter study involved unrelated men from Spain, whereas the present sample consisted of French-Canadian males and females from nuclear families. According to Martinez-Calatrava et al., ethnic homogeneity should reduce the probability of stratification within the studies [14]. Also, although the authors found an association between PAI-1 genotype and HTN independent of PAI-1 levels, they did not minimize circadian variations in PAI-1 levels which also may have influenced their results.

PAI-1 GENOTYPE AND OBESITY (Specific Aim 2)

The second aim of this dissertation was to examine the influence of gene-obesity and gene-physical activity interactions on BP. Studies of transgenic and knockout mice demonstrate that PAI-1 influences the development of obesity [16-18]. Furthermore, PAI-1 and its 4G/5G polymorphism are associated with obesity, BMI, and fat mass in adults [19-22]. In a study of healthy adults, the odds of being obese was threefold higher for individuals with the 4G/4G genotype compared to the other genotypes [20]. Approximately 27% of the subjects in the present study were classified as obese; however, the prevalence did not differ by genotype (4G/4G=25%, 4G/5G= 27% and 5G/5G=27%) nor did the mean values of adiposity differ by genotype (Table 4).

Although the interactions between PAI-1 4G/5G genotype and continuous BMI on SBP and MAP were significant (P=0.04), they did not remain significant after accounting for multiple testing (P=0.24). These non-significant results were further substantiated by partial correlations, which found that PAI-1 genotype did not significantly modify the association between BMI and BP (Table 6). Therefore, the original significant interactions between PAI-1 and BMI on SBP and MAP are probably statistical artifacts and not true physiological interactions. However, previous studies have suggested that the association between the 4G/5G polymorphism and CVD may be stronger in high-risk compared to low-risk populations [23, 24]. Thus, this interaction was further tested by grouping subjects based on overweight/obesity status as determined by BMI. It appears that normal weight subjects homozygous for the 4G allele have higher SBP than their normal weight peers of other genotypes (Figure 4). Furthermore, overweight and obese subjects were twice as likely to be hypertensive as their normal weight peers (Table 5). However, although the BP of overweight/obese subjects was higher than normal weight subjects regardless of genotype, there were no significant PAI-1 4G/5G genotype x weight status interactions (Figure 4). Based on these results, there are no viable physiological interactions between PAI-1 genotype and BMI on BP in this sample. It may be that the strong association between fatness and BP masks any possible association between PAI-1 genotype and BP.

In a previous paper from the QFS, PAI-1 polymorphisms were associated with obesity and abdominal visceral fat in women [19]. Specifically, homozygotes for the 5G allele had approximately 50% more abdominal visceral fat than carriers of the 4G allele and had the highest mean percent fat (32%) of all genotypes [19]. Adipose production of PAI-1 strongly correlates with plasma PAI-1 levels [25], and furthermore visceral fat and PAI-1 levels are also positively correlated [26-30]. Thus, it is likely that QFS women with the 5G/5G genotype may have increased plasma PAI-1 levels. However, elevated plasma PAI-1 levels are typically associated with the 4G/4G genotype as previously described [31-34]. Thus, the hypothesized increase of PAI-1 levels in women with the 5G/5G genotype in the present QFS study may attenuate any hypothesized effect of PAI-1 levels and thus the 4G/4G genotype on BP. This could help explain why no interaction between BMI and PAI-1 genotype on BP was observed. However, the production of PAI-1 in adipose tissue is complex and activated by many cytokines, growth factors, and hormones [35] and is likely to be affected by multiple gene variants.

PAI-1 GENOTYPE x PHYSICAL ACTIVITY INTERACTION (Specific Aim 3)

