β2-adrenoceptor gene polymorphisms and Hypertension in

African Trinidadians

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Title

Do β2-adrenoceptor gene polymorphisms explain ethnic variation in vascular reactivity?

Key words

Adrenoceptor, Polymorphism, African Trinidadian, Pulse Wave Analysis,

Hypertension

Abstract

Background

Essential hypertension remains a major risk factor for coronary heart disease (CHD) and stroke, and its prevalence is greater, more severe, occurs earlier, and is less well controlled among black individuals than among white individuals, at all ages after young adult hood (Cornoni-Huntley et al, 1989).

In Caucasians, studies have shown that β_2-adrenoceptor polymorphism accounts for the variability in the vascular responsiveness to the agonist isoprenaline (Cockcroft et al, 1994 and Lang et al, 1995). Individuals homozygous for Gln 27 β_2-adrenoceptor showed reduced responses due to chronic down regulation of β_2-adrenoceptor in the vasculature. Therefore, variability in response to isoprenaline was determined by β_2-adrenoceptor gene polymorphism.

Aim and Objectives

This study investigated whether there is a relationship between the Arg/Gly16 and Gln/Glu 27 β_2-adrenoceptor polymorphisms by examining whether the incidence of occurrence is prevalent in African Trinidadians.

In addition, comparison data of vascular responses with arterial compliance using pulse wave analysis (PWA) was correlated.

The study aimed to give evidence if these polymorphisms contributed fully or in part, to determine the disease severity, or response to therapy in hypertensive individuals. It also aimed to prove that PWA is a reliable and therapeutic tool, in diagnosing and treating blood pressure, as reliance on brachial artery recording of blood pressure, alone, is becoming a poor indicator and predictor of risk.

Methods

The study genotyped 408 African Trinidadian subjects for the β_2-adrenoceptor polymorphism and used the technique of applanation tonometry to analyse the central pulse wave, generating information on arterial compliance, left ventricular function and coronary perfusion. Blood pressure was measured in triplicate using a semi-automatic blood pressure meter after 15 minutes of supine rest and bloods lipids assessed using a validated portable lipid cartridge. This was achieved by subjects attending a nurse-led cardiovascular risk clinic.

Results

There is no significant association between the Arg→Gly16 polymorphism and the Gln→Glu27 polymorphism and hypertension in African Trinidadians. Interestingly, the appearance of the Glu27 polymorphism was very uncommon in African Trinidadians and this is constant with findings by Candy et al, 2000.

Conclusion

There is no difference in the frequency of β_2-polymorphisms between normotensive and hypertensive African Trinidadians, and are unlikely to be a contributing factor for essential hypertension. Therefore, hypertension would indicate that it is polygenic with complex gene to gene and gene environmental interactions, through multiple, indirect and intermediate phenotypes and interactions.

For

Randy, Marilyn and Colleen

We is We

Abbreviations

AG	augmentation
AIx (AI)	augmentation index
AMPS	ammonium persulphate
ANOVA	analysis of variance
Arg	arginine
ASO	allele specific oligonucleotide
ATP	adenosine triphosphate
AIx	augmentation
β_2-AR	β_2-adrenoceptor
β_2-ARK	β_2-AR kinase
BMI	body mass index
B/P	blood pressure
cAMP	cyclic AMP
cm(s)	centimetre(s)
CHD	coronary heart disease
CRF	clinical research form
CVD	cardiovascular disease
DBP	diastolic blood pressure
DNA	deoxyribonucleic acid
dNTPs	2' – deooxyribonucleotides
DoH	Department of Health
ECG	electrocardiograph
EDRF	endothelium-derived relaxing factor
EDTA	diaminoethanetetra-acetate acid
ET	endothelin
g	gram
Gln	glutamine
Glu	glutamate
Gly	glycine
GP	general practitioner
HCL	hydrogen chloride
HDL	high density lipoprotein
HR	heart rate
HRT	hormone replacement therapy
H-W	hardy-weinberg
H_2O	water
KCL	potassium chloride
kg	kilogram
Km^2	kilometres squared
kv	kilo volts
l	litre
LDL	low density lipoprotein
L-NMMA	L-N^G-Monomethyl-arginine
M	molar
MAP	mean arterial pressure

mg	milligram
μg	microgram
MgCl$_2$	magnesium chloride
ml	millilitre
μl	microlitre
mM	millimolar
NaCl	sodium chloride
NaOH	sodium hydroxide
ng	nanogram
nmol	nanomole
NO	nitric oxide
NOS	nitric oxide synthase
NSAIDs	non steroidal anti-inflammatory drugs
P	phosphorus
NSF	National Service Framework
p	probability
PCA	pulse contour analysis
PCR	polymerase chain reaction
PP	pulse pressure
PWA	pulse wave analysis
PWA/V	pulse wave analysis/velocity
RAS	renin angiotensin system
RAAS	renin angiotensin-aldosterone system
RFLP	restrictive fragment length polymorphism
RI	reflection index
rpm	revolutions per minute
SI	stiffness index
SBP	systolic blood pressure
SNP	single nucleotide polymorphism
SNS	sympathetic nervous system
Sig	significant
Sq. km.	square kilometre
SPSS	Statistical Package for the Social Sciences
Std	standard
Taq DNA Pol	*Thermus aquaticus* DNA Polymerase
TAE	tris acetic EDTA
TC	total cholesterol
T&T	Trinidad and Tobago
TBE	tris borate EDTA
Tx	triglycerides
U.K.	United Kingdom
UV	ultraviolet
V	volts
VLDL	very low density lipoprotein
WT	wild type

Forward

A Geographical, Cultural and a Historical Perspective of Trinidad and Tobago

Introduction

At the southernmost tip of the Lesser Antilles chain, between the Caribbean Sea and North Atlantic Ocean, just nudging the South American coastline of northeast Venezuela, lie the islands of Trinidad and Tobago (usually shortened to T&T).

These twin islands are the most influential, unexplored and uncontrived of Caribbean islands, rich in indigenous culture. At its centre, Trinidad and Tobago has a strong cultural base with the most diverse and absorbing society of all the Caribbean islands.

I was very privileged to spend my data collection period in these beautiful islands and absorb the abundance and diversity of a colourful culture and landscape, which were well beyond my work.

Oddly, few have heard or explored the island of Trinidad, most know of its' smaller sister island of Tobago as a paradise holiday destination.

Therefore, I feel the reader should have some insight into the history, background and present day influences on African Trinidadians and Tobagonians, that shape their lives, beliefs and their attitudes and possibly their health.

Geography and Climate

Trinidad and Tobago combined cover 5,128 (km^2), its terrain is that of plains and low mountains, with 48% of the land covered in tropical forest and woodland. The climate

is typical of that found on the Equator, tropical with a rainy season but with constant temperatures between 20°C and 33°C, with an abundance of natural growing fruits, flora and fauna.

Figure (I) Map of Trinidad and Tobago

History and People

Trinidad was the first inhabited island of the Caribbean, having been settled by Amerindians from South America as early as 5000 BC. The early settlers were Arawaks, peaceful farmers and fishers, however, after 1000 AD they were joined by Carib tribes with warlike cultures.

When Christopher Columbus landed in Trinidad in 1498, the population numbered around 35,000, most living in coastal areas. However, in 1592, Spanish settlers arrived in Trinidad, largely wiping out the original inhabitants of Arawaks and Caribs, and with the survivors were gradually assimilated.

Despite the Spanish Empire, it had neither the desire nor the resources to develop the island, treating the island as a convenient 'port of call' en route to the riches of South America. The island was repeatedly attacked by the French, Dutch and British, but with this constant political upheaval, brought illegal slave trading from other islands and ships off-loading African immigrants. Trinidad remained under Spanish rule until the British captured it in 1797. However, despite the British occupation, slavery continued under a ruthless military regime. Thankfully, in 1807, the British Government was facing the anti-slavery lobby and by 1834, with the passing of the Act of Emancipation, slave trading was abolished over the British occupied islands of the Caribbean.

By 1888, Trinidad and Tobago was incorporated into a single colony, as Tobago was finally ceded to Great Britain in 1814.

In 1958, the United Kingdom tried to establish an independent Federation of the West Indies comprising most of the former British West Indies. However, disagreement

over the structure of the federation with Jamaica, Trinidad and Tobago's withdrawal soon led to its collapse. Trinidad and Tobago achieved full independence in 1962 and joined the British Commonwealth.

Today, Trinidad and Tobago's people are mainly of African or East Indian decent. Virtually all speak English. Trinidad's East Indian culture came to the island in 1834 with the emancipation of the African slaves. This was to fill the labour shortage that was created by the abolition of slavery.

Other Immigrants

Several companies of [1]black' American soldiers who had supported Britain in its 1812 war against the United States were given grants of land in southern Trinidad, where they founded the villages named after the companies in which they served. Other immigrants, mainly freed slaves from other Caribbean islands, were attracted to Trinidad by high wages. Africans liberated by the British Navy on anti-slave patrols settled in urban areas, becoming craftsmen and construction workers, establishing strong cultural institutions and heritage.

Today, the African population makes up 40% of Trinidad and Tobago's 1.3 million population (U.S Department of State Statistics, 1999), with the remainder consisting of East Indian 40.3%, European 1%, Chinese 1%, mixed 14%, and other 3.7%.

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[1] The term Black is used as a political term to describe communities who have experienced discrimination and racism on the basis of skin colour, religious beliefs and cultural or ethnic identity.

Government

Trinidad and Tobago is a unitary state, with a parliamentary democracy modelled after that of the United Kingdom. From 1962 until 1976, Trinidad and Tobago, although completely independent, acknowledged the British monarch as the figurehead chief of state. In 1976, the country adopted a republican constitution, replacing Queen Elizabeth with a president elected by parliament. The general direction and control of the government rests with the cabinet, led by a Prime Minister and answerable to the bicameral parliament.

Economy

In 1995, successful economic reforms were implemented to allow foreign investment and trade to flourish. However, persistently high unemployment remains one of the chief challenges of the government. The petrochemical sector has spurred growth in other related sectors, reinforcing the government's commitment to economic diversification. Tourism is growing, especially in the Tobago region. This investment is assisting greatly in driving the economy.

Health Provision

The main hospitals in Trinidad are Port of Spain General in the capital and Mount Hope complex in St Augustine. There are also small, well equipped regional hospitals in all of the main towns. Treatment is free at the point of delivery but limited with

medical technology. Those who can afford private health care do so, and most specialist doctors have private practices.

Medication is by prescription but some drugs can be purchased over the counter. This pharmaceutical formulae list is more extensive than in the United Kingdom, and many Trinidadians will seek limited medical advice and cheaper medication for ailments due to cost implications.

As unemployment is high, taxation for health provision is limited and Trinidad is typical of a two-tier health system, not unlike that found in the United States.

Contents

Table of Figures

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Table of Tables

Chapter 5

Chapter 6

Chapter 1

Aims and Introduction

1.1 Background and Policy

In March 2000, The National Service Framework for Coronary Heart Disease (Department of Health (DoH), 2000) set out the United Kingdom government's strategy for tackling heart disease and stroke. The Secretary of State for Health announced that the government's aim was to enable everyone to enjoy better health. Thus, reduction in the rates of coronary heart disease (CHD) and stroke has become a key priority for the health of the nation. Despite a decline in death rates from CHD over the past 10-15 years, the United Kingdom still has the highest incidence of circulatory disease in Europe (WHO, 2000). This trend exists despite recent health improvements and initiatives, making the management of CHD in the UK a major challenge for health care providers and policy makers alike.

Specifically with this framework, it outlined a strategy to build healthy communities and tackling inequalities in health care with further developments of services, which are sensitive to the needs of minority ethnic people and promote a great awareness of their specific health risks. This was supported by Tackling Health Inequalities, 1998.

This highlighted the health needs of the minority ethnic group of [1]African blacks which are specifically considered in the development and implementation of policies aimed at reducing socio-economic inequalities and the reduction of coronary heart and vascular disease, hypertension and stroke. However, extensive research has been implemented to examine whether genetic predispositions to [1]African Blacks may be affected by environmental, climatic, migratory or socio-economic change.

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[1] The term 'Black' is used to describe people of an African origin.

Genetic research has enhanced the evidence base so that health care provision can be sure of what needs to be done and that progress required to reduce particular health risks and equalities in ethnic groups. However, such research is complex and involves different cultures, languages and religious considerations. This requires the full co-operation of ethnic minorities. There is a risk of token involvement, raising false expectation, and the failure to deliver long- standing change to services that maybe insensitive and inappropriate to ethnic needs (Saving Lives: Our Healthier Nation, 1999). Therefore, a specific planned approach will not only answer the questions of genetic predisposition but also address planned care and a lasting approach in health promotion to minimise risk.

1.2 'Accelerated Risk' in African Caribbean People

Hypertension is particularly common in African Caribbean blacks, with prevalence as high as fifty percent over the age of forty years (Cappuccio et al, 1997). Within This group hypertension appear to be more severe and accelerated with onset occurring at an earlier age. (British Hypertension Guidelines, 1999). Within this ethnic group, there are higher risks to complications, in particular stroke, renal failure and left ventricular hypertrophy.

The term 'Black' is used to describe people of an African origin. In the case of this study, those people in the population studied were born and resided in Trinidad in the West Indies and defined their ethnicity as African Trinidadian. For those black people in the United Kingdom, the definition is African Caribbean. As gross phenotypic descriptions, the terms 'African Trinidadians', 'African Caribbeans', and 'Caucasians' take no account of the genotypic variations within each group. Indeed,

part of the purpose of this study is to begin to unravel some of the genetic heterogeneity within and between different ethnic groups. All subjects clearly regarded themselves as African Trinidadians (or Caribbeans) or Black. These terms are used, not only as phenotypic descriptions, but also as social constructs. Nevertheless, they often appear without qualification in scientific papers.

People of African Caribbean decent and living in Western countries have long been known to have a greater disease burden in terms of essential hypertension and stroke (Cornoni-Huntley et al, 1989). However, few studies have been conducted in the Caribbean islands (Kotanko et al, 1997) where essential hypertension is prominent feature. Many people from the Caribbean migrate to and from the U.K. and this may have a genetic impact on predisposition and epidemiology. Thus, essential hypertension remains one of the major risk factors for vascular and heart disease and stroke, and together with diabetes, contributes to an even greater risk in the African Caribbean community (Cornoni-Huntley et al, 1989).

The progression of essential hypertension leads to target organ damage (heart, brain and kidneys), which is more prevalent in African Caribbeans than Caucasians (Calhoun and Opril, 1995). The frequency of co-morbidities in African Caribbean people, such as diabetes and renal disease, make treatment all the more challenging. However, the reasons for this increased severity of hypertension in this ethnic group remain obscure (Kuznar, 2000).

1.2.1 Variation of Blood Pressure

Hypertension accelerates more rapidly in African Caribbean people (Kaplan, 1998). This was first demonstrated in the CARDIA study, a longitudinal study in 5116 United States America (USA) adults aged 18-30 years (Liu et al, 1989). Also, a population-based study found higher prevalence of hypertension and severe hypertension at a younger age in black people than in whites, even after adjustment for body mass index and weight-height ratio (Lackland et al, 1992). In the United Kingdom (UK), a clinic-based study of African Caribbean people who were referred for hypertensive treatment, demonstrated that hypertensive patients were of a younger age than Caucasians (Kattar et al, 2000). This follow-up study showed significantly higher mean values for 24-hr ambulatory systolic blood pressure in Caribbean subjects, compared with Caucasians, despite similar baseline clinic blood pressures. Similarly, a study of nocturnal blood pressure (Harshfield and Trieber, 1999) using 24-hr blood pressure monitoring suggested a blunted response in blacks compared to whites.

1.2.2 Hypertension and drug therapy in African Caribbean groups

Hypertension tends to be very sensitive to dietary salt restriction and in those without evidence of any organ damage, a salt restricted diet will be sufficient to lower and control blood pressure (British Hypertension Guidelines, 1999). However, when drug intervention is needed, the use of diuretics and calcium channel blockers appears to be more responsive in this ethnic group (Sauders et al, 1990; Wright et al, 1991). Beta-blockers and ACE inhibitors have been found to be only marginally effective in monotherapy of African black patients (Sauders et al, 1990; Wright et al, 1991). This is due to the renin-angiotensin system being frequently suppressed. However, this is

overcome in some black patients by giving a combination with drugs that activate the renin-angiotensin system. For example adding a diuretic, calcium channel blocker or alpha-blocker with and ACE-inhibitor or beta-blocker. With resistant hypertension in African blacks a combination of diuretic, calcium channel agonist, ACE inhibitor or alpha- blocker is effective

1.3 Vascular Causes of Essential Hypertension

Coronary artery disease is one of the most frequent accompaniments of raised arterial pressure. Atheroma is not seen in the pulmonary arteries unless there is pulmonary hypertension, indicating a central role for pressure itself in the genesis of atherosclerotic lesions. However, the importance of large vessel coronary heart disease complicating hypertension has probably been underestimated. Modest pressure elevation is common in the population. In severe pressure elevation the problem of stroke and cardiac failure dominate.

High pressure initially induces useful compensatory hypertrophy but later decompensation results in heart failure (Berkin and Ball, 2001).

Arterial compliance is considered as an independent risk factor for cardiovascular disease and stroke and resulting vascular damage that leads to end organ damage may be a combination of structural and haemodynamic changes. What initiates and augments the development of essential hypertension remains largely unknown though extensively investigated.

1.3.1   Structural and functional changes

A feature of hypertension is the increase in size of left ventricular mass. The number of cardiac myocyte cells does not increase but with raised pressure within the

6

vasculature there is cell hypertrophy (Berkin and Ball, 2001). In addition fibroblast and interstitial change occurs. Myocytes represent nearly two thirds of cardiac mass but only a quarter of cell content. With pressure elevation, small vessel changes occur not unlike other tissue responses to raised pressure. This response produces the increase of the wall thickness and a reduction in the wall lumen (Agabiti-Rosei et al, 1988). Continued occlusion of a large vessel leads to further damage to heart muscle and precipitates overt failure in an already compromised hypertrophied heart (Tarazi, 1985, Hartford et al 1983).

With hypertension arterial compliance is reduced (Safar et al, 1992), and until recently it has been difficult to measure arterial compliance non-invasively (O'Rourke et al, 1992; Wilkinson et al, 1998a). A structural change in smooth muscle by thickening causes the vessel lumen to narrow. However, the luminal diameter of the vessel will compensate by stretching. Pulse wave analysis has revealed altered arterial compliance in hypertension (McVeigh et al, 1991), diabetes (Westerbacka et al, 1999 and 2000), even in adolescent Type 1 diabetics (Brooks et al, 1999). Moreover, pulse wave analysis provides strong evidence of an association of increased conduit vessel stiffness with stroke and total mortality.

However, these changes may not be greatly apparent in the microcirculation, as such alterations will affect peripheral vascular resistance. Moreover, it is larger vessels affected by athersclerosis and are more prominent in their contribution to stroke and myocardial infarction.

Numerous hormonal and neurogenic factors have been put forward to contribute to these changes (Blaes and Boissel, 1983; Hartford et al, 1983). The advantages in morbidity and mortality from treatment with beta antagonists and angiotensin

converting enzymes (ACE) inhibitors in patients with impaired systolic function, clinical heart failure after myocardial infarction or at high cardiovascular risk suggest at least two important adverse processes liable to partial correction with treatment. However, the reduction of blood pressure, despite drug intervention will reduce the incidence of heart failure and myocardial infarction (Hansson et al, 1998).

1.3.2 Haemodynamic changes

With established hypertension, particularly in younger adults, increased heart rate and stroke volume with normal peripheral resistance has been reported. Increased peripheral resistance and loss of compliance with increasing arterial stiffness of large arteries is apparent with increasing mean arterial pressure and pulse pressure, which is due to systolic pressure elevation. Endothelial dysfunction plays a part in flow modulation due to impaired nitric oxide synthesis by the coronary endothelium. This leads to the loss of compliance due to systolic wall stress and thus stroke volume is increased (Berkin and Ball, 2001).

1.3.3 Rarefaction

Rarefaction can be termed as either structural or functional in its vascular action. A reduction of density of vessels results in either a lack of vessels (structural) to perfuse tissue or intermittent perfusion of tissue (functional). These structural and functional aspects of rarefraction result in increased vascular resistance. Structural rarefraction resulting in essential hypertension had been identified as early as 1920s (Boas and Frant, 1922; Boas and Mufson, 1923), in the examination of conjunctival capillaries.

1.3.4 The role of vascular endothelium

Endothelium is the layer of cells that is the inner lining of all blood vessels, which plays an important role in the regulation of vascular function. The endothelium releases a number of vasoactive mediators including nitric oxide (NO) and endothelin (ET), which alters vascular tone and arterial stiffness (Cockcroft et al, 1997). Exogenous supply of NO, for example GTN, causes changes in the arterial waveform, indicating reduced arterial stiffness, without a reduction in peripheral blood pressure (Yaginuma et al, 1986). Endothelin is a known potent vasoconstrictor (Gray et al, 1996).

1.3.5 Endothelial Dysfunction

Endothelial dysfunction occurs when there is an imbalance from the production of NO to that of the vasoconstrictor such as ET (Celermajor et al, 1997). Two different mechanisms are responsible for promoting stiffness. Firstly, a direct and functional effect on vascular tone and secondly an alteration in vessel structure (Celermajor et al, 1997). Loss of mitogenic effects of NO, and the enhancement of mitogenic effects of ET will promote vascular proliferation and atheroma formation. In laboratory studies involving the arterial infusion of L-N^G–Monomethyl-arginine (L-NMMA) in the forearm of hypertensive humans, indicates a reduction in endogenous NO production leading to diminished vasoconstriction and an increase in arterial stiffness (Calver et al, 1992; Panza et al, 1993).

Other studies have shown impaired vasodilatation in the arteries of hypertensive patients with the infusion of acetylcholine (Treasure et al, 1993), but the findings of Cockcroft et al, 1994, suggests that stimulated vasodilatation in the forearm vasculature maybe selective in hypertensive patients. This suggests that endothelial

dysfunction maybe selective in its action when exposed to acetylcholine in a subgroup of hypertensive patients.

1.3.6 Constricting factors of the endothelium

The endothelium produces potent vasoconstrictors such as angiotensin II and endothelin-1 (ET-1) (Webb et al, 1990; Yanagisawa et al, 1988). ET-I is a peptitide with commanding effect. Other forms ET-2 and ET-3 have also been found. However, ET-1 is the main contributors to the maintenance of basal vascular tone (Hayes and Webb, 1994) and is altered in such diseases as hypertension.

ET-1 binds to two subtypes ET_A and ET_B. ET_A is the most prominent receptor, which mediates vasoconstriction in the arteries and ET_B mediates the release of endothelium dependent vasodilatory factors and has some resistance in vessels where it affects vasoconstriction.

With this in mind, endothelium produced contracting and dilatory factors are greatly disturbed in hypertension and may be associated with genetic predisposition.

1.3.7 Nitric Oxide (NO)

Nitric oxide maintains vascular tone throughout the cardiovascular system by counteracting vasocontricting mechanisms (Vallance et al, 1989a). The release of nitric oxide is continuous from endothelial cells (Palmer et al, 1992), which specifically maintains blood vessel tone.

In vitro studies have shown vasodilatation when acetylcholine stimulates the synthesis of nitric oxide from L-arginine, (Palmer et al, 1992; Rees et al, 1989). *In vivo* studies indicate that basal production of nitric oxide opposes vasoconstriction mechanisms in forearm vessels (Vallance et al, 1989b). This was with the arterial

administration of L-NMMA, which reduced forearm blood flow by half.

1.3.8 Endothelium and the regulation of arterial stiffness

Arterial stiffness is a key independent determinant of cardiovascular risk (Blancher et al, 1999). Structural components within the arterial wall, mainly collagen and elastin, together with transmural pressure are important determinants of large vessel stiffness (Avolio et al, 1998). However, smooth muscle tone can also influence the stiffness of the elastic and muscular arteries (Gow et al, 1972), suggesting there is also functional regulation of arterial stiffness in vivo.

Nitric oxide (NO) and endothelin-1 (ET-1) are two important mediators released by the vascular endothelium, which exert major, but opposing influences on blood pressure (Haynes et al, 1993), and basal vascular tone (Vallance et al, 1989a). Previous data has recently shown that NO regulates large artery distensibility in vivo (Wilkinson et al, 2002), and this may explain why conditions that are characterized by reduced NO bioavailability are also associated with increased arterial stiffness. However, the role of ET-1 in the regulation of arterial stiffness is, at present, still unclear.

1.3.9 Sympathetic nervous system and the regulation of arterial stiffness

The sympathetic nervous system (SNS) plays an important role in the regulation of blood pressure (BP) homeostasis and cardiac function. Elevated sympathetic activity not only plays a role in the induction of ischemia due to reflex tachycardia and coronary vasoconstriction, but also correlates with hypertension, insulin resistance and coronary risk. Therefore, the increased SNS activity is a predictor of mortality in patients with these cardio-vascular diseases (Rooke et al, 1997). Interference of sympathetic activation may reduce cardiovascular risk. Thus, antihypertensive

11

pharmacotherapy and its influence on the SNS are of great importance (Rooke et al, 1997).

1.3.10 The value of measurements of arterial stiffness as predictors of cardiovascular outcome

The measurement of arterial stiffness is a useful parameter in the assessment of cardiovascular risk or outcome (Asmar et al, 2001). With clinical assessment of large arteries by PWA/PWV the augmentation and velocity of blood flow can detect the potential etiologic role in cardiovascular disease. This assists in recognising arterial changes which constitute as an 'early risk marker' and can be useful in the assessment of drug intervention in the arteries (Asmar et al, 2001).

1.3.11 Augmentation Index as a predictor of atheromatous disease

Augmentation has been shown to correlate significantly with findings of atherosclerosis by carotid ultrasonography, the most widely used clinical measure of atherosclerosis (Yamasaki et al, 2000). Therefore, augmentation is a promising marker for screening purposes. PWV, while widely used to assess arterial stiffness, is time-consuming and provides information about compliance that is limited to a specific artery or segment. However, augmentation of central arterial pressure can simply and reliably assess systemic arterial stiffness.

With increased arterial stiffness, central systolic blood pressure rises and diastolic blood pressure falls in the central arteries as a consequence of rebound of the previous pressure wave, which shifts the augmentation pressure from diastole to systole. The importance of assessment of the central waveform is underlined by a

strong correlation between aortic stiffness and degree of coronary artery disease at angiography (Hirai et al, 1989).

High augmentation has been significantly associated with greater higher plaque score, and higher prevalence of CVD (Fukui et al, 2002). Early intervention and examination with respect to atherosclerosis is needed in patients with high augmentation, although direct cardiac catheterization and echocardiography offers a more precise assessment of augmentation. However, PWA/V is a simpler, non-invasive screening method for identifying individuals at cardiovascular risk.

Previous studies have demonstrated for the first time that central arterial augmentation pressure was a reliable marker of atherosclerosis in type 2 diabetes (Fukui et al, 2002). Therefore, this simple, non-invasive determination would permit large-scale, early screening for atherosclerosis in patient groups, who are at increased risk for cardiovascular disease.

1.4 Hypercholesterolemia

Hypercholesterolemia is a major precursor of atheromatous arterial disease (Dart et al, 1991). This arterial disease can vary from atheroma to less advanced lesions such as intimal thickening, increased collagen deposition and foam cell infiltration. The precise mechanism is not completely understood but hypercholesterolemia is associated with endothelial dysfunction (Panza et al, 1993; Noon et al, 1996). Endothelial dysfunction is associated with abnormal serum lipid profiles (Goode et al, 1995) in particular oxidised LDL (low-density lipoprotein) cholesterol (Chin et al, 1992; Salonen et al, 1992).

All lipids in human plasma are transported as complex proteins. Except for fatty acids, lipids are carried as lipoproteins. Atherosclerotic plaques consist of foam

cells, which are transformed macrophages and smooth muscle cells that have become filled with cholesteryl esters. This is the result of the endocytosis of chemically modified lipoproteins via scavenger receptors. The accumulation of cholesteryl esters is due to the inability of those receptors to be down regulated by high intracellular levels of cholesterol. The most important chemical transformation of lipoproteins that creates ligands for the scavenger receptors is free radical mediated oxidation that occurs in the artery wall (Katzung, 1998).

The entire vascular tree is surfaced with a monolayer of endothelial cells. The endothelium senses changes in the vascular tone and reacts by generating mediators (Wilkinson and Cockcroft, 1998). There are many mediators in the endothelium that can cause vasoconstriction or vasodilatation of the smooth muscle in the arteries. For example, endothelin and angiotensin II that cause vasoconstriction and nitric oxide and endothelium derived hyperpolarising factor that causes vasodilatation. Nitric oxide (NO) is synthesised from the conversion of the amino acid L-arginine to L-citrulline by NO-synthase. NO freely diffuses to the smooth muscle where cGMP levels are increased. This causes relaxation of smooth muscle. In normal vascular tone, there is a basal NO release. NO may act as an antioxidant blocking the oxidation of LDL (Low density lipoprotein) and thus preventing the formation of foam cells in the vascular wall by inhibiting scavenger receptors to form ligands (Davis and Hagen, 1993).

Vascular plaque formation in hypercholesterolemia leads to reduced NO formation and endothelium-dependant vasodilator responses. Oxidisation of LDL may impair the L-arginine/nitric oxide pathway and eventually inhibits relaxation of the smooth muscle. This causes endothelial dysfunction that can turn subsequently to increased arterial stiffness and atherosclerosis by the following mechanism. In atherogenesis,

the arterial endothelium is chronically damaged by serum lipids, turbulent blood flow and other factors. This injury leads to lipid accumulation and the adhesion of monocytes and platelets. The aggregation leads to the formation of a fatty streak. With time the fatty streak may progress to an atheromatous plaque. This progression is dependent on the location of the fatty streak and the presence of risk factors (Davis and Hagen, 1993).

Atherosclerosis is the pathway to cardiovascular disease and risk factors contributing to that are diabetes, hyperlipidemia and hypertension (Chobanian and Alexander, 1996; Glasser, 1997).

Hypertensive and hypercholesterolaemic individuals are at an increased risk of developing clinically significant target organ damage. Few studies have used arterial techniques to assess dysfunction of the vascular system in essential hypertension and hypercholesterolaemia. Hypertension has a multi-factorial aetiology and it may be certain that certain sub-populations of people are more at risk than others of developing target organ damage. Patients with hypercholesterolaemia also have impaired basal (Casino et al, 1993) and stimulated (Chowienczyk et al, 1992; Casino et al, 1993, Noon et al, 1998a) release of nitric oxide in the forearm vascular bed, and in the coronary circulation (Drexler & Zeiher, 1991). Hypercholesterolaemia leads to atherosclerosis, a process that is accelerated if hypertension is present. Thus, the capacity of the endothelium to maintain adequate tone and to prevent thrombus formation in hypercholesterolaemic patients may have important implications for atherogenesis.

It is unclear whether impaired nitric oxide generation in hypertension and hypercholesterolaemia is confined to skeletal muscle resistance vessels and coronary vessels, and whether the defect is specific to nitric oxide generation or whether, in

humans, the impaired function also affects other mediators such as prostacyclin. Moreover, it seems that caution should be taken in assessing endothelial dysfunction using the acetylcholine test, as some patients with hypertension (previously treated or untreated) have no difference in cholinergic vasodilatation in the forearm compared with normotensive controls (Cockcroft et al, 1994). Alternatively, the invasive methodology of forearm perfusion studies has not allowed sufficient numbers of patients to be examined to establish the epidemiology of endothelial dysfunction. Therefore, central to this thesis, is the development of a simple, non-invasive technique for investigating endothelial function both in health and in diseases such as essential hypertension and dyslipidaemia. The technique of applanation tonometry to assess the central pulse wave will be used to assess arterial stiffness as an independent risk factor for cardiovascular disease. No studies have assessed arterial stiffness in African Caribbean groups of people, despite accelerated risk in these populations.

As well as essential hypertension, other risk factors for cardiovascular disease and stroke have been defined by various groups (Cameron et al, 1995; Cooper et al, 1997; Gulnick et al, 2001). Unfortunately, the lack of consensus among these groups and periodic changes in risk factor listings have led to confusion among health professionals. As so many risk factors are inter-related, it is difficult to isolate the effect of a specific risk factor such as dyslipidaemia. For example, hypertension is known to exacerbate atherosclerosis (Ross, 1995). The known effects of physical inactivity, obesity, cigarette smoking, age, hypertension and diabetes mellitus all play a part on lipids and lipoprotein levels. Secondary to salt, perhaps no other factor has featured more frequently in the pathogenesis of cardiovascular disturbances and hypertension than fats.

In addition to the vascular structural alterations, the role of functional abnormalities of the vascular endothelium focuses on the presence of hypertension and hypercholesterolaemia *in vivo* (John et al, 2000). Potential mechanisms underlying impaired endothelial function and decreased bioavaliability of nitric oxide lead to the reduction of elasticity and the development of atherosclerotic lesions (John et al, 2000). However, in relation to ethnicity and lipid profile, African Americans tend to have a better risk profile than whites for cardiovascular disease, despite their higher propensity to hypertensive illness (Tyroler et al, 1980).

The development of hypertension in African Caribbeans has been related to insulin resistance, which is a frequent feature of essential hypertension (Zeiher et al, 1991; Boden et al, 1996; Pitre et al, 1996). The magnitude of insulin resistance has been related to systemic blood pressure. Of particular relevance to the problem of vascular tone is the capacity of insulin to produce vasodilatation. Therefore, the action of insulin on the vascular system can be greatly impaired if there is endothelial dysfunction as a result of another pathogenesis.

1.5 Genetic Causes of Essential Hypertension

A review of original and published data (Halbertstein, 1999) yields the consistent finding that chronic hypertension is endemic and prevalent throughout the Caribbean area, and the prominent involvement of genetic predisposition is suggested in several lines of evidence (Rigat et al, 1990; Caulfield et al, 1994; Barley et al, 1996; Kaplan 1998) including significant ethnic differences in blood pressure values.

1.5.1 Gene Mapping Techniques

Since the early 1990s, strides have been made in developing simple genetic tests to assess individual risk for a variety of disease processes. The detailed Human

Genome Project will clarify understanding of the importance of genetic mechanisms in the targeting, assessment, prevention, and treatment of disease. Many Mendelian disorders have now been mapped and the genes responsible found. This is now technically straightforward. However, Mendelian diseases are rare compared to complex diseases such as essential hypertension. This is due to multiple interacting genes, possible low penetrance, external non-genetic factors and problematic diagnosis. So far there has been limited success in finding 'complex disease genes' that are highly penetrative and give clear definition to complex disease process. Gene mapping, to find genetic markers can be found by numerous techniques.

Linkage analysis determines genetic markers to disease that are passed through ethnicity along with the disease interest (Xu et al, 1999). This is the collection of DNA from individuals in an ethnic group. Genotyping a series of microsatellites spread across the genome would test whether the transmission of any these is correlated with the transmission of phenotype (Lander et al, 1995). Alternative approaches involve direct testing of DNA polymorphisms for *association* with the disease. Association mapping is the identification of a particular mutation that increases susceptibility to a disease of interest. It is more common among affected individuals than among random controls. Linkage disequilibrium mapping is random genetic markers near a disease susceptibility mutation may be associated with the disease (Terwilliger, 1995). These processes can be used in several contexts; testing for association at a candidate gene; examining regions of suggestive linkage from ethnic studies; genome screens for association, in particular for the future and narrowing down the location of a mutation. Mapping complex disease loci is a major challenge for human genetics and powerful tests and methods are required for population genetic problems. For example, the identifying of allelic heterogeneity

and the location of disease mutation (Risch, 2000; Risch et al, 1996).

1.5.2 Environmental 'Triggers'

Rarely is a genetic disease attributed to a single gene mutation. The overwhelming evidence is that essential hypertension is a polygenic disease, with environmental interactions and 'triggers' being just as important as the genetic predisposition (for review (Joly, 1999).

Examples of environmental factors that may influence genes include:

❖ The slavery hypothesis of natural selection favouring a salt-conserving physiology in ancestral populations, suppressing plasma renin activity, predisposing genetic variance (Halberstein, 1999).

❖ Climatic change on migration is thought to influence genetic expression (Halberstein, 1999).

❖ Fear of racial provocation, increased levels of stress, and hormonal changes (McNaught, 1987).

❖ Traditional use of medicinal plants was successful because of the beneficial bioactivity of many antihypertensive phytochemical components, which have been supplemented, with widespread introduction of synthetic biomedical drugs (Halberstein, 1999).

Genetic data has reinforced these concepts by demonstrating a link between

hypertension and particular alleles of candidate genes in some population groups (Gavras et al, 1999).

Essential hypertension in the vast majority of patients is believed to be a complex, polygenic, multifactorial disorder resulting from the interaction between several genes with each other and with the environment. Clinical strategies used to enhance searches for 'candidate genes', such as subgrouping of populations into relatively homogenous groups according to heritable anthropometric, clinical or biochemical characteristics. In this study, it will be investigated whether particular mutant genes are associated with phenotype, i.e., arterial stiffness. Previous findings have concluded that the survivors of vascular diseases are at an increased risk of carrying a particular genetic variation (genotype), and that some health professionals have accepted higher levels of blood pressure as normal when they occur in ethnic populations (Torkington, 1991). This may be a rationale for poor intervention and management. However, genetic markers have caused concern, and that testing for a recessive gene that can identify whether the individual has a higher risk of a disease process, may lead to discriminating policies by corporate companies.

1.5.3 The β_2-adrenoceptor gene

In Caucasians, it has been shown that there are different forms of a gene that plays a part in the regulation of blood flow (the β_2-adrenoceptor gene). Individuals with a particular mutant form have impairment in the ability of blood vessels to relax (dilate) (Cockcroft et al, 2000). It may be that this particular form of the gene is more prevalent in African Caribbean people, as preliminary evidence from South Africa suggests (Candy et al, 2000).

A recent study involving the infusion of the agonist drug isoprenaline, an agonist at the β_2-adrenoceptor, demonstrated that African Caribbean subjects exhibit a blunted vasodilatory response to the drug compared to Caucasian subjects (Lang et al, 1995). These findings suggest a contribution of particular genes to enhanced vascular reactivity in African Caribbeans and may play a part in a pathogenesis of hypertension in these subjects. Moreover, recent evidence (Svetkey et al, 1995) linking the β_2-adrenceptor gene type with hypertension, may well be important in explaining racial differences in blood pressure.

A genetic mutation of the β_2-adrenoceptor gene has been identified (Riehaus et al, 1993). The polymorphism resulted in amino acid Gln 27 becoming Glu 27. The Glu 27 type of receptor gene mutant fails to downregulate after exposure to an agonist to the same extent as the wild type, (Green et al, 1995). It has been demonstrated that individuals with the gene types Glu 27 or Gln 27 exhibit different responses to the inhaled β_2-agonist salbutamol (Hall et al, 1995). In the vascular bed in healthy Caucasian subjects, β_2-adrenoceptors downregulate to an extent following stimulation with an agonist that is dependent upon the form of the gene (Cockcroft et al, 1997; Noon et al, 1998), demonstrating vascular effects that may contribute to essential hypertension.

Published data (Candy et al, 2000) suggests that the frequency of the Glu 27 allele is considerably lower in South African people than in Caucasians. Large cohorts of subjects need to be recruited before a sufficient number of this polymorphism could be detected. In addition, the frequency of the prodownregulatory Gly 16 allele of the β_2-adrenoceptor is higher in hypertensives with increased body mass index (Candy et al, 2000) than in normotensives, and is also associated with essential hypertension in

African Caribbean people (Kotanko et al, 1997).

This genetic predisposition is more pronounced in African Caribbean people with a family history of hypertension and is associated with poor vascular response (Eichler et al, 1990; Lang et al, 1995). Thus, in addition to the mutation at codon Gln/Glu 27, the present study will investigate the codon Arg/Gly16 mutation in an African Caribbean population.

Similarities in some phenotypic expressions between hypertension in blacks and patients with Liddle's syndrome (Shimkets et al, 1994), a monogenic form of hypertension, have suggested that abnormalities of the distal tubular epithelial sodium channel, a major regulator of the overall control of sodium balance that may underlie the development of high blood pressure in African Caribbean's (Schild et al, 1995). A number of variants of the sodium channel β-subunit coding sequences have been identified in subjects with hypertension (Chang & Fujita, 1996; Dong et al, 1997; Persu et al, 1998). These lead to a single amino acid change rather than a major truncation as seen in Liddle's mutations and are much more frequent in blacks than in whites, especially the most commonly identified T594M and G442V polymorphisms (Persu et al, 1998).

1.6 Other Risks - Renal Differences

Hypertension in ethnic groups is often sensitive to dietary salt intake and in those without evidence of organ damage, thus, a low salt diet may occasionally be sufficient to control blood pressure (British Hypertension Society, 1999). Both normotensive and hypertensive African Caribbean individuals are known to be more salt-sensitive than whites, (Fray et al, 1993; Calhoun & Oparil, 1995; Clark 1999). This suggests that African Caribbeans' have an excessive retention of sodium and water causing volume overload, thus, contributing to sustained high blood pressure. Salt-sensitivity occurs in that salt potentiates sympathetic nervous system-induced vascular reactivity. Moreover, neurohormonal responses are altered in salt-sensitive hypertension (Obeifuna et al, 1991; Ono et al, 1997), with aberrations in the serum renin-angiotensin-aldosterone system (RAAS). This may account for most salt excretion irregularities, as hypertensive patients often have a more gradual decline of plasma and urinary concentration of aldosterone after salt loading than occurs normally (Mills et al, 1995).

Safar et al, (2000) suggested that high serum sodium levels are associated with aortic hypertrophy. These alterations often correlate with increased stiffness and secretory properties of vascular smooth muscle. Such mechanisms are reversed with lowering

sodium levels. In African Caribbeans', the RAAS is impaired when sodium levels are high, thus, decreasing artery diameter and tone, resulting in increased blood pressure (Ergul, 2000).

Racial differences in renal physiology, influenced by either genetic or socio-economic factors have been suggested as possible causes; however, these differences have not been made clear or explored fully. Figure 1.2 is a schematic representation

adapted from Fray and Douglas (1993) of the interaction between psychological and physiological factors that contribute towards the development of hypertension in blacks. Recurrent exposure to social and environmental stressors cause chronic activation of the sympathetic nervous system and other vasoactive substances, such as ET-1 and Angiotensin II, which then increase peripheral vascular resistance. In this model, genetic factors may play a role in the regulation of the stress response (i.e. catecholamines and ET-1), which influences both the renal function and vascular activity. ACTH indicates adrenocorticotrophic hormone.
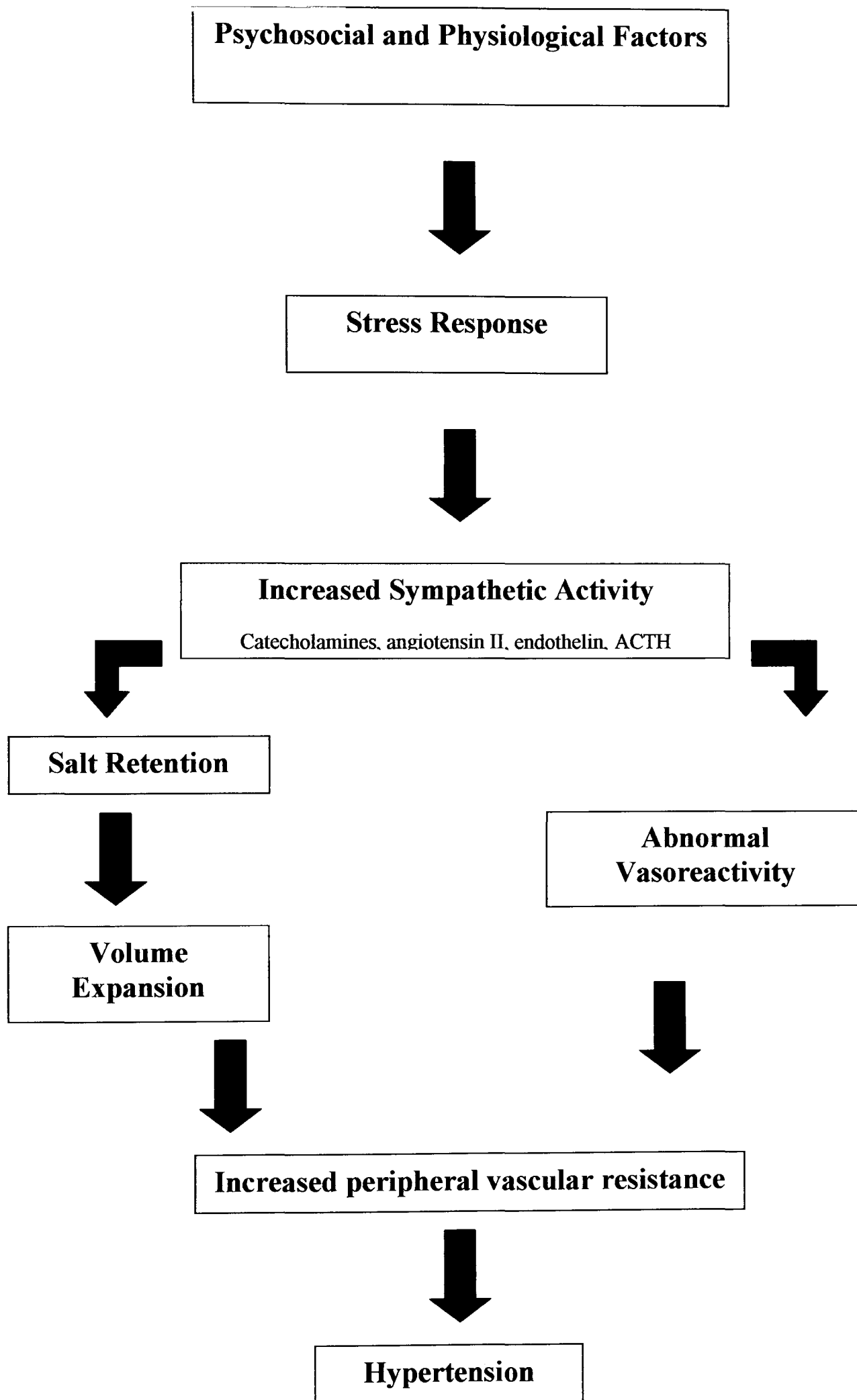
Figure 1.1 A schematic representation adapted from Fray and Douglas (1993) of the interaction between psychological and physiological factors that contribute towards the development of hypertension in blacks.