PAI-1 levels have been shown to decrease in response to acute and chronic (i.e., exercise training) bouts of exercise [36, 37], and this response may be mediated by the 4G/5G polymorphism [38]. In the present study, the interaction of habitual, free-living total PA and MVPA with PAI-1 4G/5G genotype did not influence BP. However, subjects with the 4G/4G genotype within the lowest tertile of total PA had the highest mean SBP (Figure 5). When BMI was removed from the model, the P-value for the interaction of PAI-1 genotype x tertile of total PA on SBP decreased from P=0.06 to P=0.02 with BMI in model, but was not significant after accounting for multiple testing (P=0.12). Furthermore, in the model without BMI, the mean SBP of subjects with the 4G/4G genotype in the low tertile of total PA (128.5 ± 2.0 mmHg) was significantly higher than every other group regardless of genotype or tertile (mean 122-124 mmHg). The interactions of PAI-1 genotype with MVPA on BP were also examined. There were no significant interactions between PAI-1 genotype and MVPA for any BP measure. The lack of an overall PAI-1 genotype x PA interactions seems appropriate as the correlations between total PA and MVPA and BP were very low in this sample (r= -0.006 to -0.05, respectively, P>0.05). Thus mean differences in BP by PA level tertiles would not be expected to be found in this sample.

Although PA level and PAI-1 genotype did not have any overall main effects on BP, the lowest tertile may represent individuals who would benefit the most from increased PA levels as the effects of PAI-1 genotype on BP may not be seen until an individual with high genetic risk enters a high risk environment. The results suggest that individuals homozygous for the 4G allele (e.g., high risk gene) participating in low levels of PA (e.g., high risk environment) have elevated SBP compared to the other genotypes. A RCT found regular, moderate-intensity PA did not decrease PAI-1 levels in the overall group, but individuals homozygous for the 4G allele experienced a 36% reduction in PAI-1 levels [38]. Thus, 4G/4G individuals may benefit the most from regular PA (i.e., decreased PAI-1 levels); however the lack of regular PA (i.e., low tertile) could be more detrimental to this group as well. Therefore, although there were no overall interactions between PAI-1 genotype and PA in the present sample, it is possible that 4G/4G subjects in the low tertile of total PA may have increased PAI-1 levels and thus increased SBP. However, this was a cross-sectional study and causes of BP or PAI-1 levels can not be determined from these results.

As previously mentioned, it was also found that overweight/obese (e.g., high risk) subjects had the highest SBP levels. Therefore, it can be hypothesized that being either overweight or obese and having low levels of PA would result in the highest BP levels. A post-hoc sub-analysis was performed to examine the influence of the PAI-1 genotype on this association. The post-hoc analysis indicated that 4G/4G homozygotes who were overweight or obese and in the lowest tertile of total PA (n= 26) had the highest overall mean SBP (131.5 ± 2.8 mmHg) compared to the other genotype-weight status-physical activity groups. However, these results should be interpreted as preliminary since the stratifications divided the subjects into 18 groups with cell sizes ranging from 13 to 91 subjects.

PAI-1 AND ACE GENOTYPE INTERACTION (Specific aim 4)

Since BP is a polygenic trait, the differences in BP between PAI-1 homozygotes and heterozygotes may be a result of interactions with other genes. It is well-known that the renin-angiotensin and fibrinolytic systems interact [39]. It has been hypothesized that adipose tissue, as a source of Ang II, may be the link between elevated PAI-1 levels and HTN, especially in obesity [40]. ACE converts Angiotensin I into active Ang II, thus it may play a role in the association between PAI-1 and BP. This association may be dependent on the epistatic effects of the ACE I/D and PAI-1 4G/5G polymorphisms [41]. It has been shown that individuals homozygous for the risk alleles of both polymorphisms (ACE DD and PAI-1 4G/4G) were 3-times more likely to be diagnosed with early onset of coronary heart disease [42]. The synergistic effect of the ACE and PAI-1 polymorphisms, specifically the interaction of the ACE DD and PAI-1 4G/4G genotypes, has also been associated with clinical conditions such as type II diabetic nephropathy and macroangiopathy [43, 44].

The results of the present study indicate that the epistatic effects of the ACE I/D and PAI-1 4G/5G genotypes on BP are only significant for DBP (Figure 5). Those with the ACE II and PAI-1 5G/5G genotypes had the highest mean DBP, whereas the combined ACE ID and PAI-1 5G/5G mean DBP value was the lowest (Figure 5). These results are in contrast to the hypothesis that individuals homozygous for both risk alleles (i.e., ACE DD and PAI-1 4G/4G) would have the highest BP values. However, a previous study on the risk for myocardial infarction found that the pattern for high and low risk for the ACE genotype differed depending on the associated PAI-1 genotype (i.e., epistasis) [45]. The authors found that the high risk groups were ACE DD with either