1.7 Pulse Wave Analysis

As arterial stiffness has become recognised as an independent risk factor in cardiovascular disease (McVeigh et al, 1991; O'Rourke, 1999), measurement of stiffness could provide a better stratification for treatment, and for monitoring therapeutic response. Traditionally, arterial compliance was measured *in vitro* using excised arteries, but can be measured *in vivo* by ultrasound imaging of arterial diameter change, or by the simpler technique of pulse wave
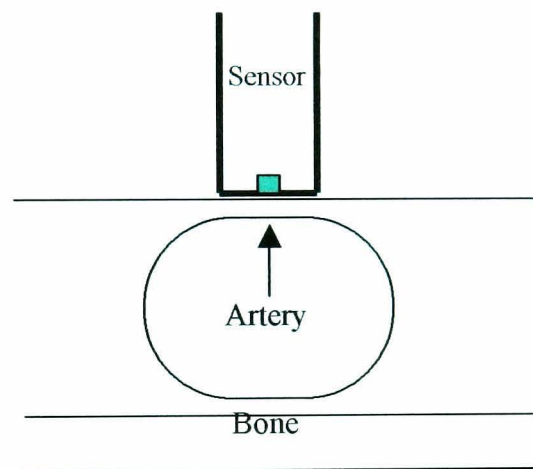
Figure 1.2 Applanation tonometry (see text below).

analysis (PWA) (Arnett et al, 1994). This non-invasive, well-validated technique for measuring arterial compliance (Arnett et al, 1994; Wilkinson et al, 1998), involves holding a sensor on the skin over the point of maximal arterial pulsation of a large artery (such as, radial, brachial or carotid) and pressing down against the underlying bone (Kelly et al, 1989). By flattening the curved surface of a pressure-containing structure, the stresses inherent in the wall of the curved structure may be balanced,

allowing accurate recording of the intra-arterial pressure. This is calibrated against brachial blood pressure to produce a peripheral pressure waveform, from which the central arterial pressure wave is generated using a computerised transfer factor based on invasive studies (O'Rourke, 1999).
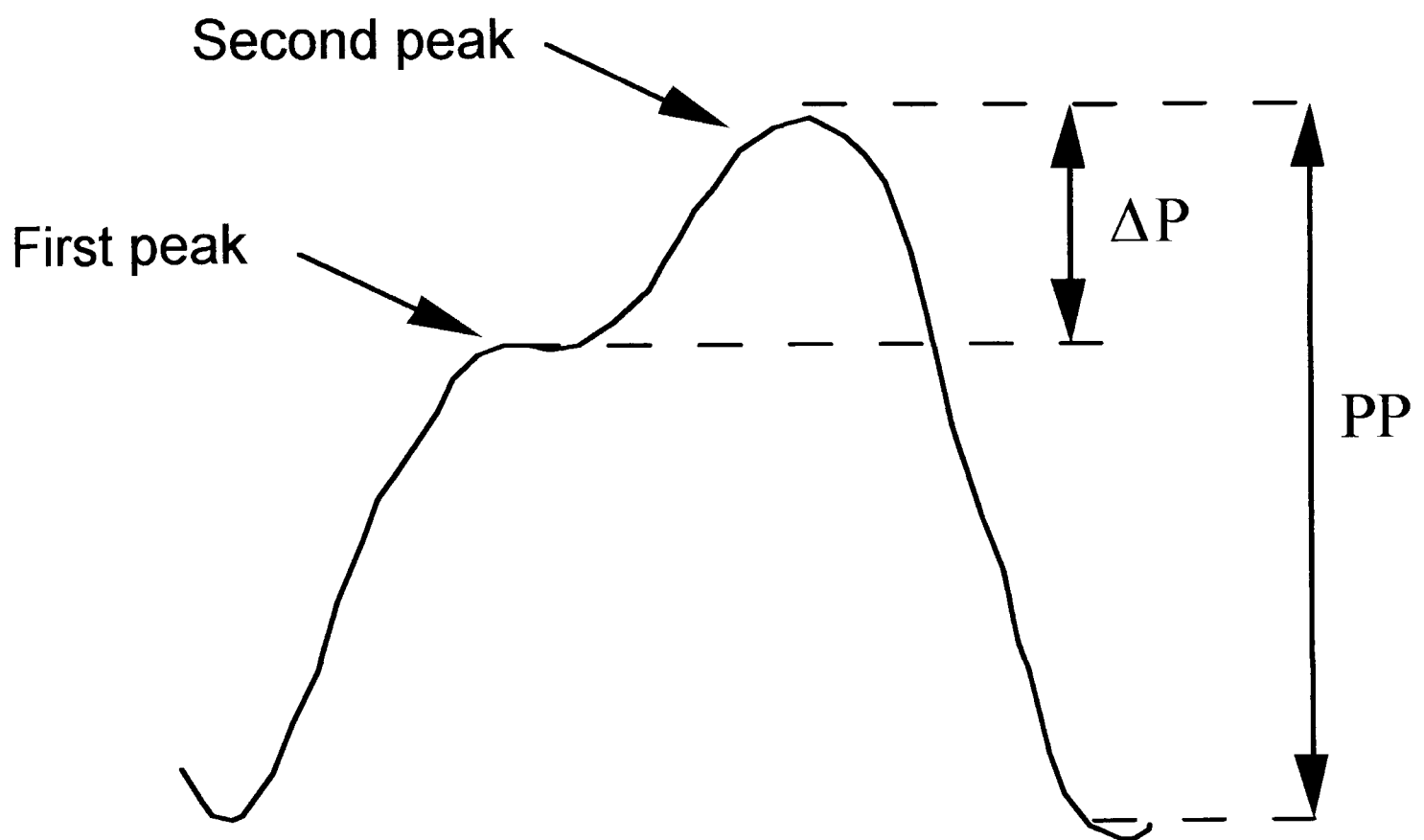
Figure 1.3 A typical central aortic pressure waveform in a middle-aged subject. The second peak becomes more prominent with age or as the arteries stiffen, and is caused by wave reflection. The augmentation index is the difference between the second and first systolic peaks, expressed as a percentage of the pulse pressure (O'Rourke, 1999).

1.7.1 Assessing arterial stiffness

Increased arterial stiffness is an important, independent risk factor for cardiovascular disease (Arnett et al, 1994). Pulse wave analysis (PWA) provides an ideal method for assessing arterial stiffness and central aortic pressure (SphygmoCor® AtCor Medical). It is a non-invasive, highly reproducible technique, easily applied in the clinical setting. The measurement of stiffness may provide for better risk stratification of patients, targeting treatment and monitoring therapeutic responses. Endothelial dysfunction may be partly responsible for increased stiffness seen in-patients with traditional cardiovascular risk factors. Indeed, arterial stiffness is becoming a therapeutic target as more of the underlying mechanisms are dissected.

1.7.2 Applanation Tonometry

Accurate non-invasive assessment of the arterial pulse is possible in the radial, brachial, femoral or carotid artery using PWA. PWA uses applanation tonometry to detect these peripheral waves, which are recorded into the PWA software. This technique is used in ophthalmology to measure intra-occular pressure. If two curved surfaces can be flattened (like an artery) the circumferential pressures balance the internal pressure, and an accurate waveform can be recorded (O'Rourke, 1992, 1996; Wilkinson, 1998). A probe with a high fidelity ultrasound micromanometer at its tip is used to flatten the artery. Superficial arteries overlying bone, like the radial artery, are practicably best to use. The peripheral waves are transformed into a central waveform by a validated transfer factor (Chen, 1997; Wilkinson, 1998). This is based on invasive intra-arterial pressures, and shown to be highly reproducible. From the

derived ascending pressure, the effects of wave reflection can be determined and central aortic pressure waveform is obtained.

1.7.3 Augmentation Index

Augmentation index (AIx) is a measure of arterial compliance and is generally negative in young and healthy people, but will become positive around the age of 40 years. The learning period for PWA is short, and most operators begin to obtain good quality recordings after brief instructions in the technique. The variability of central AIx determined from applanation tonometry of the radial artery is small.

The absolute value of AIx (%) depends upon age, sex, height and a number of other variables. In this instance the augmentation index was measured and recorded in P^2/P^1, and represented as a percentage.

__Equation 1__

AIx (%) = P^2/P^1

Augmentation index (AIx (%)) is the scientific calculation and therefore considered the most appropriate measure for analysis for this study. The index is calculated by the two peaks as shown in chapter 1, figure 1.3. The first peak (P^1) is the pressure generated by ventricular ejection and the second peak (P^2) is due to wave reflection. The incisura is the closing of the aortic valves. The degree of the arterial stiffness is given by the augmentation and augmentation index. Augmentation index (AIx) is defined as the difference between the second and the first systolic peaks divided by the pulse pressure in percentages. Augmentation is the difference in height between the first and second peak and is expressed in mmHg.

1.7.4 The Pressure Waveform and Arterial Stiffness

The contour of the arterial pressure waveform varies along the arterial tree. This is due to differences in vessel stiffness, and the phenomenon of wave reflection (O'Rourke, 1992). Arteries are compliant structures and buffer the pressure changes caused by intermittent ejection of blood from the left ventricle. Pressure waves are reflected back from the periphery, most likely from the sites of impedance mismatch, and summate with the forward-going wave. Thus, the waveform at any one site will be a combination of the forward and backward-going waves (Segers et al, 2001). Normally, the reflected wave arrives back at the aortic root in diastole, thus, helping to maintaining coronary artery perfusion. However, with age, or conditions which stiffen the arterial tree, such as diabetes, hypercholesterolaemia and hypertension, (Chobanian et al, 1996; Glasser et al, 1997; Iannuzzi et al, 1999), thus pulse wave velocity (PWV) and the amplitude of the reflected wave both increase (Avolio et al, 1985; Kelly et al, 1989). Thus, a larger reflected wave returns to the aorta earlier, and adds to, or augments, central systolic pressure. The magnitude of peripheral pressure changes much less, although the waveform itself does change. Therefore, AIx is a useful index of arterial stiffness; providing information which cannot be obtained by measurement of brachial artery pressure with a sphygmomanometer. In particular, AIx reflects the manner in which the arterial tree interacts as a complete unit. Clearly, arteries do not function in isolation, and the value of measurement of stiffness in a single, short arterial segment with other techniques is, therefore, likely to provide less information. Moreover, ultrasonic measurements are more observer-dependent, and the technique takes longer to learn.

The sphygmocor system has configuration settings to set quality control values to ensure accurate readings of waveforms. This is to ensure that the beat-to-beat pulses

in each waveform are similar, and that there is no marked drift of the signals outside the boundary of the quality control configurations. This allows a visual guide to the operator as to how well the individual pulses can be overlay to form an averaged pulse/waveform.

1.7.5 Alternative method to detect Arterial Stiffness

The assessment and measurement of arterial stiffness is a relatively new concept and it was only recently that the reproducibility of an ultrasonic technique for the assessment of dynamic vessel wall properties (Glasser et al, 1997) was studied.

In addition to high-resolution ultrasound, another non-invasive method is pulse wave velocity (PWV), (Glasser et al, 1997). PWV is applicable to large arterial segments only, and ultrasonic techniques are limited by the ability of the method to image accurately the anterior and posterior wall of the vessel under investigation. Thus, there are only applicable to large accessible arteries. Similarly, most techniques to date only provide data on stiffness of singular vascular beds and are invasive with certain risks. However, one increasingly used method is pulse wave velocity (PWV). It is a sophisticated system for estimating the pulse wave velocity between two superficial artery sites. This system is not too dissimilar to pulse wave analysis (PWA) and the use of a tonometer with electro-cardiograph (ECG) leads, connected to the electronics module, to non-invasively record a patients peripheral artery pressure and ECG waveform. To perform a measurement two sites are chosen. Site A would be the proximal artery (i.e. closest to the heart) and site B is the distal artery (i.e. furthest from the heart). The distance between the two is measured and recorded into the system in millimetres. A mean blood pressure is also entered. The tonometer is then placed at site B and recordings taken. Then the tonometer is placed at site A

and ECG leads placed on the patient and a trace is commenced. Readings are then taken from site A. From these measurements, the system software is able to calculate the pulse wave velocity between the two artery sites. However, this procedure was not used for this study as it would have been too time-consuming and there is a risk of carotid rubbing, (most commonly used site for A) which can lead to cardiac arrhythmias, despite its non-invasive approach. Although the data on such a large cohort would have been very interesting, it was difficult to process with precision and medical assistance was not always readily available to cover any emergency. Pulse wave analysis has minimal intervention and risk with equal results. Presently, there is no accepted 'gold standard' for the assessment of vascular stiffness. Before this can be achieved two main aspects must be achieved. Firstly, techniques must be validated in the clinical setting and secondly large-scale studies not unlike this thesis need to be undertaken to discover whether arterial stiffness is a strong predictor of mortality than current risk factors.

1.7.6 Pulse Contour Analysis (PCA)

Pulse Contour Analysis (PCA) or Pulse Trace is relatively new concept that is not too dissimilar from PWA. PCA is a powerful non-invasive device that measures large artery wall stiffness (SI) and vascular tone (RI). PCA uses pressure wave transmission and reflection theory to evaluate the speed with which the pressure pulse travels through large arteries, and estimate vascular tone, determined by the diameter of the smaller and medium arteries. The derived pressure pulse waveform changes as it travels through the arterial system (Nichols et al, 1998). The speed or velocity at which the pressure pulse travels through the arteries is directly related to their stiffness (Nichols et al, 1998). Other influencing factors are blood vessel diameter,

blood pressure and blood density. By measuring the speed by time it takes the pressure wave to travel through the arterial system, gives a simplistic and accurate way of measuring arterial stiffness (Millasseau et al, 2002). This technique would have been an ideal and even more simplistic application had it have been available and validated at the time of the data collection stage.

1.7.7 Alternative method for assessing blood flow by venous occlusion Plesthysmography

This technique is widely employed but for this study it was impractical to study a large cohort of people quickly and carried certain risks with arterial cannulation and availability of vascular acting drugs. The equipment is bulky and could not be easily transported overseas, in view of electrical and computer compatibility.

However, this is an ideal method for smaller cohorts because it provides more information than pulse wave analysis.

1.8 Aims

The aim of this study is to provide information on the causes of the 'Accelerated Risk Syndrome' in African Caribbean people. This information will reveal pathophysiological data that will aid a better understanding of risk, and to form a basis on which prevention or intervention can be instigated.

1.9 Objectives

This project will:

- ❖ measure arterial stiffness in African Trinidadians;

- ❖ correlate stiffness with blood pressure;

- ❖ quantify incidence of β_2-adrenoceptor mutations;

- ❖ correlate stiffness with β_2-adrenoceptor mutations;

- ❖ investigate whether stiffness tracks with degree of dyslipidaemia.

A secondary aim of the study is to determine whether genetic screening or pulse wave analysis are useful tools in early detection of risk in African Caribbeans' who have an 'accelerated risk' of cardiovascular disease.

1.10 Original hypothesis

The evidence presented in this literature review suggests an explanation for the "accelerated risk syndrome" in African Caribbean people, and that the onset of this syndrome occurs earlier than in Caucasians. Already, I have used pulse wave analysis to detect altered central pulse pressure, indicating stiffened arteries in a Trinidadian population. I will also assess blood pressure and genetic factors (*see* below). In addition, compared to Caucasians, I hypothesise that black *normotensive* subjects will have higher augmentation indices and increased blood velocities (stiffer arteries).

Previous work by our group in Caucasians showed a relationship between the Gln27Glu form of the β_2-adrenoceptor polymorphism and vascular reactivity to isoprenaline (Cockcroft et al, 2000), and that the Glu-27 allele is much rarer in Black South African populations than in Caucasians (Candy et al, 2000). Recent data have shown that the effect of β_2-adrenoceptor polymorphism is more dependent upon haplotypes across the region of chromosome 5q containing the β_2-adrenoceptor gene than single short nucleotide proteins (SNPs). At least 15 SNPs exist in the Caucasian and African Caribbean populations within the 31 kb region containing the β_2-adrenoceptor gene and its 5′ regulatory regions (Drysdale et al, 2000). Four common haplotypes exist which appear in part to predict clinical responsiveness in the airways (Drysdale et al, 2000). These haplotypes can be adequately defined by genotyping for the 4 SNPs (-367, -47, codon 16 and codon 27) due to strong linkage disequilibrium between these SNPs and other SNPs forming the extended haplotypes. No studies to date have used haplotype-based approaches to delineate contribution of this locus to vascular reactivity.

1.11 Summary

In summary, the aims of this study are to detect if;

* ❖ African Trinidadian normotensive subjects have a higher augmentation (stiffer arteries) than normotensive Caucasians.

* ❖ African Caribbean's with the Glu-27 polymorphism have stiffer arteries.

* ❖ Individuals carrying the haplotype within the Gln27Glu polymorphism (Arg-16, Gln-27) have increased vascular stiffness.

* ❖ Data on lipid profiles of each individual in the African Trinidadian cohort will determine that dyslipidaemia is associated with arterial stiffness.

Combined, this data will provide information on the aetiology of the risk syndrome, on severity of disease, and may lead to better prevention and treatment.

Chapter 2

Methods, Materials and Methodology

2.1 Research Design

Research Design is determined by the research question, and precision, power and minimal bias, reflect good research (Polit and Hungler, 1999). Therefore, the research design and methods employed to undertake this study are quantitative. Quantitative methods are often associated with identifying and explaining causal relationships. However, quantitative methodology has been criticised for isolating phenomena from their contexts and leading to the development of general laws that are expected to be applicable in all cases (Duffy, 1985). Despite this, the establishment of a general law is appropriate when assessing a population's need for screening. Context can be applied to a causal relationship, factors influencing arterial compliance have been researched and the literature will explore these factors in discussion when applied to the care and management of cardiovascular risk in ethnic groups. The use of a quantitative methodology should isolate factors or a factor that influences arterial compliance and thus blood pressure, the literature used is to assist the context in which the findings of the study can be applied.

As quantitative methodologies are either experimental or non-experimental, some experimental designs involve random assignment of subjects to a control or experimental group manipulating the independent variables and controlling the research situation (Polit and Hungler, 1999). To identify a causal relationship, the cause must be found to precede the effect, and an empirical relationship found cannot be explained as a result of chance or a third variable (Polit and Hungler, 1999). Due to a high degree of research control, experimental designs are the most powerful method of testing causal relationships (Bowling, 2000). A quantitative experimental methodology is employed for this study. The design involves the random assignment

of subjects to a control or experimental group, manipulating the independent variables and controlling the research situation.

In the present study, the experimental and control groups were moderately equivalent, and were investigated systematically under conditions that were identical, in order to minimise variation between them.

This feature is described as a *true* or *classic* experiment ensuring that the investigator has control over the independent variable as well as the power to place participants into groups. The design included a *pre-test*, which included an interview and self-administered questionnaire. This was necessary in order to measure the effects of intervention in the experimental group and the direction of any associations.

As an experimental design can only be performed when the independent variable can be brought under control of the experimenter in order that it can be manipulated, and when it is ethically acceptable for the investigator to do so. Consequently, it was not possible to investigate important social issues within the confines of the experimental design.

2.2 Instruments and Data Collection

2.2.1 Validity and Reliability

The concept of validity and reliability are major components to the strength and credibility of any research. When critically examined, it is not only the quality and quantity of the research findings that are considered, but those aspects that contribute to the authenticity of evidence, and to the plausibility of the conclusions and findings. However, research evaluation models have emerged as focusing on multiple data collection techniques and, thus, the stakeholders accommodating different values (Guba and Lincoln, 1989). Therefore, what is judged and that what is regarded in

terms of validity and credibility is based on the values and perspectives of individuals reviewing the research. The issue of validity and reliability has more contentions when the qualitative evaluation approach is used, and faced with many threats. Conventional scientific methods, on the other hand, are viewed as being more objective than subjective but similarly can be viewed as too rigid and repetitive as a 'hard' science (Duffy, 1985). The following account will firstly look at the issues of reliability and validity in quantitative research and then will critically address the threats to reliability and validity in the present quantitative scientific study, and will identify strategies for dealing with them.

2.2.2 Defining Validity and Reliability

Numerous researchers have stressed the importance of validity and reliability of findings in research. (Field and Morse, 1985; Guba and Lincoln, 1985; 1989; Polit and Hungler, 1999).

Validity can be defined as either *internal* or *external*. Internal validity refers to the extent to which the instrument is really measuring what it purports to measure, and external refers to research findings that are generalised and can be applied to a wider population of interest and different settings (Bowling, 2000). This means that the concept of validity is justifiable by its claim to knowledge and has relevance, and strength of evidence used to support such claims.

Conventionally, in quantitative research, as a measure of truth and accuracy, validity is of great significance as it is the measure of truth and is imperative to the research design. Internal validity, which is referred to more frequently in experiential studies, examines causality to the extent whereby a true reflection of reality is greater than the result of extraneous variables (Cormack, 2000). Therefore, internal validity is able to

ascertain the 'true value' of the inquiry and how it works. Robson, (1993) identified a number of supposed threats to internal validity. These included maturation, instrumentation, statistical regression, history, testing, experiential mortality and a selection for which the inquiry design must compensate by controlling and/or randomising processes.

Alternatively, external validity addresses whether or not findings from a study can be generalised beyond the sample from which it was derived (Polit et al, 2001). Just as there are threats to internal validity, in conventional inquiry, also there are threats to external validity, which include selection effects, the setting, history and construction (Robson, 1993). If addressed early on, any study should be able to be applied to the larger population from which the smaller sample was drawn. The study's constancy, stability, predictability, dependability and accuracy of results define reliability. This is determined by the research instrument, reproducing similar results when applied in identical situations at different times or occasions. However, in conventional investigation, reliability can have several threatening factors, these include careless measurement and assessment of the research process, poor instrument measurement or decay, human error, longevity of assessment, and by ambiguities of various sorts.

2.3 Ethnicity of Trinidad and Tobago

Over the years there has been great perplexity and debate over the terminology of race and ethnicity and its inconsistency of measurement (Sheldon and Parker, 1992). Race has been defined as a biological concept, distinguishing groups of people by their phenotypical features passed on through generations. However, race has been criticised for its limited biological value (McKenzie and Crowcroft, 1996), due to its poor marker for genetic variation. The existence of definable racial groups is not so

self evident and the concept has been opposed for imposing fixed differences on groups of people. More palatable epidemiological research prefers the term ethnicity to describe social concepts, national identity and shared cultural characteristics (Sheldon and Parker, 1992). Since boundaries can change according to context, it is also difficult to measure with precision (Bhopal, 1997). Both concepts are confounded by socio-economic status and the diversity within racial and ethnic groups makes research findings susceptible to faulty interpretation. Therefore, genetic makeup is the most powerful determinant of biological difference and investigation was undertaken.

2.3.1 Ethnic Background

If clinical and genetic data had been collected in the United Kingdom, the cohort would have been smaller and phenotype broader. In African Trinidadians the genetic variation is nominal as subjects all originated from Trinidad and the population was widely available. This in view of time, and non-diversity of race, this was considered more appropriate for study than in the United Kingdom. However, the external validity of the research can be generalised to the wider population and in particular African Caribbean's who have migrated to the United Kingdom.

2.3.2 Subjects and Sample size

For findings to be generalised, the sample needs to be representative of its population. Random probability sampling ensures that each element of the population has an equal chance of selection (Polit and Hungler, 1999). This was possible as there was a large diverse population within Trinidad. A representative sample was strengthened by choosing a homogenous target population and controlling extraneous variables through exclusion criteria and group matching. The power of a study is the ability to detect relationships among variables. This is increased through precision of

measurement, reduction of variability caused by extraneous variables and an increased sample size (Polit and Hungler, 1999). Power analysis offers a statistical method of determining the sample size that is needed to reject a false null hypothesis or accept a true null hypothesis. This requires knowledge of the likely strength of relationship between variables.

Previous work in Caucasians showed a relationship between the Glu/Gln 27 form of the β_2-adrenoceptor polymorphism and vascular reactivity to isoprenaline (Cockcroft et al, 2000), and that the Glu-27 allele is much rarer in Black South African populations than in caucasians (Candy et al, 2000). The mutant polymorphism in the South African sample was greater than 5%, therefore, a power calculation indicated that 500 African Trinidadians were needed to be recruited to examine the genetic incidence, and magnitude of effect of other variables, such as, lipid profiles, blood pressure, and augmentation index.

2.3.3 Sample Recruitment

To access the sample of African Caribbean people, Dr. Clifford Thomas, Consultant Cardiologist from the Department of Clinical Medical Sciences at Eric Williams Medical Complex (ERMC), University of the West Indies, Trinidad was contacted and collaboration for the study established.

2.3.4 Pilot Study

Once ethical approval was obtained from the University of Nottingham Medical Ethics committee and from the University of the West Indies, a pilot study was initiated. The pilot study was to carry out a small-scale version of the main study and assess its feasibility. This revealed any revisions that needed to be made before the main study. The principle focus of the pilot study was the assessment of the adequacy of the data collection plan. It was important to know whether, for example, technical equipment was compatible with the electrical current supplied, whether the respondents understood questionnaires, or whether any questions were objectionable in any way. Primarily the pilot subjects were chosen from the same population and ethnic background for the major study. By monitoring reactions and overall impressions of the project, feedback was given to whether the study was viable. This established whether the main study group would have any objection to participating in the study. By assessing difficulties and obtaining feedback from participants and staff identified weaknesses and strengths of the study. For example, any comments on completing forms, transport availability, appointment flexibility, patient information and comprehension of information sheet, the importance of consent and inclusion and exclusion criteria, finding the clinic in the hospital, health education and other logistic aspects. Data were collected and scrutinised with minor refinements to the questionnaire and appointment bookings of time extended and minimised to ten people per morning. In view of the insignificant nature of the revisions, no second trial run incorporating these revisions was thought necessary.

2.3.5 Ethics

Ethical approval had been obtained from the University of Nottingham, Medical School Ethics Committee, and the faculty of Medicine Ethics Committee at St Augustine, Campus Trinidad, University of West Indies. Subjects were given written and verbal information about the nature of procedure benefits and risks of the research. Time was given to consider whether or not to participate, and subjects were asked if they understood the information given to them and whether they had any further questions. The questionnaire was then filled out by the participant and assistance given if needed. Details were then scrutinised for inclusion criteria and, if compatible, the subject was then asked to complete the signing of a consent form, witnessed and countersigned by the investigator. All information gathered was kept confidential and each subject was given an identification number for correlation.

Essential hypertension is a widespread and common feature among African-Trinidadians. The investigating procedures used were safe and non-invasive but carried the risk of causing anxiety and *'labelling'* of asymptomatic subjects. To minimise this, procedures were explained and subjects were informed that the investigations were for research purposes only. The results for lipid profile and pulse wave analysis were disclosed to the participant on completion along with health education and promotion if required, and if needed, they were referred to their general practitioner.

2.4 Selection Criteria of Subjects

2.4.1 Inclusion Criteria

The selection criteria used for the study were as follows:

- Aged 18 or above.

- Of strong African-Trinidadian descent going back three forefathers.

- For control group: Normotensive.

- For case group: Hypertensive. (WHO definition of hypertension (Zanchetti et al, 1993)).

- Abstained from alcohol and caffeine containing products for 24 hours.

- Fasted for approximately 8 hours prior to appointment.

2.4.2 Exclusion Criteria

The exclusion criteria used for the study were as follows:

- Under 18 years of age.

- Renal or any organ failure that results in extensive and existing complications.

- Pregnant women.

- Patients taking large quantities of medication for acute or chronic conditions, which may affect blood pressure.

- Not of African-Trinidadian decent.

- Non-steroid anti-inflammatory drugs or aspirin for approximately 7 days prior to study.

2.5 Experimental protocol, variables and control of research environment.

To prevent confounding data it is essential to control any extraneous variables as carefully as possible. All participants were asked to arrive at clinic having fasted for at least six hours and having abstained from caffeine and alcohol for 24 hours and aspirin and non-steroid anti-inflammatory drugs for seven days prior to screening. This would prevent 'masking' the effect of blood flow and dilatation of blood vessels, as caffeine and alcohol are stimulants and have a dilatory effect on blood vessel function (Kaplan, 1998). To control this variable, a letter or verbal instruction by telephone was undertaken, prior to their appointment, so that potential subjects were compliant to study criteria. They were asked at the screening appointment if they had adhered to this instruction. Each participant was seen in the morning at the clinic (EWMC) for a single appointment. Assistance was provided, where necessary, in with filling out the questionnaire, providing detailed, accurate information on ethnicity, smoking, family and personal history of hypertension, cardiovascular disease (including stroke) and diabetes and any current medication. This was to determine if the potential participant met the inclusion criteria.

Before employing PWA, height and weight and blood pressure were measured. Height was measured without shoes to the nearest centimetre (cm), using a height chart attached to a wall. Weight was measured without shoes and outdoor clothing, to the nearest 0.1 kilogram (kg). Subjects were rested in the supine position in a quiet room maintained at a temperature of 22°C to 23°C, controlled by air conditioning. A

quiet atmosphere also helped to reduce anxiety that could elevate blood pressure or heart rate readings.

Three recordings of blood pressure were taken using the Takeda UA-751 semi-automated sphygmomanometer on the participants non-dominant arm, using the correct size cuff size, taking blood pressure readings over a fifteen-minute period, with the mean of the readings used for the analysis (British Hypertension Society (BHS), 1999). The first reading was disregarded, if in some individuals it were found to be abnormally raised, in comparison to their previous blood pressure readings, undertaken elsewhere. Applanation tonometry (see section 2.6 and figure 1.3) was then performed to assess arterial compliance using information derived from the central pulse wave. Pulse wave analysis was then performed using a tonometer placed on the participants' non-dominant arm, radial artery, whilst the subject was recumbrant. Three subsequent recordings, separated by two to five minutes were taken with quality control parameters calibrated and set.

Three, ten second periods of good quality waveforms in each subject were recorded.

The average of the three blood pressure measurements and the average of three PWA measurements were used for analysis. The investigator performed all recordings, and the two groups were intermixed to prevent bias. Once completed, the fasted participant had a venous blood sample taken for lipid profiling, glucose level and genotyping. Results of blood pressure, and fasting lipid and glucose levels (Cholestech; Bayer) were given to the participant at the end of the session and appropriate health advice was offered, with referral if required. Each appointment lasted between thirty minutes and one hour.

Blood pressure and arterial compliance has been found to be preserved at night (Harshfield et al, 1988). For this reason, and given that the subjects were fasted,

arterial compliance was assessed in the morning. This controlled for any diurnal variation in these measurements (Kool et al, 1992). Environmental stimuli can explain changes in vascular reactivity and play an important role in leading to an increased vascular tone. Pre-capillary arterioles throughout the body might play a part in vasoconstriction triggered by exposure to environmental stresses (Folkow, 1982). Thus, temperature, electrical interference and noise control were required for comfort and to reduce anxiety levels in the subjects. Less potent environmental stimuli, including relatively minor irritations that occur from intermittently, could, under experimental conditions, lead to substantially greater vasoconstriction and subsequent blood pressure response than would ordinarily occur. Caffeine and alcohol intake increases blood pressure chronically, influencing arterial compliance (Kaplan, 1998). Data from a large Kaiser-Permanente database (Klasky et al, 1977) which included over 10,000 'black' patients, showed substantially higher blood pressures in subjects who drank three to five alcoholic drinks per day than those who drank less. Cause and effect relations are not proven. However, the consensus recommendations for limiting alcoholic drinks appear to be generally prudent for 'black' as well as white populations.

2.5.1 Summary of Blood Pressure Measurement and reasons

The following principles were adhered to in measuring blood pressure are as follows:

❖ Follow the British Hypertension Guidelines on technique (Ramsey et al, 1996).

❖ Use blood pressure measurement devices that are validated, maintained and regularly calibrated. This was the SphygmoCor® (AtCor Medical) and Takeda UA-751 semi-automated sphygmomanometer.

❖ Measured blood pressure was taken lying down, but could be taken sitting, as long as the participant is relaxed. All participants were lying down.

❖ Removal of tight clothing, so it does not restrict the cuff, support the arm and ensure the hand is relaxed. This allows accurate readings to be recorded.

❖ Use an appropriate size of cuff. This should encompass the arm 1.5 to 1.8 times the circumference of the participant's non-dominant arm. This ensures full occlusion of blood flow to the forearm and record accurate blood pressure on release.

❖ A minimum of three blood pressure readings was taken at the visit. The first reading was disregarded in calculation if the participants' blood pressure measurement served as an assessment for 'white coat' or 'office' hypertension (Mancia et al, 1995). This guideline was adapted to compensate that the participant had assessment of blood pressure on only one visit and not four clinic visits to determine blood pressure threshold (British Hypertension Guidelines 1999).

❖ The average of the following blood pressure readings were used and data entered into the SphygmoCor® to assess central cardiac indices.

❖ Pulse wave analysis was then performed using a tonometer placed on the participants' non-dominant arm, radial artery, whilst lying down and three subsequent recordings were taken with quality control parameters calibrated and set.

❖ The average of three blood pressure and PWA measurements were used for analysis. This was to ensure that all readings conformed and were consistent with the recommended experimental technique to ensure reliability, accuracy and validity.

❖ Muscle strength and mass will have an effect on blood pressure. The dominant arm is more likely to have greater muscle mass and strength due to continual isometric use and thus enhanced aerobic characteristics. Therefore, the dominant arm would not be a true enough reflection of blood pressure systemically (BHS, 1999). People from African decent have physiological differences in muscle fibre that makes them more resistant to muscle fatigue and have proved to be more athletic (Noakes, 1993). However, despite people of African origin have greater endurance in exercise, muscle strength is lower compared to caucasians (Noakes, 1993), and that the key differences in strength and mass lies within the muscle tissue and not the cardiovascular system (Noakes, 1993).

2.5.2 Questionnaire and Interview

In the planning and piloting stage, the construction of a questionnaire was thought appropriate to capture personal details including gender, date of birth, ethnicity going back three forefathers, educational background, personal and family medical history, present medication and smoking status. This was used to validate ethnicity, inclusion and exclusion criteria, personal and contact details, medical history, and socio-economic status. Questions were designed to be simple and familiar, so that all subjects could understand them. Questions were closed and dichotomised. The advantage of this was that they were quicker to analyse and easier for the investigator to validate at interview, as well as the subjects comprehension. The closed questions were not felt to force the respondent into inappropriate categories as the topics and background was commonly known. No clues were evident about the answers that were expected for inclusion or exclusion criteria. The format of the questionnaire was easy and basic, with non-threatening questions. However, some questions did refer to personal and family medical history and technical terminology was used. Opening and closing statements on the questionnaire offered assistance to those who had difficulty in interpreting medical jargon. Care was given when the respondent had difficulty choosing a response possibly through lack of knowledge of their personal or family medical history. The questionnaire had an open question at the end of the questionnaire to allow the respondent to write in their own words any other details they thought appropriate about themselves and their participation in the study.

A closed question was thought unwise when asked about ethnicity. This was thought to give the respondent prompts about the type of answer that was expected (Bowling 2000). Therefore the respondent was required to describe their ethnicity and their background in their own words, to avoid form and prompts. Included with the

questionnaire was an information sheet, describing the format of questions asked followed by the tests performed. After the respondent completed the self-administered questionnaire, the investigator conducted a personal interview. This was short and factual, lasting for approximately five minutes. Closed questions were scrutinised for errors and ethnicity verified verbally, checking background with the subject. It was important to establish a good rapport, as this had a positive motivation factor on the respondent. Being friendly, positive, trustworthy, committed, preserving and adopting a neutral manner were essential in avoiding bias of the investigator. In contrast, the investigator was aware that racial and cultural differences, for example language, which might result in inconstancies and less reliable results (Cosper, 1972). To minimise language difficulties an African Trinidadian member of staff was available in the clinic to assist. However, this problem was rare. See figure 2.1 for copy of the questionnaire used in the study.

2.5.3 Ethnic Classification

Ethnic monitoring was achieved by a self-classification system, based on the 1991 census guide to ethnic definition. This would ensure findings were comparable and applicable to practice (Bhopal, 1997). Subjects were asked what their racial origin was, but no applicable categories were expressed for classification. The subject was asked to define themselves and their forefathers in their own words. Those identified as black African, black Caribbean, Black Trinidadian or Tobagian were included in the study. Those identifying him or her as mixed (specified) or other (specified) were excluded.

Screening Checklist for Trinidad Blood Pressure Study

Name ……………………………………………………………………………………

I.D Number ……………………………………………………………………………………

Age/ d.o.b ……………………………………………………………………………………

Generation and ethnic origin …………………………………………………………………

Date of Visit ……………………………………………………………………………………

- Height (cms) ……….

- Weight (kg) …………..

- BMI ………………….

PWA/V

Augmentation (%) 1……. Index 1…..
2……. 2…..
3……. 3…..

Mean ……… ……...

Central Pressure B/P ………   **Pulse Pressure** 1………

2……… Mean……

3………

Blood Pressure

1……………

2……………

3……………

Mean B/P ………….

Lipids (mmols/l)

1. Total Cholesterol ……….

3. HDL ……………

5. VLDL ……………….

7. Fasting glucose ………….

2. Triglycerides………….

4. LDL ………………….

6. HDL Ratio ……………

Cardiovascular Risk Assessment Form

If you are unsure about any of the questions, please ask a member of the medical team who will gladly help you complete this form.

1. Personal Details

Name _____

Date of Birth _____

Address _____

_____

Contact telephone number _____

Name of Referring Doctor or G.P_____

(Please circle or tick that which applies to you)

Gender: Male /Female   Education: Primary/ Secondary/ Tertiary

2. Ethnicity

Please fill in below your parents and grandparents ethnic background

Parents:

Mother _____   Father _____

Grandparents Maternal:

Mother _____   Father _____

Grandparents Paternal:

Mother _____   Father _____

How do you define your ethnicity? _____

3. Medical History

Have you been diagnosed with any of the following conditions?

Please circle or tick which applies.

A) High blood Pressure	Yes/No	B) Diabetes	Yes/No
C) High Cholesterol	Yes/No	D) Angina	Yes/No
E) Stroke	Yes/No	F) Heart Attack	Yes/No
G) Cardiac Failure	Yes/No	H) Renal Problems	Yes/No

4. Family History

Has anyone in your family suffered from?

A) High blood Pressure	Yes/No	B) Diabetes	Yes/No
Who and at what age _____		Who and at what age _____	
C) High Cholesterol	Yes/No	D) Angina	Yes/No
Who and at what age _____		Who and at what age _____	
E) Stroke	Yes/No	F) Heart Attack	Yes/No
Who and at what age _____		Who and at what age _____	
G) Cardiac Failure	Yes/No	H) Renal Problems	Yes/No
Who and at what age _____		Who and at what age _____	

5. Medication

Are you taking any medications and if so what is it and how much per day?

Tablets/Drugs   Amount taken each day (in mg if possible)

_____   _____

_____   _____

_____   _____

6. Smoking

Do you smoke or have you in the past?

(please circle or tick that which applies)   Yes/No

If yes, how many per day   _____

How long in years   _____

Do you smoke now?   Yes/No

When did you give up?   _____

7. Any other information

Is there anything else you would like to tell us about your health?

Thank you for filling out this questionnaire.

In collaboration with the University of the West Indies, Medical Sciences Faculty and the University of Nottingham, Health and Medicine Department, United Kingdom

Figure 2.1 Sample Copy of Screening Checklist and Participant Questionnaire

2.5.4 Test of Hypothesis

To test a number of hypotheses empirically, concepts are defined and explanation required on how they will be measured (Bowling, 2000). This is to enable correct interpretation and comparison to other similar studies. For example, when the independent variable was ethnicity the dependent variables were arterial compliance, blood pressure, fasting lipid and glucose levels, and genetic polymorphism.

2.6 Pulse wave Analysis-Technique (1)

2.6.1 Introduction

Pulse wave analysis allows the generation of the ascending aortic pressure waveform from the arterial pulse pressure. It is recorded non-invasively by applanation tonometry (Chapter 1, section 1.7.2) in the radial or carotid artery. This is made possible by the use of high fidelity tonometers, by characterization of hydraulic properties of the artery in an upper limb or the neck, and through the employment of mathematical engineering techniques in advanced computer languages or systems (O'Rourke et al, 1996).

The description of application of PWA is described in section 2.5 and 2.5.1 of this chapter.

2.7 Screening Subjects

2.7.1 Screening for Aspirin and Non-steroid anti-inflammatory drugs.

Subjects were asked if they had taken aspirin or any non-steroidal, anti-inflammatory drugs (NSAID) in the past seven days. Such drugs improve acetylecholine-mediated vasodilatation in patients with atherosclerosis (Noon et al, 1998). This would influence arterial compliance and not give a true reflection of arterial stiffness. If

subjects had taken aspirin or non steroidal anti-inflammatory drugs (NSAID's) they were asked to return at a later date to allow a *'wash out'* period of time for the drugs not to be effective.

2.7.2 Screening of Lipid Profile

All subjects had a fasted, venous blood sample, taken for lipid profile at the end of the clinic visit and fat intake was assessed through dietary questions. As the cost of testing for hypercholestesterolaemia was high in the West Indies, the application of Cholestech (Bayer) slides for lipid profile and glucose was found to be more cost effective, portable for clinic use and efficient with instant results, and well validated in comparison to laboratory analysis.

2.7.3 Method of lipid analysis

Subjects had a tourniquet applied to the upper arm and blood drawn from a brachial vein using a 21g needle (Leur-lock Terumo) and 5ml syringe (Terumo). A small drop was applied to the well area of the Cholestech slide and placed within the Cholestech (Bayer) analyser for analysis. This process took approximately 5 minutes. The remaining whole blood was placed in a Vacutainer (BD Systems) for genotyping. The results would appear on a digital screen. The results were recorded on the clinical research form (CRF) and a duplicate form was made for the subjects' reference. The used slide was then removed from the analyser and disposed of in a designated disposal unit for sharps and blood product waste.

2.8 Genetics-Technique (2)

2.8.1 DNA extraction method.

2.8.2 Introduction

The swiftness and proficiency of DNA (deoxyribonucleic acid) extraction has been advanced by the availability of commercially prepared extraction kits and the choice of method depends on the material available for analysis. The general principle of DNA extraction is the lysing of cells, followed by separation of the DNA contaminants of whole blood lysate, which could interfere with further reactions such as PCR (polymerase chain reaction).

2.8.3 DNA extraction from clotted blood (whole blood)

Purification of DNA from 200µl of clotted blood (whole blood) was achieved using the QIAmp blood kit from Qiagen. 5mls of whole blood were collected into sodium EDTA (diaminoethanetetra-acetate acid) tubes, to provide material for DNA extraction. Samples were then stored at -20°C until DNA extraction was performed.

2.8.4 Lysis and preparation of whole blood sample

A 5ml clotted blood sample was defrosted carefully at laboratory temperature (22°C ± 1°C) for approximately one hour and 200µl was pipetted into a 1.5ml eppendorf tube. 25µl Qiagen protease (as supplied by the kit) was added, followed by 200µl lysis buffer AL (as supplied by the kit), the cap closed and vortexed for 15 seconds to promote efficient lysis. The sample was then placed in a water bath at 70°C and incubated for 10 minutes. 210µl isopropanol (100% v/v solution) was added and the sample vortexed briefly to mix. This helps increase the yield of DNA extracted from the blood.

2.8.5 Absorption of DNA

A QiaMP spin column was placed into a 2ml collection tube (supplied in the kit) and 600µl of the dilution was carefully pipetted into the spin column without moistening or staining the rim of the column. The column cap was closed and centrifuged at 13,000rpm for 1 minute. The spin column was then removed and placed into a clean collection tube. The remaining filtrate from centrifuging was disposed of carefully in accordance to guidelines for the disposal of blood products.

2.8.6 Washing of DNA

The spin column cap was gently opened and 500µl of Wash buffer AW (supplied in the kit) was added. The column was centrifuged at 13,000rpm for 1 minute. Again the spin column was removed and placed in a clean collection tube and a second 500µl of wash buffer AW was added. This time the sample was centrifuged at 13,000rpm for 3 minutes to remove all buffer AW from the column. All filtrate from the washing process was discarded safely.

2.8.7 Elution of DNA

Following washing, the spin column was placed in a 1.5 eppendorf tube. 200µl of sterile water heated to 70°C was pipetted into the column. The cap was closed and incubated at 70°C for 5 minutes. This increases yield compared to room temperature incubation. The sample was then centrifuged at 13,000rpm for 1 minute to elute the DNA from the column. The DNA sample was then marked with the appropriate reference number and the 1.5ml eppendorf tube was then placed in the -20°C freezer to prevent DNA breakdown.

2.9 Polymerase chain reaction (PCR)

2.9.1 Introduction and PCR amplification conditions

In order to generate fragments of DNA spanning the specific region of interest (in this case the β_2 AR gene) PCR was used. The basis for the PCR reaction is to use oligonucleotide primers to hybridise opposite strands and target the DNA sequence that is to be enlarged and extended. The heat-stable enzyme *Taq* DNA polymerase catalyses the elongation of the primer.

A series of temperature changing cycles allows template denaturation, primer annealing and extension of the annealed primers by the *Taq* polymerase, resulting in an exponential increase in the target DNA. See figure 2.2

5' A C T A A 3'

←

Reverse Primer

5' A C T A A G G A G G T A G T T 3'

━━━━━━━━━━━━━━━━━━━━━━━

3' T G A T T C C T C C A T G A A 5'

Template DNA

3' A T G A A 5'

→

Forward Primer

↓

5' A C T A A G G A G G T A G T T 3'

Heat -stable *Taq*

‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑

DNA **polymerase**

‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑

3' T G A T T C C T C C A T G A A 5'

DNA strand separates at 95°C -Denaturation

↓

5' A C T A A G G A G G T A G T T 3'   **Cool to annealing temperature 60°C**

‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑‑━━━━━━

← ▬▬ ✦ 3' A T G A A 5'

↑   ↱   5' A C T A A 3' ✦ ▬▬▬ →

━━━━━━━‑‑‑‑‑‑‑‑‑‑

3' T G A T T C C T C C A T G A A 5'

Heat to 75°C, *Taq* **polymerase extends the complimentary DNA strand**

Repeated cycles result in amplification of the region of DNA between the two primers.