PAI-1 4G/4G or 4G/5G, and ACE II or ID with PAI-1 5G/5G [45]. Although those with the ACE II and PAI-1 5G/5G genotypes had the highest DBP (i.e., high risk group) in the present study, no gene-gene interactions for SBP or MAP were observed. However, using nine distinct genotype groups decreased the power to detect BP differences between groups. Therefore, analyses were also performed by further grouping the genotypes as ACE D carriers and/or PAI-1 5G carriers. Results indicated no significant gene-gene interactions for any BP measure (data not shown). Overall, there is no substantial evidence to support a role of epistasis between the ACE I/D and PAI-1 4G/5G genotypes on BP in this sample. However, BP is known to be a complex multi-factorial phenotype influenced by a number of susceptibility genes and multiple environmental determinants. Amidst a plethora of data, additional studies are needed to better understand the genetic architecture of BP and identify the relevant candidate genes and functional gene variants associated with BP levels and their interactions with obesity, physical activity and other possible environmental triggers.

STRENGTHS AND LIMITATIONS

A strength of the present study is its fairly large sample size, along with a well-controlled study design and methodology. Also, the homogeneity of the subject population is a strength of this cross-sectional genetic association study. All of the subjects were Caucasian French-Canadian adults, which should reduce the probability of stratification.

A major limitation of this study is that measures of PAI-1 and ACE were not available. Although gene polymorphisms are associated with plasma levels of PAI-1 and

ACE, polymorphisms explain only about 50% of the total variance/heritability of PAI-1 or ACE levels [46, 47]. Furthermore, results linking the PAI-1 4G/5G polymorphism to plasma levels of PAI-1 have been mixed [15, 34, 48]. Therefore, simply using the 4G/5G genotype as a marker for PAI-1 levels may not capture the entire association between PAI-1 and BP as subjects with any genotype may have elevated PAI-1 levels.

Since this is a cross-sectional study, it does not allow for the establishment of a cause-effect relationship. The mechanisms by which impaired fibrinolysis may cause HTN are still unknown, although shear stress and endothelial dysfunction are thought to play a role [49-51]. It has been suggested that adjusting for CVD risk factors should not be performed in association studies involving PAI-1 because of the possible intermediary role of PAI-1 in the biological pathway of these risk factors and CVD [52]. However, this may be avoided by examining the 4G/5G polymorphism rather than PAI-1 values as done here.

Although BP medication was controlled for in the analyses, a limitation may be that some subjects were currently taking BP medication which artificially lowers BP levels. However, when removing these subjects from the analyses (n=108) the results did not change in terms of overall significance, but the p-value for the interaction between PAI-1 and ACE genotype on DBP went from P=0.03 to P=0.05. A potential limitation is that families were examined, which may influence phenotypic patterns. However, controlling for relatedness amongst family members in separate analyses did not change the results of the present study.

The method of estimating PA levels by the 3-day diary may also be a limitation of the present study as it is subject to recall bias. However, this method has shown good reliability and validity [53, 54]. A recent validation study found the mean intra-individual correlation of estimated total energy expenditure between accelerometry and the 3-day diary was 0.74 [54].

CONCLUSIONS

Overall, the results of this study showed that the 4G/5G genotype was not associated with BP, nor was this association influenced by interactions with adiposity or PA. However, post-hoc analyses indicated that the mean SBP was the highest in overweight and obese adults with the 4G/4G genotype that participated in low levels of PA. Thus, the 4G/4G genotype may be associated with elevated SBP, especially when combined with low levels of PA and overweight. Lastly, no epistatic affects of the ACE I/D and PAI-1 4G/5G genotypes were found on BP. It may be that actual PAI-1 (and ACE) levels were needed to show associations with BP in this sample, rather than the genetic polymorphisms alone. Further studies are needed to better understand the association of the PAI-1 4G/5G polymorphism with BP in adults (see Chapter 6).