Figure 2.2 A Schematic of a PCR reaction, resulting in the amplification of region of DNA, using two primers. A. Kornberg; *DNA Replication* 1980

A typical amplification reaction for PCR was as follows:

- ❖ 5μl   10x PCR reaction buffer

- ❖ 3μl   MgCl₂ (25 mM stock, 1.5 mM in reaction)

- ❖ 2μl   dNTPs (5Mm stock, 0.2Mm dCTP, dGTP, dATP, and dTTP in the reaction)

- ❖ Xμl   5' Primer (0.1 nmol)

- ❖ Xμl   3' Primer (0.1 nmol)

- ❖ Xμl   target DNA (50-100ng)

- ❖ Xμl   sterile water to make up to 48μl volume total

- ❖ 1μl   *Taq* DNA polymerase

The combined volume (48μl) reaction was overlaid with one drop of light white mineral oil (Sigma). This prevented the constituents of the reaction from evaporating during the reaction cycles.

The reactions were placed in a Biometra thermocycler. A 'Hot start' PCR was used. The sample was heated to 95°C for 5 minutes, (which allows the DNA to denature fully), before the addition of 1μl *Taq* DNA polymerase, pipetted into the reaction below the overlaid mineral oil. The first cycle was then 60°C annealing for 90 seconds followed by 72°C for another 90 seconds for DNA extension. For subsequent cycles, denaturation was performed for 90 seconds at 95°C, followed by annealing for 90 seconds at 60°C and the extension for 90 seconds at 72°C. The complete reaction consists of 36 cycles.

At the end of the reaction, a final 10 minutes at 72°C, extension was performed. Once complete the PCR reaction was placed in placed in the refrigerator at 4°C before use for the next stage.

Table 2.1 PCR conditions for amplification of β-2 Adrenoceptor Fragment.

Gene polymorphism	Primer sequence	Annealing Temperature	Cycle Number	Fragment Size
β-2 Adrenoceptor ARG16→GLY & GLN27→GLU	**Forward primer** 5'CCCAGCCAGTGCGCTTACCT3' **Reverse primer** 5'CCGTCTGCAGACGCTCGAAC3'	60°C	36	234bp

2.10 Agarose Gel Electrophoresis

2.10.1 Electrophoresis

The standard gel used for the studies described in this thesis was composed of 1% agarose dissolved by microwave in 100mls of 1xTAE buffer and 5 µl (10mg/ml) ethidium bromide. This was left to cool in a glass jar. Space combs and dividers were placed in position in the electrophoresis tank and the cooled gel solution was gently poured into the tank. Once set the combs and dividers were removed and the set gel was covered with 1 x TAE buffer. 1 µl of loading dye was added to 6µl PCR product, and steadily pipetted into one of the wells on the gel. Each gel had the capacity to perform 24 PCR samples. The tank was then attached to the voltage conductor (power unit) and a current of 35 volts (40 milliamps) was passed through the gel for approximately 30 minutes. This allows separation of the PCR product on the basis of fragment size. Once complete the buffer solution was poured off and the gel examined under UV illumination.

2.10.2 Visualisation of DNA fragments

The gel was removed carefully from the electrophoresis tank and placed gently onto a short wave (302nm) UV transilluminator. The ethidium bromide in the agarose gel imbeds within the DNA, enabling the fragments to be viewed under UV illumination. A Polaroid photograph was taken for identification and verification of PCR products. See figure 2.3.
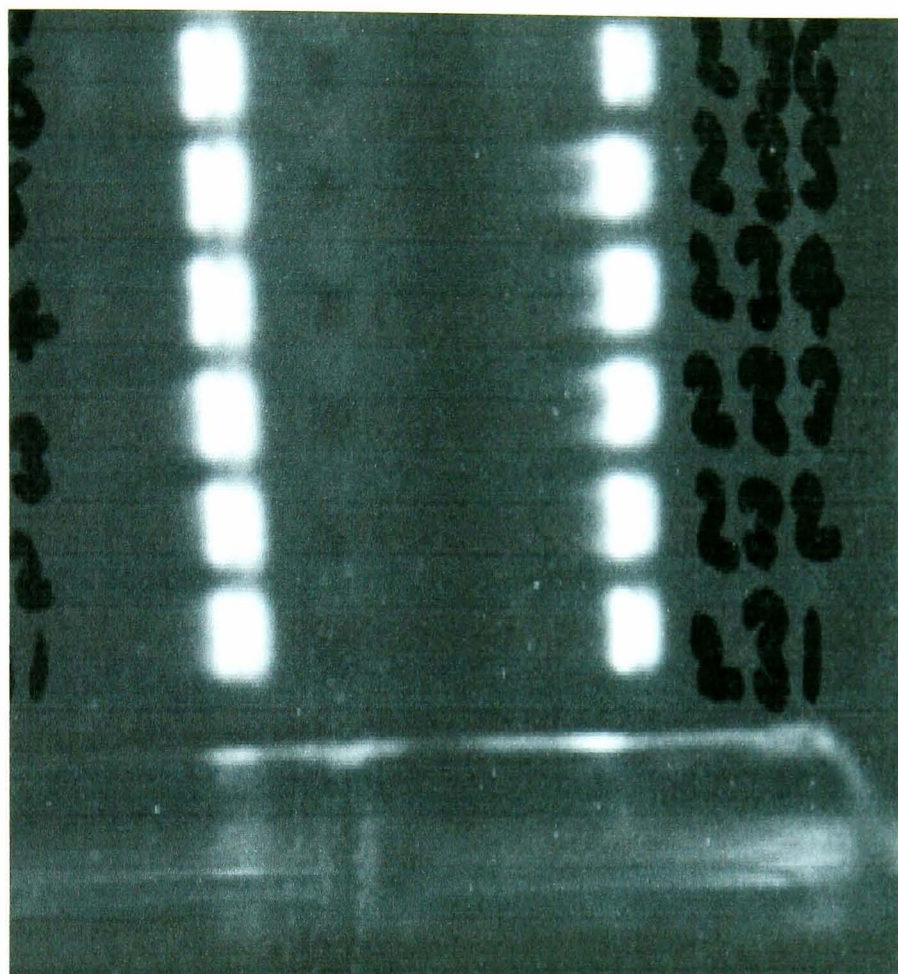
Figure 2.3 A 1% agarose gel representing PCR fragments spanning Arg→Gly16 and Gln→Glu 27.
All lanes marked 5-1 underwent PCR conditions of 60°C through 36 cycles.
(Produced by Dr. Amanda Wheatley).

2.11 Allele specific oligonucleotide (ASO) hybridisation

2.11.1 Introduction

Allele specific oligonucleotide (ASO) hybridisation is a technique (Gunneburg et al, 1993), that enables genotype to be determined by using allele-specific oligonucleotides to compete for binding to sequences containing single nucleotide polymorphisms (SNPs). It is an efficient and effective assay for screening large numbers of subjects or populations.

2.11.2 Genomic DNA, Polymerase chain reaction (PCR) and Allele specific oligonucleotide hybridisation (ASO)

PCR fragments spanning the region of interest were generated as described in Table 2.2 and figure 2.4, used for ASO.

Table 2.2 Oligonucleotide sequences of the probes used for ASO detection.

SNP Position	Oligonucleotide probe sequence
β_2-Adrenoceptor ARG 16	5' GCACCCAATAGAAGCCATG 3'
β_2-Adrenoceptor GLY 16	5' GCACCCAATGGAAGCCATG 3'
β_2-Adrenoceptor GLN 27	5' CACGCAGGAAAGGGACGAG 3'
β_2-Adrenoceptor GLU 27	5' CACGCAGCAAAGGGACGAG 3'

2.11.3 ASO filter preparation

Hybond N+ membranes (Amersham) and blotting paper (Whatman) were cut to size, to fit the 96 well dot blot apparatus (Gibco BRL Life Technologies). Two pieces of blotting paper and one Hybond N+ membrane were soaked in 2x SSC (see materials), before being placed on the dot blotting apparatus. Two pieces of blotting paper were placed over the 96 holes making sure all the well holes were covered and then the Hybond N+ membrane placed on top. The apparatus was then assembled by placing the lid and tightened into place by 8 screws. A vacuum was then applied to draw off excess fluid. Two filters were prepared for genotyping each SNP, one filter for each allele. Each sample was prepared by adding 10µl of the PCR product to 400µl of 0.4M NaOH with Bromophenol blue dye, (enough to colour the solution), 400µl of 4x SSC was then added and the contents mixed in a 1.5ml eppendorf tube. 200µl of each sample was pipetted into duplicate wells on the dot blotting apparatus, and the remainder was saved for the second membrane for the other polymorphism being assayed. The filter was drained gently by vacuum and when complete, the 8 screws were loosened and the membranes were left to dry on a piece of absorbent paper. Blue dots from the dye were evident suggesting that the PCR product had successfully permeated the filter and thus the next stage of hybridisation could be undertaken.

2.11.4 Labelling of Probes

Two oligonucleotides containing the two polymorphic forms of the particular SNP were designed for each polymorphism for ASO analysis. Table 2.2 demonstrates the oligoneucleotides for the β_2 Adrenoceptor polymorphisms.

The following components were pipetted carefully into a 1.5 microfuge tube and in the following order.

1. 5.5 µl of H_2O

2. 1µl 10x polynucleotide kinase buffer

3. 1µl Oligo (either Arg 16, Gly 16, Gln 27 or Glu 27 dependent on which probe is being used).

4. 2µl $[\gamma-^{32}P]$ ATP

5. 0.5 µl T_4 polyneucleotide kinase (Strategene)

The combined components were then placed on a hot block and incubated for 1 hour at 37°C and then for 10 minutes at 70°C to denature the enzyme. The labelled probes were then either used immediately for hybridisation or were stored at -20°C.

2.11.5 Methodology of Hybridisation

The oligonucleotide employed is a segment of synthetic DNA, short in length designed to enhance the area of DNA containing the specific polymorphism. A single mismatch between an oligonucleotide probe and template DNA will substantially decrease annealing capacity, which leads to discrimination between different alleles. Allele specific oligonucleotide (ASO) is a competitive assay, which strives to amplify DNA products that are applied to duplicate membranes (X and Y) with the dot blotting apparatus. Membrane X is incubated firstly with unlabelled oligonucleotide probe ('cold') homologous to the mutant form of the receptor, and membrane Y with unlabelled oligonucleotide probe ('cold') homologous to the wild-type form of the receptor. Secondly, the membranes are hybridised with 32P-(Phosphorous) labelled

probes (radioactive, 'hot') in the opposing order. Membrane X is exposed to the homologous probe of the wild-type form receptor, and membrane Y to homologous probe of the mutant form of the receptor.

2.11.6 ASO Hybridisation

The dried filters were carefully labelled and placed between two pieces of gauze. If more than one filter was hybridised, an additional piece of gauze was over-laid, so each filter was separated. They were then rolled up and placed in a tight fitting screw top hybridisation bottle. The bottles were labelled and 20mls of 5x SSPE 1% SDS buffer wash added with 10-50µl of COLD unlabelled oligonucleotide probe was pipetted carefully into the bottle containing the membranes. The bottles were placed in an incubator at 52°C in a hybridisation oven, which gently rotates for 1 hour. Once incubated the bottles were opened and the liquid poured off and disposed of. Another buffer wash of 20mls of 5x SSPE 1% SDS was added and the process repeated, this time with the HOT probe (^{32}P labelled). This time the HOT probe was added to the corresponding bottles, for example Arg 16 probe was added to the Arg 16 membranes and bottle. Once the incubation cycle was completed the radioactive probe and solution was poured off in the appropriately designated disposal unit. The filters were washed x 4: Firstly, 20mls 2 x SSPE 0.1% SDS was added to the bottles and rotated in the incubation oven at room temperature for 30 minutes, then the solution poured off. This wash was repeated using 20mls 2 x SSPE 0.1% SDS for another 30 minutes. The third and fourth wash consisted of 20mls 5 x SSPE 0.1% SDS being added to the membranes and bottle and rotated in the incubation oven at 52°C for 15 minutes.

The filters were then placed between x-ray plates, covered by cling film and placed in an X-ray cassette with intensity screens and placed in the -80°C freezer overnight.

2.11.7 X-ray film development

Once the x-ray film (Fuji) had been exposed on the filter membranes, usually overnight, they were processed for development. A 1:5 dilution of developer (Kodak) and rapid fixer (Ilford Hypam) were made and each placed in a plastic container tray. Distilled sterile water was also placed in a tray. In a dark room with nominal exposure light (red lamp) the film was lifted out of the x-ray plate cassette and the right hand corner creased to orientate the image. The film was placed in the developer and gently rocked for 2 minutes to allow full coverage, but minimal disturbance of the image. The film was transferred to the water tray rinsing and then to the fixer tray and gently rocked for 2 minutes to fix the image. A final rinse in the water tray was undertaken before the film was hung to dry. This development process was used for all genotyping imaging.

2.11.8 Reading of filters to determine genotype

The two membranes were read in parallel, with one membrane displaying signal for wild-type homozygotes, and heterozygotes, and the other displaying the mutant homozygotes and heterozygotes. Control samples with known genotype were used on all membranes to confirm determination of homozygote and heterozygote signals. See Figures 2.5 and 2.6.
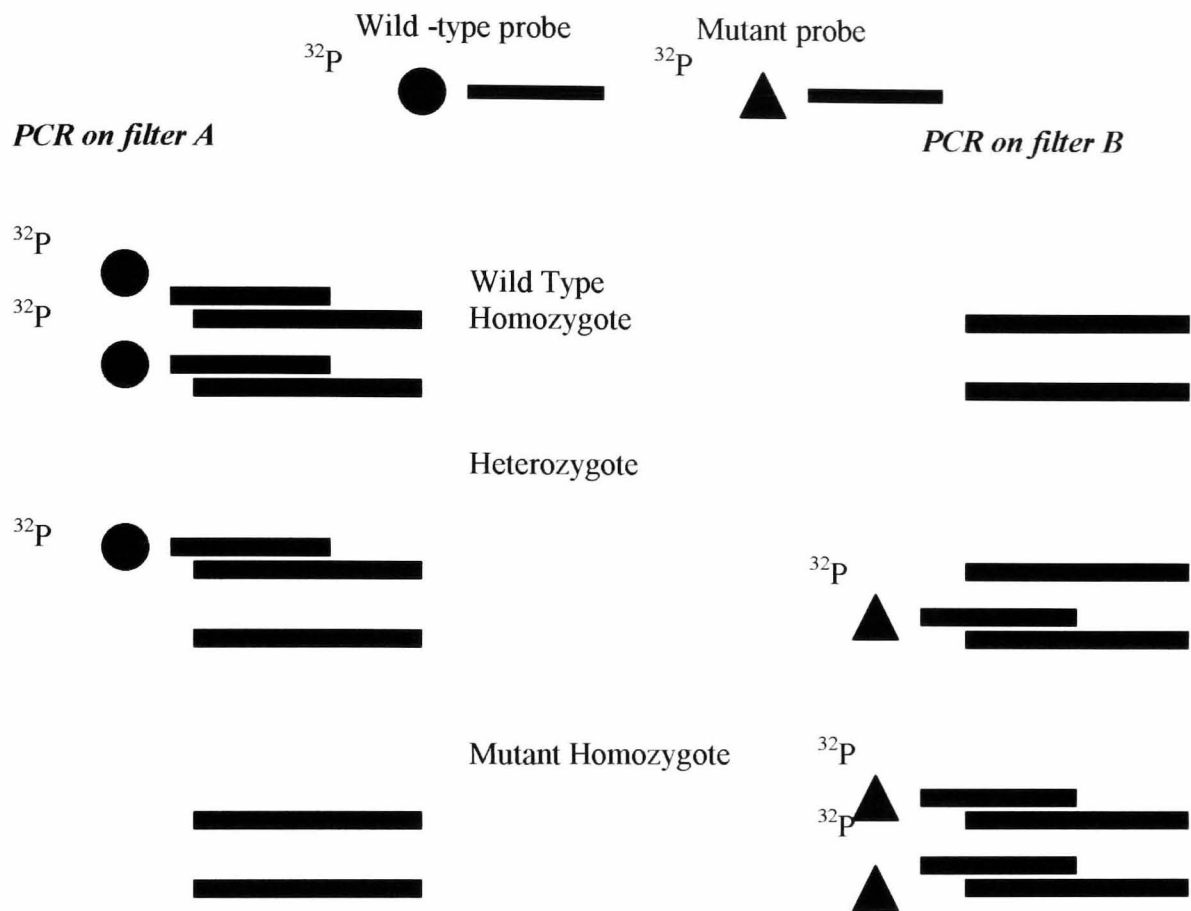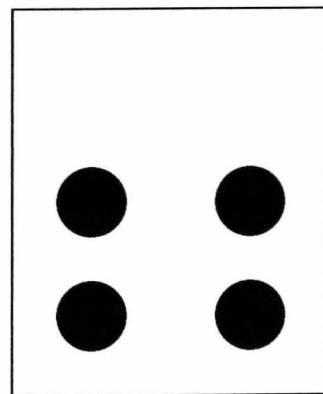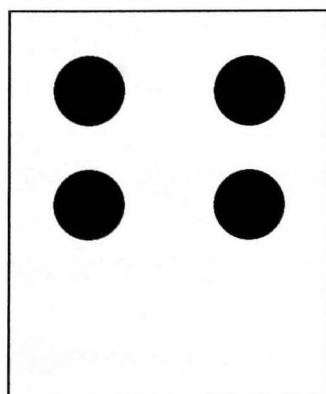
Figure 2.4 Allele specific oligonucleotide hybridisation. The PCR spans the polymorphism. This is denatured and spotted onto two separate filters (A and B as shown above) These are placed in hybridisation tubes with a 'Cold' probe. Hybridisation with the 'Hot' probe binds the specific alleles on each filter. The results are read from an autoradiograph due to ASO.
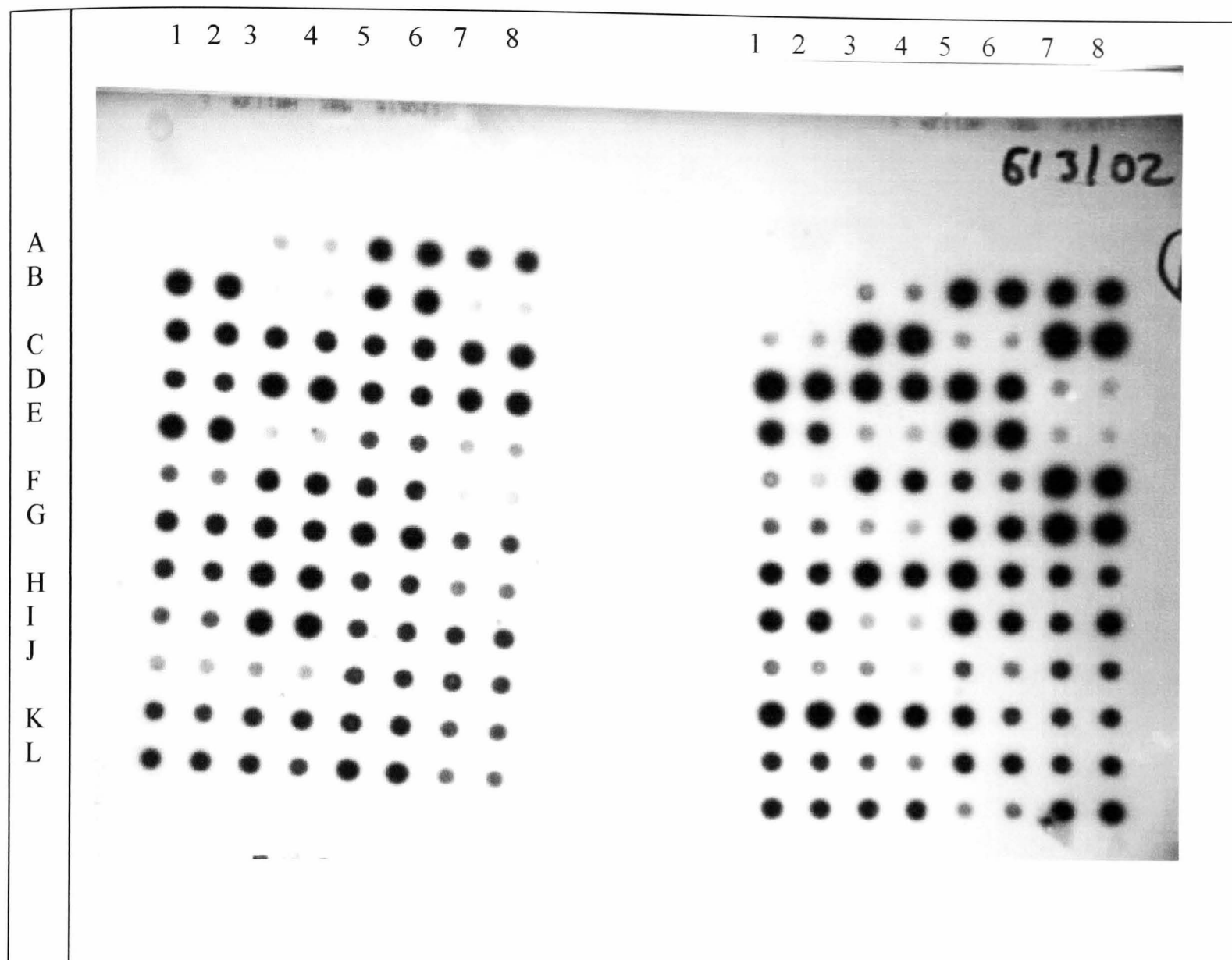
Figure 2.5

Arg16 and Gly16 ASO Hybridisation filter plates. Duplicate spots represent a single subject. A1 + 2 is a negative control and A3 + 4 is a water control sample. Comparison of filter plates would determine the subject as being either heterozygote or homozygote. For example, the positive signal in the left hand filter for B1 + 2 indicates a homozygous Arg16 individual. B3 + 4 indicates a homozygous Gly 16, and C1 + 2 indicates a heterozygote.
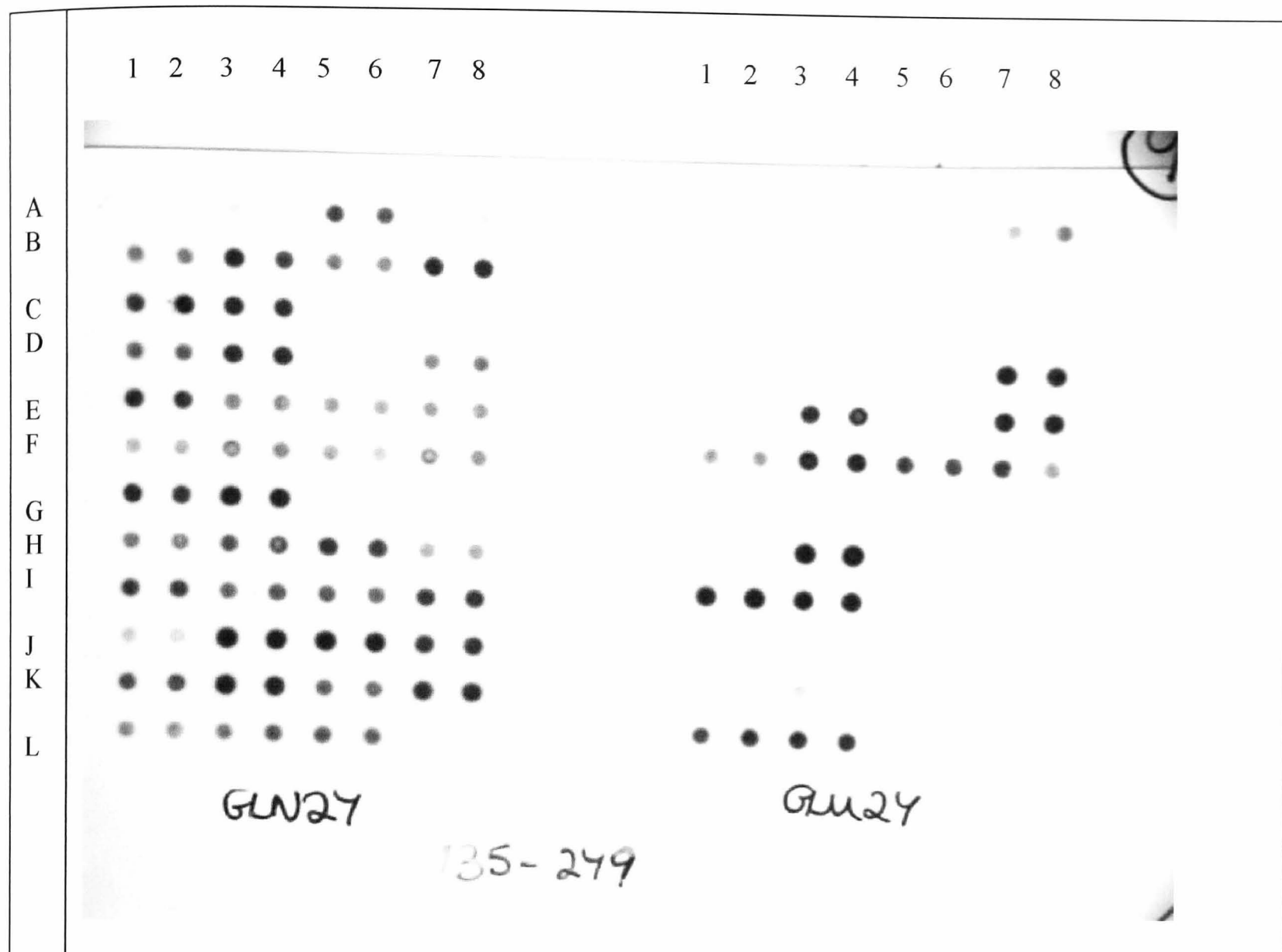
Figure 2.6

Gln27 and Glu27 ASO hybridisation filter plates. Duplicate spots represent a single subject. A1 + 2 is a negative control and A3 + 4 is a water control sample. Comparison of filter plates would determine the subject as being either heterozygote or homozygote. (See previous figure for explanation).

2.12 Verification of Polymorphisms

Automated sequencing has advanced at a rapid rate over the past decade with SNPs being easily detectable in double peaks of chromotograms (See Figures 2.7, 2.8, 2.9, 2.10, 2.11, 2.12). Sequencing is used to define and confirm the mutation once it has been found to exist by one of the other scanning or genotyping techniques. This process is very expensive and was only used to verify some polymorphism of each type to conclude the accuracy of hybridisation.
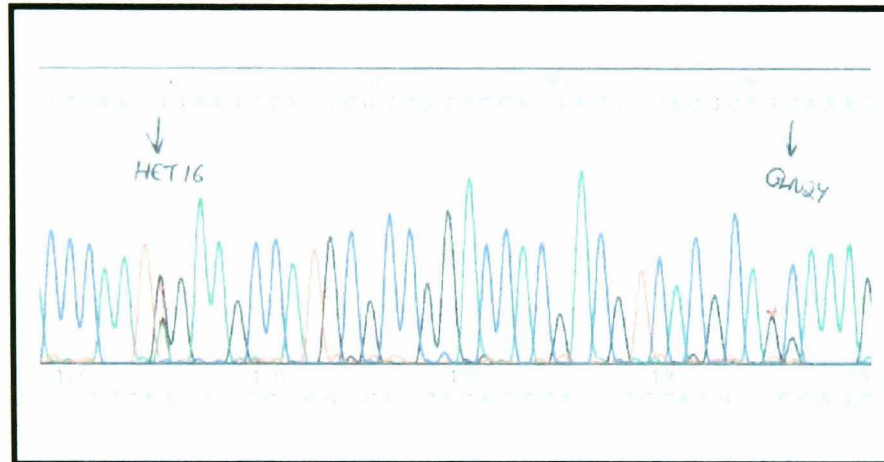
Figure 2.7

Automated sequencing of Gln27 and Arg/Gly16.

The single peak represents alleles of Gln 27 and the ununiformed double peaks are representing one allele from the mutation and wild type, thus being heterozygous on the sequencing probe of Arg/Gly16.
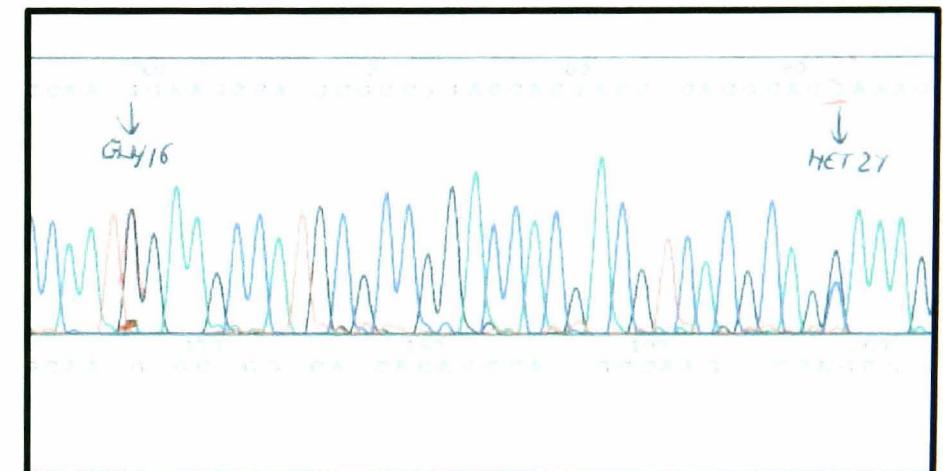
Figure 2.8

Automated sequencing of Gly16 and Gln/Glu27.

The single peak represents alleles of Gly 16 and the ununiformed double peaks are representing one allele from the mutation and wild type, thus being heterozygous on the sequencing probe of Gln/Glu27
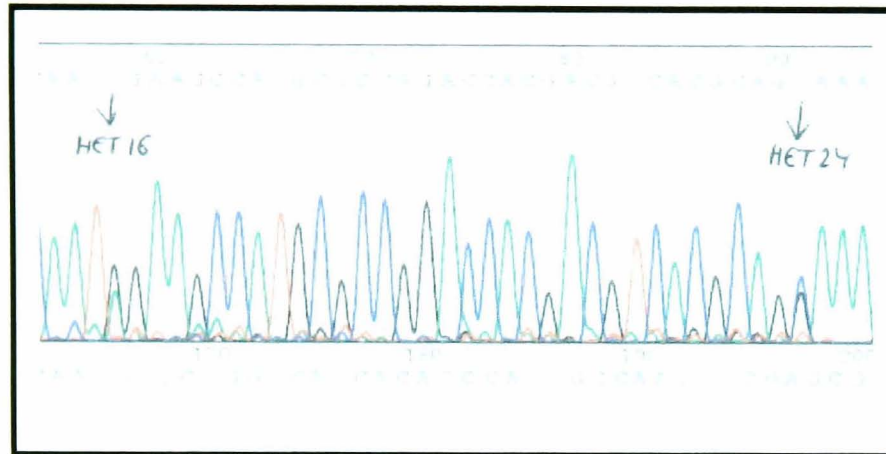
Figure 2.9

Automated sequencing of Arg/Gly16 and Gln/Glu27

The ununiformed double peaks are representing one allele from the mutation and wild type, thus being heterozygous on the sequencing probe of Arg/Gly16 and Gln/Glu27.
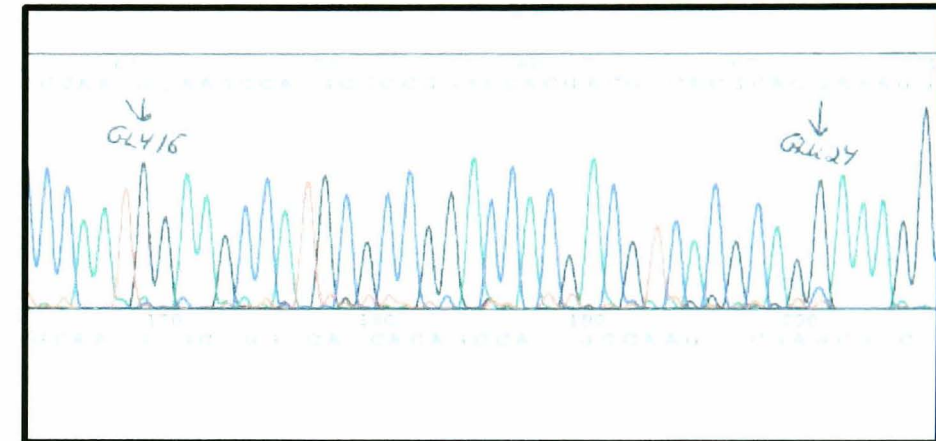
Figure 2.10

Automated sequencing of Gly16 and Glu27.

The single peak represents alleles of Gly 16 and Glu27. This represents a double mutant homozygote.
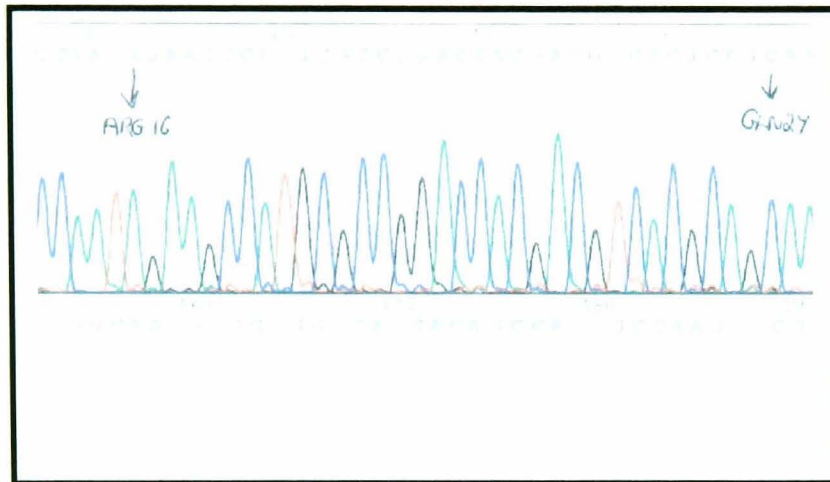
Figure 2.11

Automated sequencing of Arg16 and Gln27.

The single peaks represents alleles of Arg16 and Gln27 Sequencing probe represents wild type or dominant homozygotes.
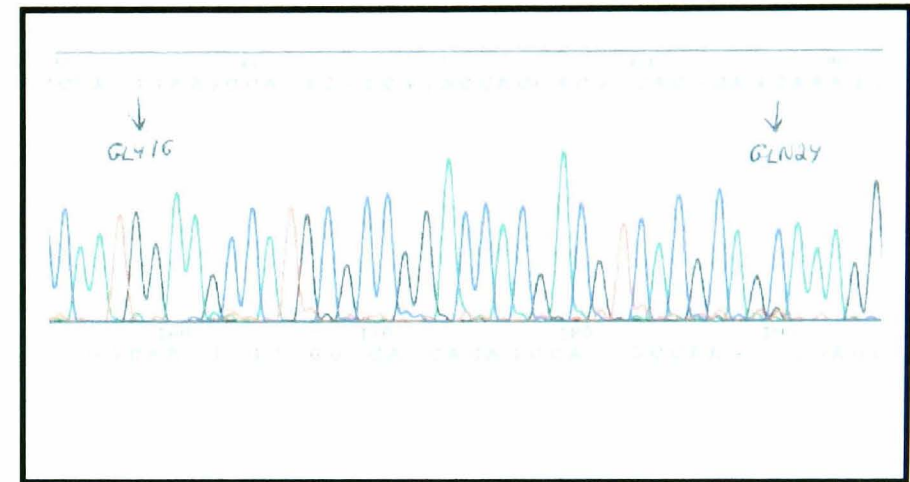
Figure 2.12

Automated sequencing of Gly16 and Gln27.

The single peaks represents alleles of Gly16 and Gln27 Sequencing probe represents a mutant and wild type homozygotes.

2.12.1 Sequencing

The technique used to distinguish band sizes without using ^{32}P is automated sequencing. A PCR product is produced and purified, amplified using electrophoresis then cut from agarose gel. This is then melted and prepared so it can be reloaded onto a polyacrylamide gel and electrophoresis can be repeated. This time, a laser beam is then passed through the strands at the bottom of the gel. The laser detects which fluorescent tag has been excited depending on which primer is present in the band. This information is directly sent to a computer display, producing a read out of different coloured peaks, one colour for each base. Automated sequencing was achieved on an ABI prism (377) sequencing system. This was kindly performed and produced by Ms. Ingrid Davies of the Biochemistry Department and Dr. Amanda Wheatley of the Department of Therapeutics, University of Nottingham.

2.13 Statistical and Data Analysis

This is a brief outline of the statistical analysis, which is used for this thesis. The following chapters provide the analysis of the data collected.

2.13.1 Chi-squared (χ^2) statistic

This test is used for comparing overall patterns of performance using frequency scores. Categories are identified and thus frequencies of scores fall into cells. Cells contain the counted items and can test by association only. They are useful in particular when comparison and distribution between two groups are unequal.

In this case it is used to test whether the numbers of subjects with specific genotypes is different between hypertensive and normotensive groups.

This is formulated and tested using the equation:

Equation 2

$$\frac{\sum (\text{Observed - Expected})^2}{\text{Expected}} \qquad \frac{\sum (O\text{-}E)}{E}$$

A p value is determined using the χ^2 table and degrees of freedom are (n-1) where n is the value or number of parameters in the equation. A p value that is less than 0.05 is significant and therefore a null hypothesis can be rejected. If not, the null hypothesis remains true and thus no significant difference is determined between the groups.

2.13.2 Hardy-Weinberg Equilibrium

In a large random-mating population with no selection, migration or mutation the gene frequencies and the genotype frequencies are constant from generation to generation; and furthermore, there is a simple relationship between the allele frequencies and the genotype frequencies. Their properties are described by a theorem known as the *Hardy-Weinberg law*. A population with a constant allele and genotype frequency is said to be in *Hardy-Weinberg equilibrium*. The relationship between allele and genotype frequencies is important as population and quantitative genetics rest upon it.

For example if the allele frequencies of two alleles among the parents are p and q then the predicted genotype frequencies among offspring are p^2, $2pq$ and q^2. (See table 2.3).

	Genes in parents		Genotype in offspring		
	A_1	A_2	A_1A_1	A_1A_2	A_2A_2
Expected	p	q	p^2	$2pq$	q^2

Table 2.3 Represents the predicted frequencies among offspring to calculated Hardy Weinberg equilibrium.

2.13.3 Test of Hardy-Weinberg equilibrium

If data are available for a locus where all the genotypes are recognisable, the observed frequencies of the genotypes can be tested for agreement with a population in Hardy-Weinberg equilibrium. According to this Hardy-Weinberg law, the genotype frequencies of offspring are determined by the allele frequency of the parents. If a population is in equilibrium, the allele frequency is the same in the parents and their offspring. Therefore, the allele frequency **observed** in the offspring can be used as if it were the parental gene frequency to calculate the genotype frequency **expected.**

2.13.4 Hardy-Weinberg Equation

Equation 3

$$p^2 + q^2 + 2pq = 1$$

p = observed frequency of the wild type allele

q = observed frequency of the mutant type allele

p^2 denotes the anticipated frequency of homozygous wild type individuals and q^2 represents the homozygous mutant individuals in a population where the alleles are in equilibrium whilst $2pq$ is the number of heterozygous subjects .

2.13.5 Linkage disequilibrium analysis

Linkage disequilibrium is the phenomenon of non-random assortment of alleles at loci which are physically close to each other. A given mutation will arise in a particular background of other mutations or polymorphisms, will only become randomly distributed in a population as generations pass.

The closer together in physical terms the two polymorphisms, the less likely that crossover will occur between them, and therefore, the longer it will take for linkage disequilibrium between two sites to disappear. In practice, most SNPs in close approximation (e.g. within a gene) are in at least partial linkage disequilibrium.

2.13.6 GraphPad Prism

GraphPad Prism® was used to combine linear regression (curve fitting), analyse biostatistics, and produce the scientific graphing. It was felt that this software was most appropriate to efficiently assist in the analysis of data, produce high quality graphs and organise the experimental data.

2.14 Suppliers and Solutions

Molecular biology kits were supplied by Scot-lab Nucleon II and Qiagen for genomic DNA extraction from whole blood. Promega Wizard® was used for PCR DNA purification and Gibco BRL Life Technologies for dsDNA cycle sequencing. Biochemicals were from Sigma, Gibco BRL Life Technologies, and Fisher

Pharmaceuticals. DNA manipulation enzymes were from Promega, Roche, and Stratagene. Amersham provided hybridisation membranes and radio chemicals. Oligonucleotides were supplied by Oswell. Lipid and glucose cartridges and analyser were supplied by Cholestech (Bayer). Photographic equipment was from a number of suppliers; Film and camera for agarose gel photographs were from Polaroid and developer from Kodak. Rapid fixer from Ilford Hypam and X-ray film from Fuji. SphygmoCor®, Pulse Wave Analysis System was developed and supplied from AtCor Medical and analysis of data by GraphPad®, Prism Inc. version 3.0.

2.14.1 Materials and Solutions

Nucleon II DNA extraction kit components

Reagent A:

10 Mm TRIS-HCL,

320 Mm Sucrose,

5mM $MgCl_2$

1% Triton X-100

Adjust to pH 8.0 using NaOH

Reagent B:

400mM Tris-HCL, pH 8.0 using 40% NaOH

60mM EDTA

150mM NaCl

1% SDS

2.14.2 Enzymes and Buffers

T$_4$ Polynucleotide Kinase storage buffer (Stratagene)

50mM Tris-HCL, Ph 7.6

25mM KCL

0.1mM EDTA

1 mM DTT

0.1μM ATP

50% glycerol (v/v)

T$_4$ Polynucleotide Kinase 10x reaction buffer (Stratagene)

500mM Tris-HCL, pH 7.5

70mM MgCl$_2$

10mM DTT

T$_4$ Polynucleotide Kinase storage buffer (Promega)

20mM Tris-HCL, pH 7.5

25mM KCL

0.1mM EDTA

2mM DTT

0.1μM ATP

50% glycerol (v/v)

T$_4$ Polynucleotide Kinase 10x reaction buffer (Promega)

700mM Tris-HCL, pH 7.6

50mM MgCl$_2$

50mM DTT

Taq DNA Polymerase storage buffer (Promega)

50mM Tris-HCL, pH 8.0

100mM NaCl

0.1mM EDTA

1mM DTT

1% Triton X-100

50% glycerol

Taq DNA Polymerase 10x reaction buffer (Promega Storage buffer A, without Mg Cl₂

500mM KCL

100mM Tris-HCL, pH 9.0 at 25°C

1% Triton X-100

2.14.3 General use solutions

10% AMPS

1g Ammonium pursulphate

10mls H_2O

6x Agarose gel loading dye

0.05% bromophenol blue

0.05% xylene cyanol

40% sucrose

0.5M EDTA (pH 8.0)

186.1g EDTA (disodium ethylenediaminetetra-acetate.$2H_2O$)

dissolve in 800mls H_2O, adjust to pH 8.0 with NaOH (approx. 20g of NaOH pellets).

Autoclave to sterilise.

Ethidium Bromide (10mg/ml)

1g ethidium bromide

100mls H_2O

Stir for several hours until dye has dissolved. Wrap container in aluminium foil and

store at room temperature.

10% SDS

10g sodium doecyl sulphate

100ml H_2O

20X SSC

175.3g NaCl

88.2g Sodium Citrate dissolved in 800ml H_2O to pH 7.0 using 10N solution NaOH

and make up to 1 litre. Autoclave to sterilize.

20x SSPE

175.3g NaCl

27.6g $NaH_2PO_4^-$-H_2O

7.4g EDTA

dissolved in 880ml in H_2O, adjust to pH 7.4 using 1M NaOH and make up to 1 litre. Autoclave to sterilize.

50X TAE

242g tris base

57.1ml acetic acid

100ml 0.5M EDTA pH 8.0 make up to 1 litre with distilled H_2O, autoclaved to sterilize.

5X TBE

54g tris base

27.5g boric acid 20ml 0.5M EDTA pH 8.0 make up to 1 litre with distilled H_2O, autoclaved to sterilize.

Chapter 3

Demographics of African Trinidadians

3.1 Results

3.1.1 Statistical Analysis

GraphPad Prism® (GraphPad Software Inc., 1999) was used to analyse biostatistics, and produce the scientific graphing. All data since summarised using mean and standard deviation (± SD). Comparison was by analysis of variance, with Bonferroni's correction for multiple comparisons.

All the following data is summarised in Tables 3.1, 3.2, 3.3 and 3.4.

3.1.2 Mean Age and gender of subjects

The mean age was higher in the hypertensive subjects (HT=53.15 ± 10.47 (n=156)). There were fewer hypertensive subjects than normotensives (NT=43.41±11.55 (n=252)). There were a larger proportion of female subjects in both groups, and the age ranges were lower in the NT subjects compared with the HT group.

Figure 3.1 bar charts represent the frequency and mean of NT and HT African Trinidadians with varying age ranges measured in years.

3.1.3 Body Mass Index

On the basis of mean values for BMI, both NT and HT groups were categorised as being *'overweight'* (BMI >25.0 kg m^{-2}).

In the HT subjects the mean BMI value was 29.2 ± SEM 5.5 kg m^{-2}. NT subjects had a BMI 27.6 ± SEM 5.2 kg m^{-2} and in the whole population the mean BMI was 27.6 ± SEM 5.2 kg m^{-2}. The NT subjects had a higher proportion of subjects within the BMI ranges of 20 to 30 kg m^{-2}, whereas the HT group had a higher proportion of BMI in the 26 to 35 kg m^{-2} ranges. This suggests that hypertensive African Trinidadians have

a higher BMI and this may be a contributing factor to high blood pressure. Figure 3.2 represent the frequency of NT and HT African Trinidadians with varying body mass index ranges.