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CHAPTER 6: SUMMARY AND RECOMMENDATIONS FOR FUTURE RESEARCH

SUMMARY

This dissertation consists of a study examining the association of the PAI-1 4G/5G polymorphism with BP, including its interactions with adiposity, PA, and the ACE I/D polymorphism. A major strength of this cross-sectional study is the large and homogeneous sample attained from the QFS. In brief, QFS is a longitudinal family study aimed at examining the genetics of health-related physical fitness. This allowed for the inclusion of anthropometric measures such as BMI and percent fat, quality BP measurements, and an estimation of PA levels through a 3-day activity diary. These measures along with the PAI-1 4G/5G and ACE I/D polymorphisms were selected based on their known associations with BP as outlined in Chapters 1 and 2.

The present study had four specific aims which will be summarized herein. Specific aim 1 involved examining the association of the PAI-1 4G/5G genotype with BP and HTN. Results from this study indicate that the PAI-1 4G/5G genotype was not associated with BP or HTN in Caucasian adults. However, PAI-1 genotype may have an effect on BP through interactions with other risk factors such as adiposity and physical activity. For this reason, specific aims 2 and 3 examined the interactions of PAI-1 genotype and adiposity and PAI-1 genotype and physical activity, respectively, on BP. Significant PAI-1 genotype x continuous BMI interactions were found for SBP and MAP, but significance was not maintained after adjusting for multiple testing. Furthermore, the partial correlations between BMI and BP were not significantly different between PAI-1 genotypes. Since the association between PAI-1 genotype and BP may be stronger in high-risk subjects, the interaction between PAI-1 genotype and overweight/obesity status was also examined. The BP of overweight/obese subjects was higher than normal weight subjects regardless of genotype, thus no significant interactions were found. However, among normal weight subjects, those with the 4G/4G genotype had the highest mean SBP. No significant interactions were found between PAI-1 genotype and percent fat on BP. Furthermore, no significant interactions were found between PAI-1 genotype and total PA or MVPA on BP. Once again, high-risk subjects were examined using tertiles of PA, and this also resulted in no interactions with PAI-1 genotype on BP. However, subjects with the 4G/4G genotype within the lowest tertile of total PA had the highest mean SBP. Post-hoc analyses indicated that 4G/4G subjects who were overweight or obese and in the lowest tertile of total PA had the highest overall mean SBP. Lastly, as complex traits such as BP are effected by multiple genes, specific aim 4 sought to examine the interaction between the PAI-1 4G/5G and ACE I/D polymorphisms on BP. A significant interaction was found for DBP only. Subjects with the PAI-1 5G/5G genotype combined with either the ACE II or DD genotypes had the highest overall mean DBP values. For any PAI-1 genotype, DBP was the highest when combined with the ACE II genotype as compared to the other ACE genotypes. Overall, these results demonstrate that the PAI-1 4G/5G genotype is likely to have minimal effects on BP in Caucasian adults.

RECOMMENDATIONS FOR FUTURE RESEARCH

Although this dissertation has added insight into further understanding the association of PAI-1 genotype with BP, additional research is needed in the following areas:

- As the distribution of PAI-1 genotype is known to vary by race and ethnicity, further studies are needed to confirm these results in other racial and ethnic groups.
- Given that PAI-1 and ACE levels are positively associated with BP, actual measures of PAI-1 and ACE levels need to be included in future association studies.
- The preliminary results based on the post-hoc analysis suggesting that individuals with the 4G/4G genotype who are overweight or obese and participate in low levels of physical activity have the highest SBP levels are quite intriguing. However, the present study did not have enough power to statistically detect this association and did not use an objective measure of PA. Thus, population-based association studies implementing objective measures of PA levels are needed to confirm these results in a larger sample.
- PAI-1 may not have an effect on resting BP but may influence other BP phenotypes such as exercise BP, BP response to stress, or BP response to exercise training. Thus, similar genetic studies should be performed from the endpoints of exercise training studies that include these measures as the dependent phenotype.
- Since BP is a multi-factorial phenotype, many genes are likely to be involved in its regulation. Thus, association or linkage studies are needed to identify nearby or other variants and additional mechanisms underlying the possible association between PAI-1, ACE and their polymorphisms and BP.
- Lastly, studies in children and adolescents are needed in order to examine the association of PAI-1 levels and PAI-1 genotype with BP during early life as this

population is likely to be more susceptible to genetic influences. Thus, studying the influence of genetic variants on the interaction between adiposity and physical activity levels with BP in children might bring insight into the early development of BP.