3.1.4 Systolic Blood Pressure

The mean systolic blood pressure (SBP) was higher in the HT subjects (146.0 ± 21.96) than the NT subjects (118.2±12.89). The HT group SBP was classified as Stage 1 hypertensive, as defined by the Hypertension Society (1999).

Figure 3.3 shows that in the NT group, SBP was most commonly in the 101-120-mmHg range (optimal classification SBP ≤ 120) followed by 121-140 mmHg range (normal to high normal classification SBP ≤ 139). Some subjects did present with a SBP of higher or equal to 140 mmHg (SBP ≥ 140 mmHg), however were not diagnosed as hypertensive as blood pressure readings were based on two or more readings taken at one or more visits and after initial screening. There was the possibility that some subjects may have been presenting with isolated systolic hypertension as defined as SBP of 140 mmHg and a DBP below 90 mmHg. However, on the basis of average blood pressure levels, the absence of target organ disease and additional risk factors, the subjects were advised to recheck their blood pressure in six months to one year in accordance with recommendations for follow up based on initial blood pressure readings (Hypertension Society, 1999).

Figure 3.3 shows a higher frequency of SBP in groups 121-140 and 141-160 mmHg ranges. These groups are categorised as stage 1 and 2 of hypertension. The 121-140mmHg range represents African Trinidadians who were previously diagnosed with hypertension and were receiving anti-hypertensive drug therapy. This is shown in

Table 3.1. However, despite 82 subjects receiving medication for blood pressure, the mean SBP was still classified as hypertensive.

3.1.5 Diastolic Blood Pressure

The mean diastolic blood pressure was higher in the HT subjects (89.95 ± 9.69) than the NT subjects (73.2±7.87). The HT group was categorised as borderline stage 1 diastolic hypertensive and the NT category for DBP defined as optimal.

Figure 3.4 shows the highest frequency of DBP in the 61-80 mmHg range and the highest frequency of DBP in the 81-100 mmHg range. Again, 82 subjects had been previously diagnosed as hypertensive and were receiving anti-hypertensive medication.

3.1.6 Pulse Pressure

The mean pulse pressure was lower in the NT subjects (45.0 ± 9.8) than the HT subjects (56.0 ± 17.0), with the overall total for the combined groups being 49.3 ± 14.1.

However, both groups were within acceptable limits and below 60 mmHg. This suggests that isolated systolic blood pressure was not common, despite elderly subjects being represented within the cohort.

Figure 3.5 represents the frequency of normotensive African Trinidadians with varying ranges of pulse pressure, and demonstrates that there was a small group with pulse pressure that was > 60 mmHg. These may have been subjects may have been elderly, indicating reduced vascular compliance in large arteries, or subjects who were exceptionally small in height, female and with varying or increased heart rate, and

were thus classified as normotensive according to the Hypertensive Society (1999) guidelines.

There is a wider spread of pulse pressure in the hypertensive group. The frequency is greater in the 41-60mmHg range, followed by the 61-80-mmHg range. This shows vascular stiffness, despite subjects being treated on anti-hypertensive medication, an aspect of which routine blood pressure measurement from conventional brachial blood pressure measurements would not disclose.

Interestingly, pulse pressure was not higher in the NT female group compared to NT males which maybe due to the NT females being younger and pre-menopausal. This picture is reversed when compared to the HT females who were older and had a higher pulse pressure compared to HT males.

3.1.7 Mean Arterial Pressure

As expected, the mean arterial pressure was higher in the HT group (108.6 ± 12.6) compared to the NT group (88.2 ± 8.7) (Figure 3.6, also see sections 3.1.4 and 3.1.5 of this chapter.

3.1.8 Heart Rate

There was minimal variability between the NT and HT groups, with HT males having the highest mean reading, (73.1 ± 51.6). There were no significant differences in heart rate between groups based on genders or the classification of blood pressure, ($P > 0.05$). Refer to figure 3.7. However, three individuals were on monotherapy of beta blockers and nineteen individuals on combined hypertensive therapy with beta blockers.

3.1.9 Augmentation index

The mean augmentation index was higher in the HT subjects (144.32 $\pm$ 25.0) than the NT subjects (128.1$\pm$ 21.57). Females in both NT and HT groups had a higher augmentation index, in particular the HT females compared to both groups of males. This will be due to the HT females being older (54.6 $\pm$ 10.16) than any other group in this study.
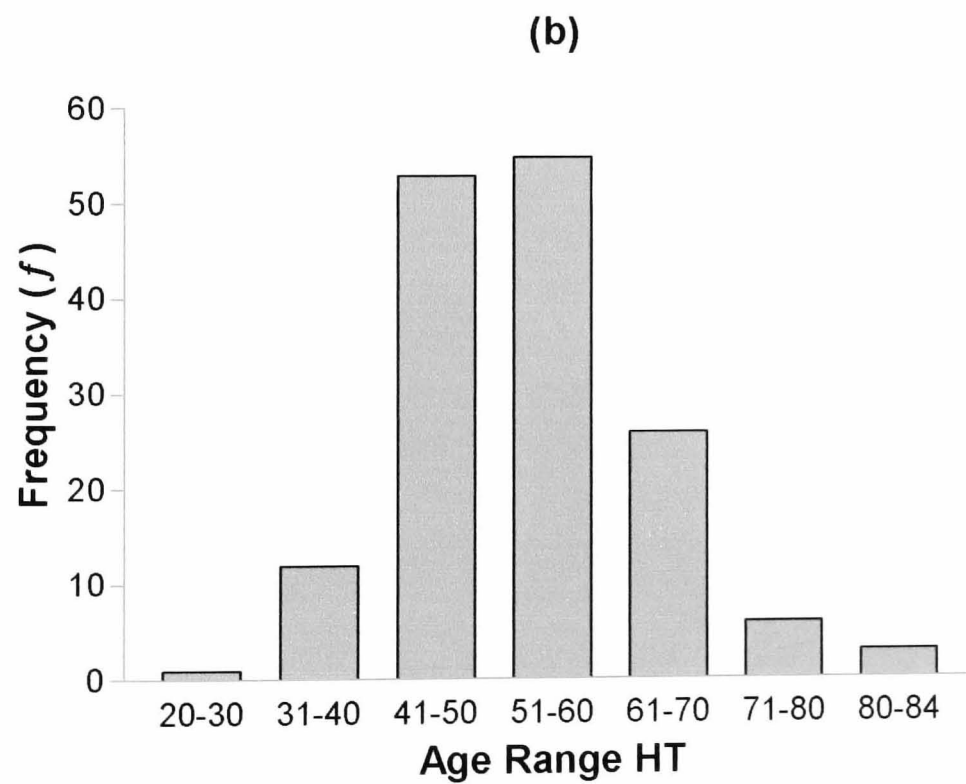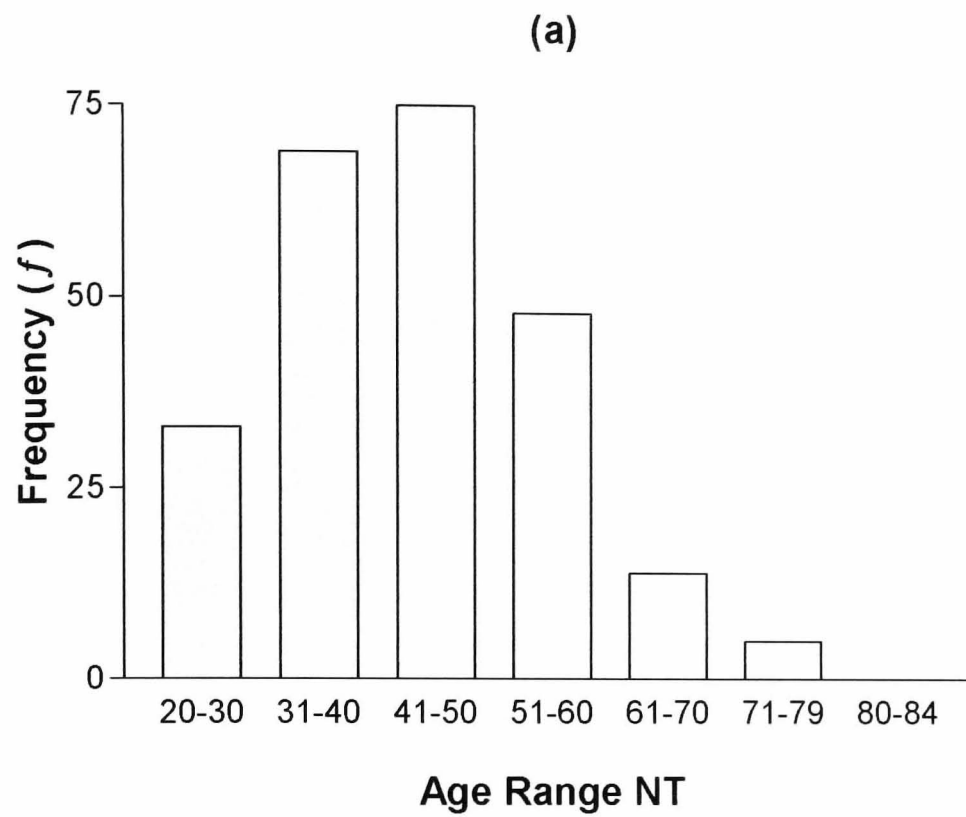
Figure 3.1 Frequency of age ranges of (a) normotensive (n= 252; mean 43.41 ± 11.0) and (b) hypertensive (n= 156; mean 53.15 ± 10.47) African Trinidadians.
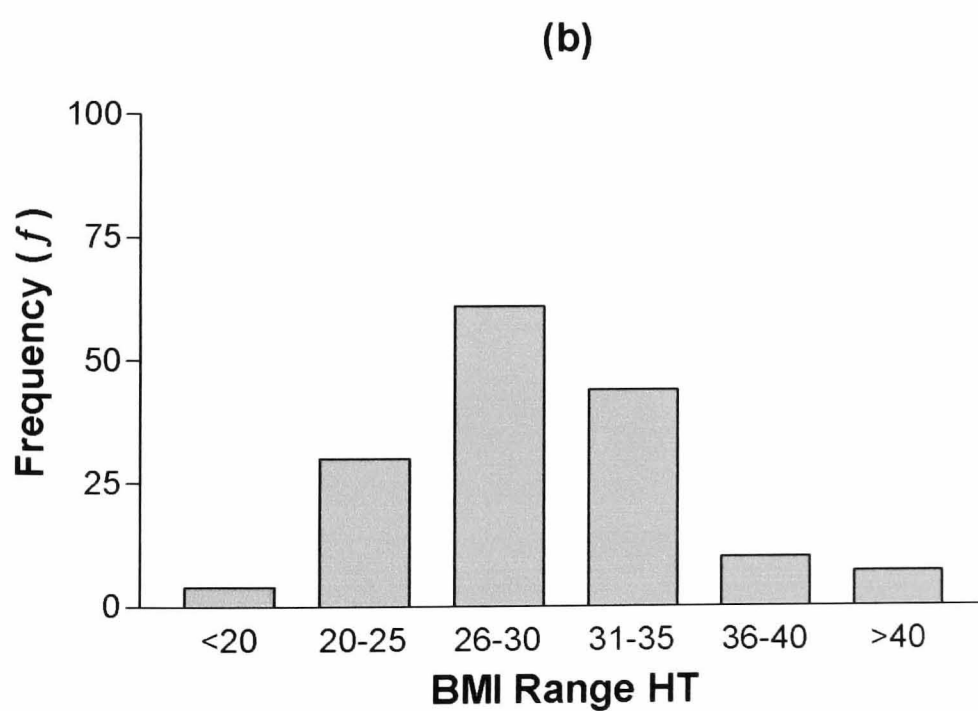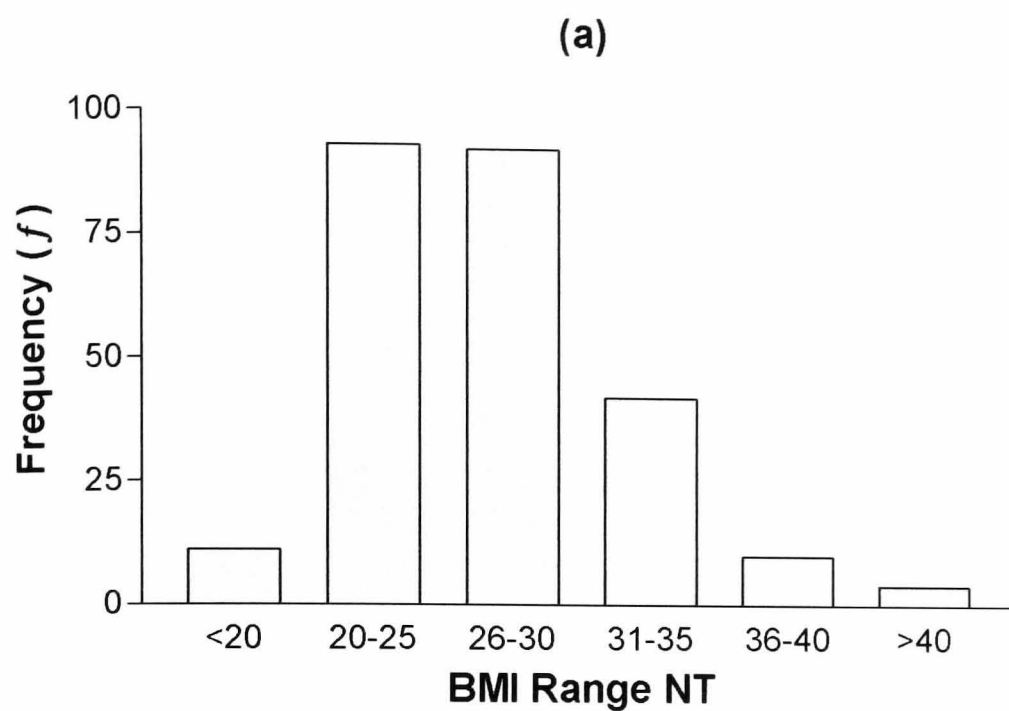
Figure 3.2 Frequency of body mass index ranges of (a) normotensive (n=252; mean 26.6 ± 4.74) and (b) hypertensive (n=156; mean 29.2 ± 5.5) African Trinidadians.

(a)

(b)
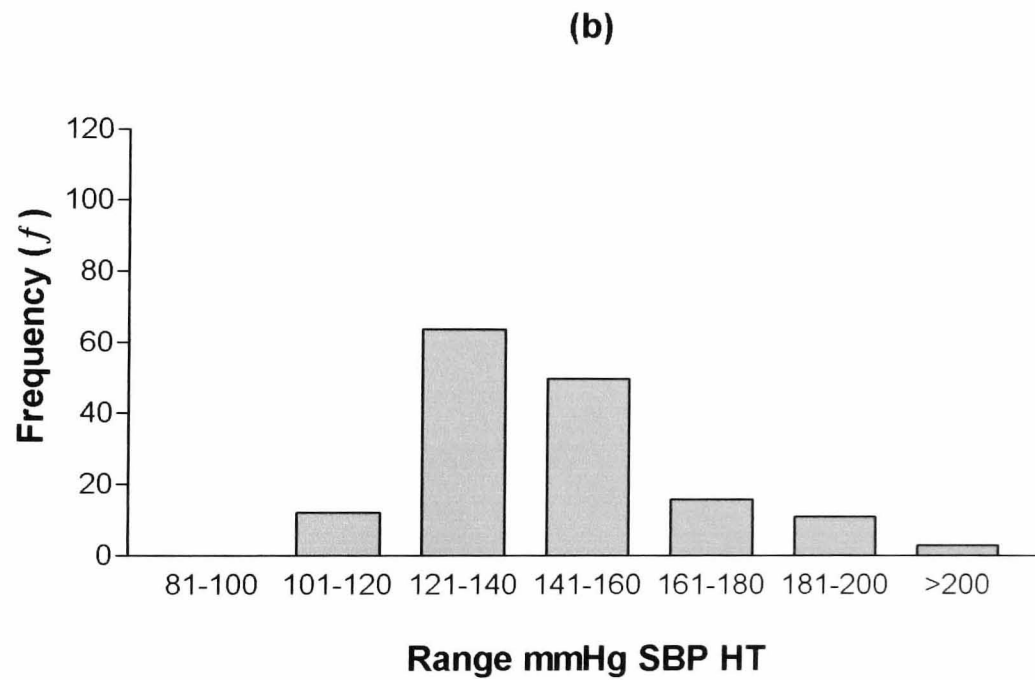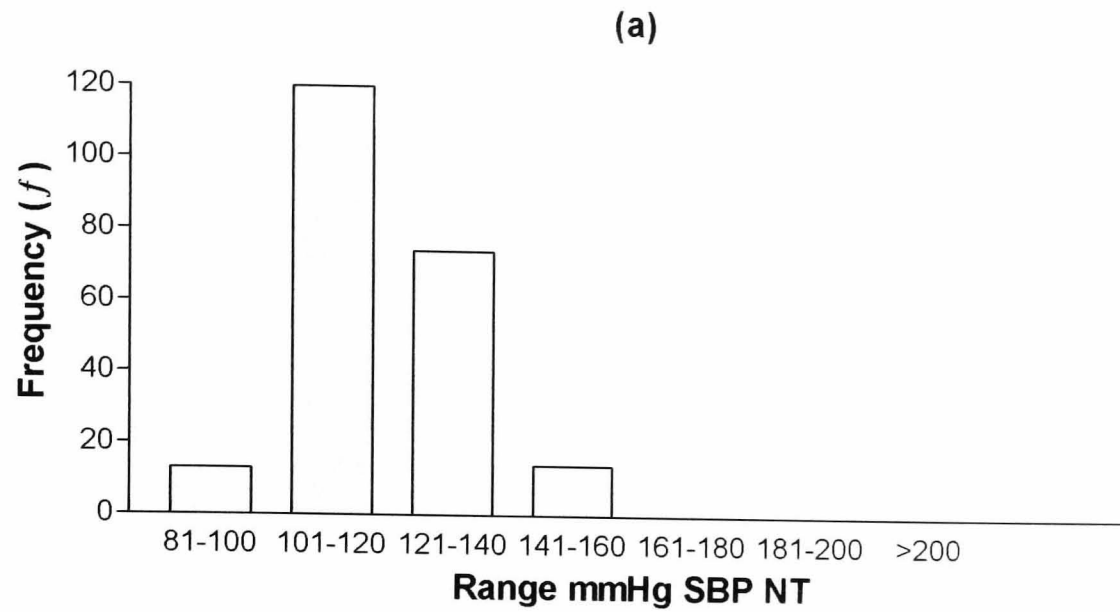
Figure 3.3 Frequency of systolic blood pressure of (a) normotensive (n=252; mean 118.2 ± 12.89) and (b) hypertensive (n=156; mean 146.0 ± 21.96) African Trinidadians.

(a)

(b)
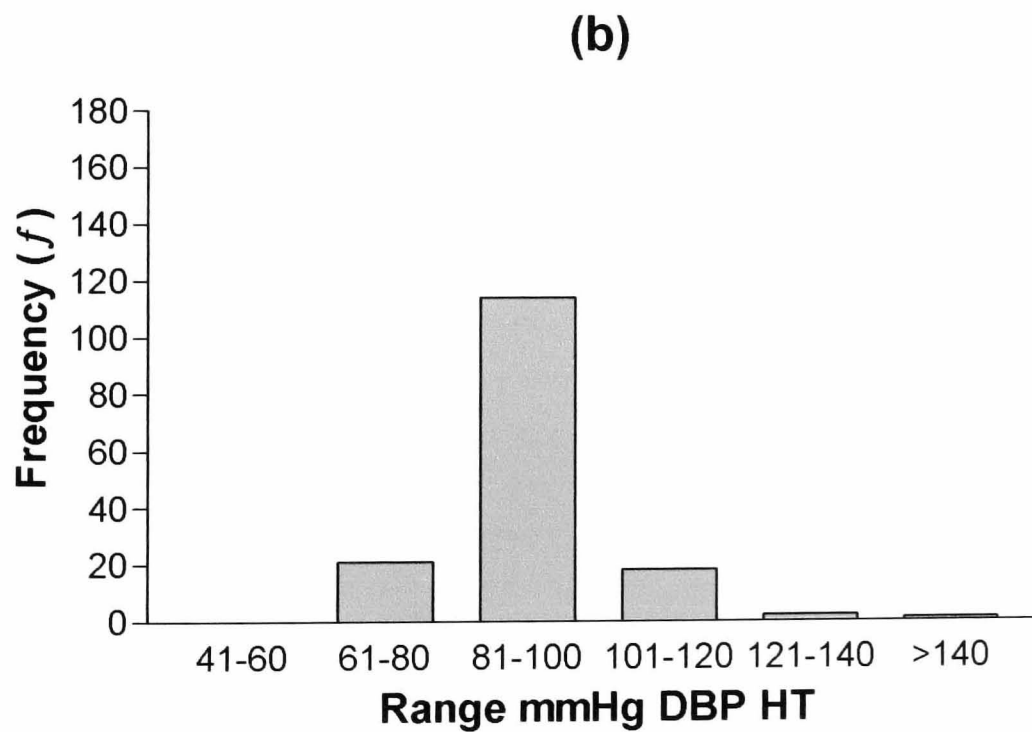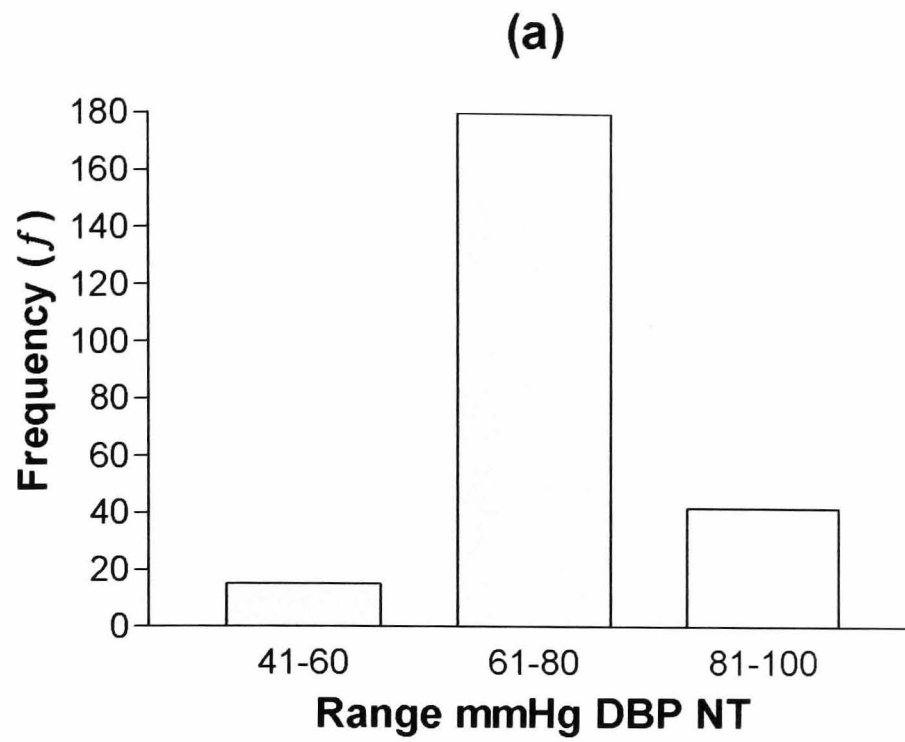
Figure 3.4 Frequency of diastolic blood pressure of (a) normotensive (n= 252; mean 73.2 ± 7.87) and (b) hypertensive (n= 156; mean 89.95 ± 9.69) African Trinidadians.

(a)

(b)
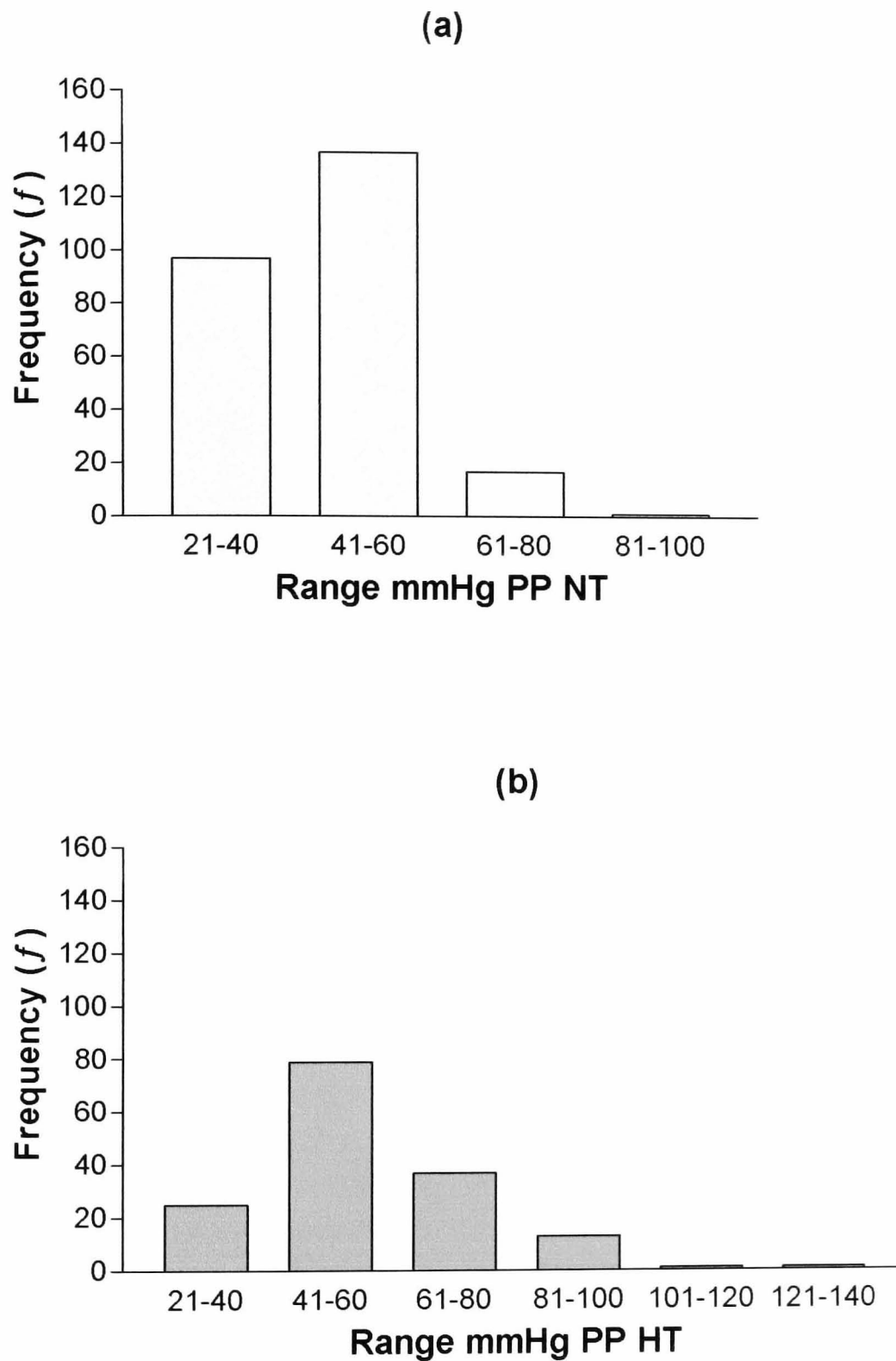
Figure 3.5 Frequency of pulse pressure of (a) normotensive (n= 252; mean 45.0 ± 9.8) and (b) hypertensive (n= 156; mean 56.0 ± 17.0) African Trinidadians.

(a)

(b)
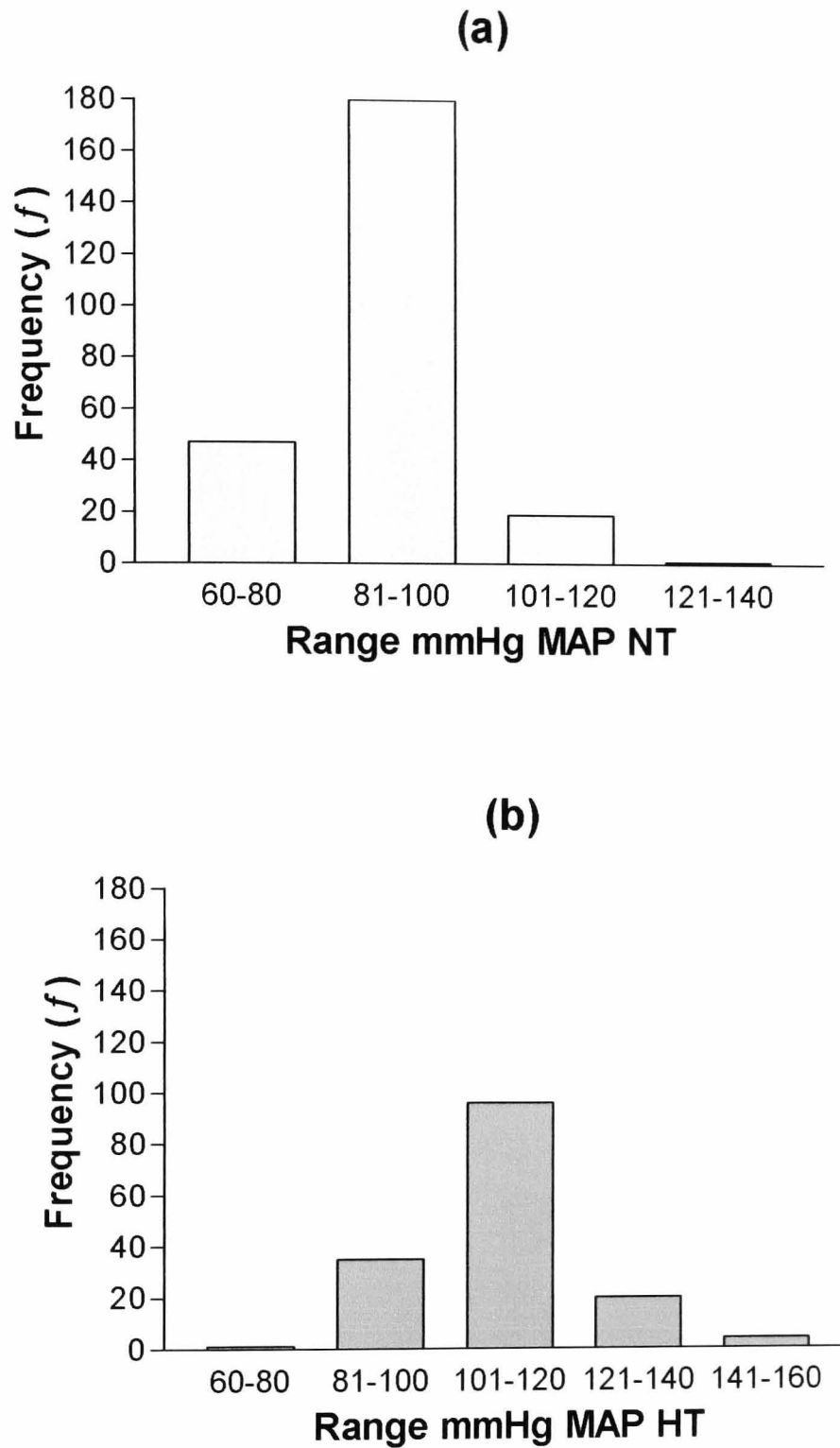
Figure 3.6 Frequency of mean arterial pressure of (a) normotensive (n= 252; mean 88.2 ± 8.7) and (b) hypertensive (n= 156; mean 108.6 ± 9.69) African Trinidadians.
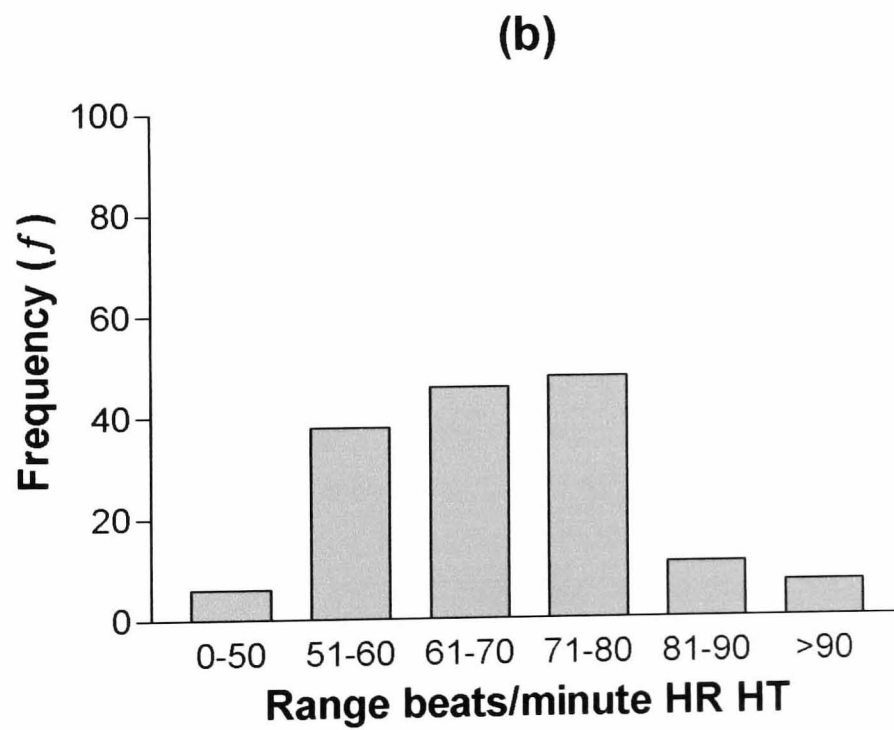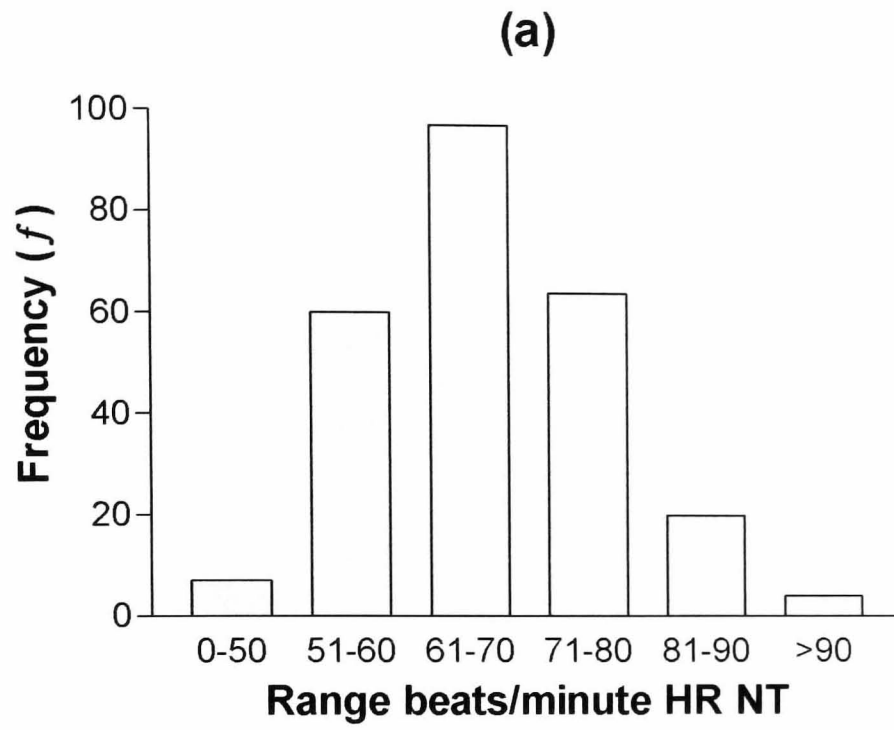
Figure 3.7 Frequency of heart rate of (a) normotensive (n= 252; mean 67.02 ± 9.91) and (b) hypertensive (n= 156; mean 71.0 ± 34.2) African Trinidadians.

(a)

(b)
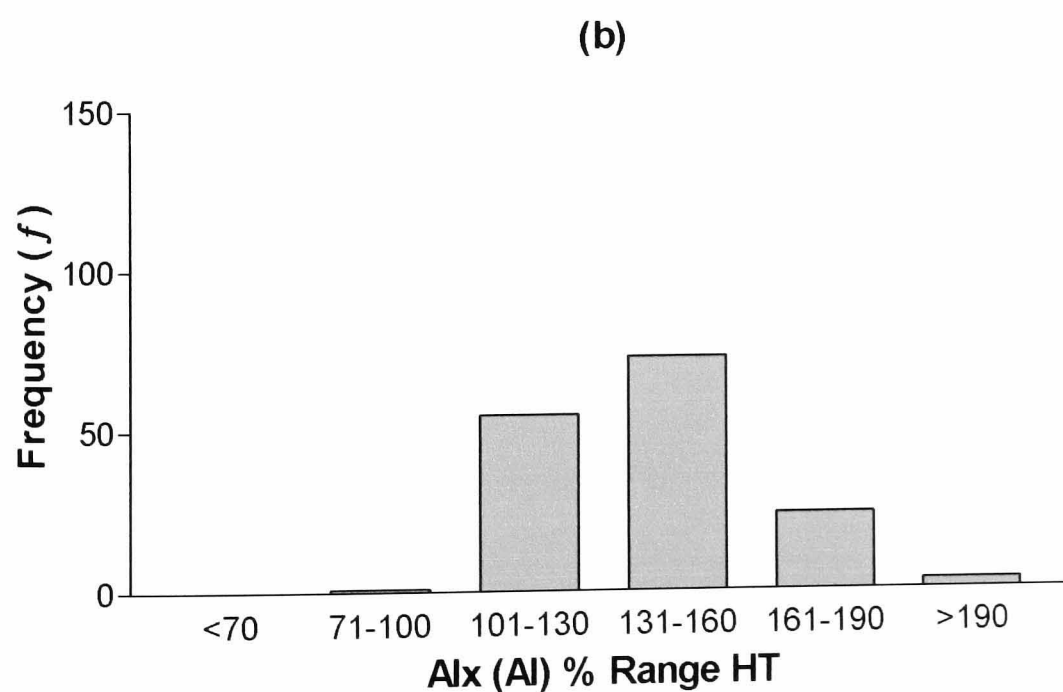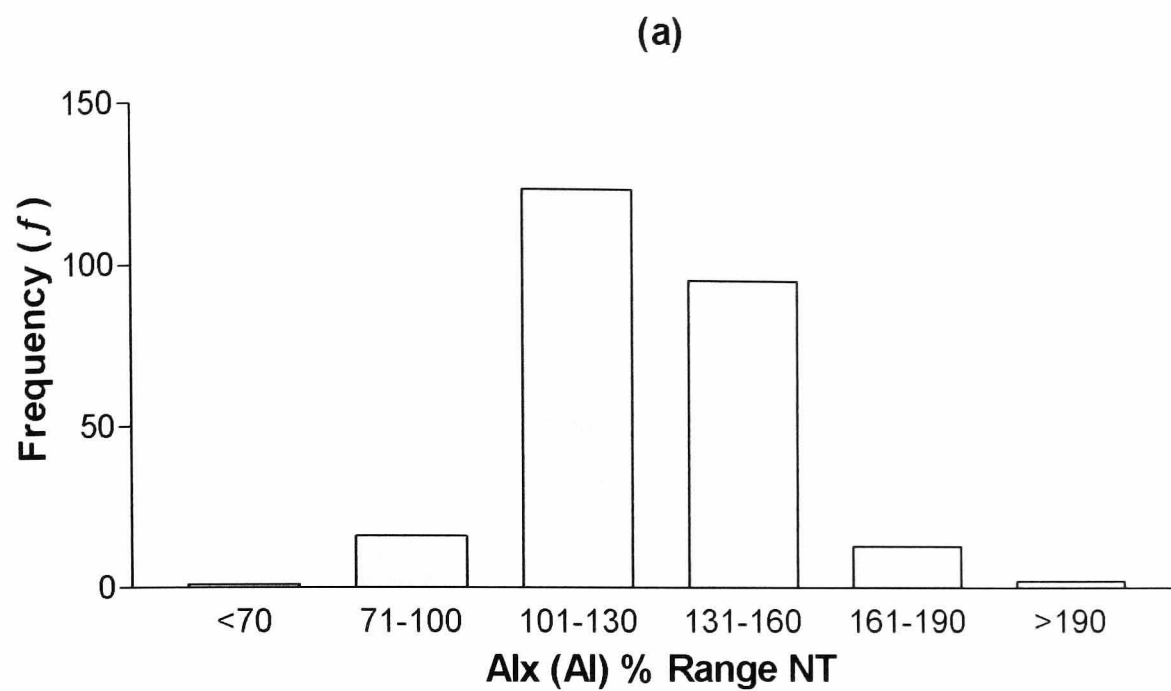
Figure 3.8 Frequency of augmentation of (a) normotensive (n=252; mean 128.1 ± 21.57) and (b) hypertensive (n=156; mean 144.32 ± 25.0) African Trinidadians.

3.2 Lipids

3.2.1 Total Cholesterol

The mean total cholesterol was higher in the HT subjects (5.29 ± 1.03) compared with NT subjects (4.55 ± 1.24). Figure 3.9 shows bar charts of the frequency of NT and HT African Trinidadians with varying ranges of total cholesterol.

3.2.2 Triglycerides

The mean triglyceride level was higher in the NT subjects (2.01 ± 1.11) compared with the HT subjects (1.38 ± 0.73). Figure 3.10 represents bar charts of the frequency of NT and HT African Trinidadians with varying ranges of triglycerides.

3.2.3 High Density Lipids

With reference to Figure 3.11 there was no significant differences between the NT and HT groups for high-density lipoprotein levels. However, NT male subjects did present with a marginally lower level of HDL (1.06 ± 0.34). See table 3.1

3.2.4 Low Density Lipids

The mean low-density lipoprotein was higher in the HT subjects (3.32 ± 0.97) compared to the NT subjects (2.82 ± 1.13). Refer to figure 3.12.

3.2.5 Very Low Density Lipids

The mean of very low-density lipoprotein was higher in the HT subjects (0.52 ± 0.29). compared to the NT subjects (0.40 ± 0.28). Refer to figure 3.13.

3.2.6　High Density Lipid Ratio

The mean high-density lipoprotein ratio was higher in the HT subjects (4.32 ± 1.28) compared to NT subjects (3.84 ± 1.07) See figure 3.14.

3.2.7　Glucose

The mean glucose was higher in the HT subjects (4.98 ± 1.55) compared to NT subjects (4.55 ± 1.32) See figure 3.15. Interestingly, the male HT group did present with the highest mean glucose (5.06 ± 1.97). Refer to table 3.1

(a)

(b)
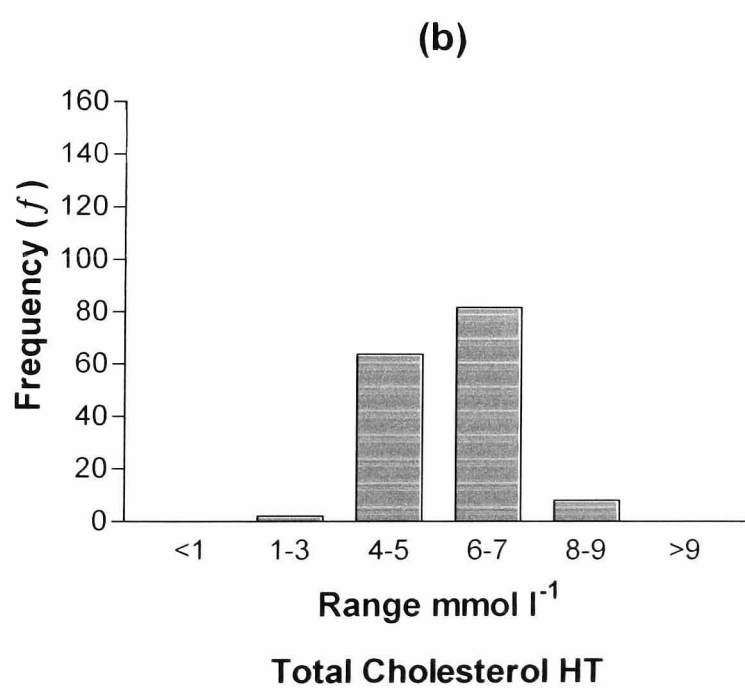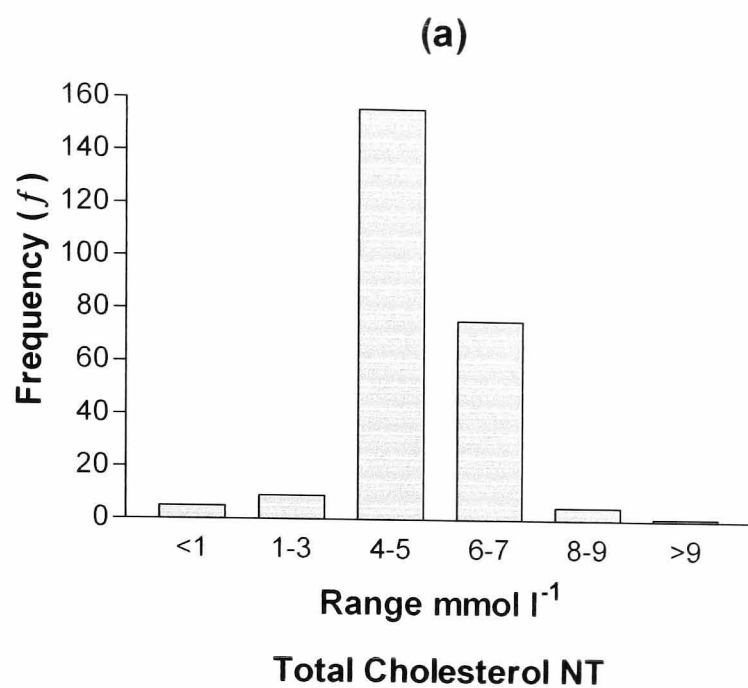
Figure 3.9 Frequency of total cholesterol of (a) normotensive (n=252; mean 4.55±1.24) and (b) hypertensive (n=156; mean 5.29±1.03) African Trinidadians.

(a)

Triglycerides NT

(b)
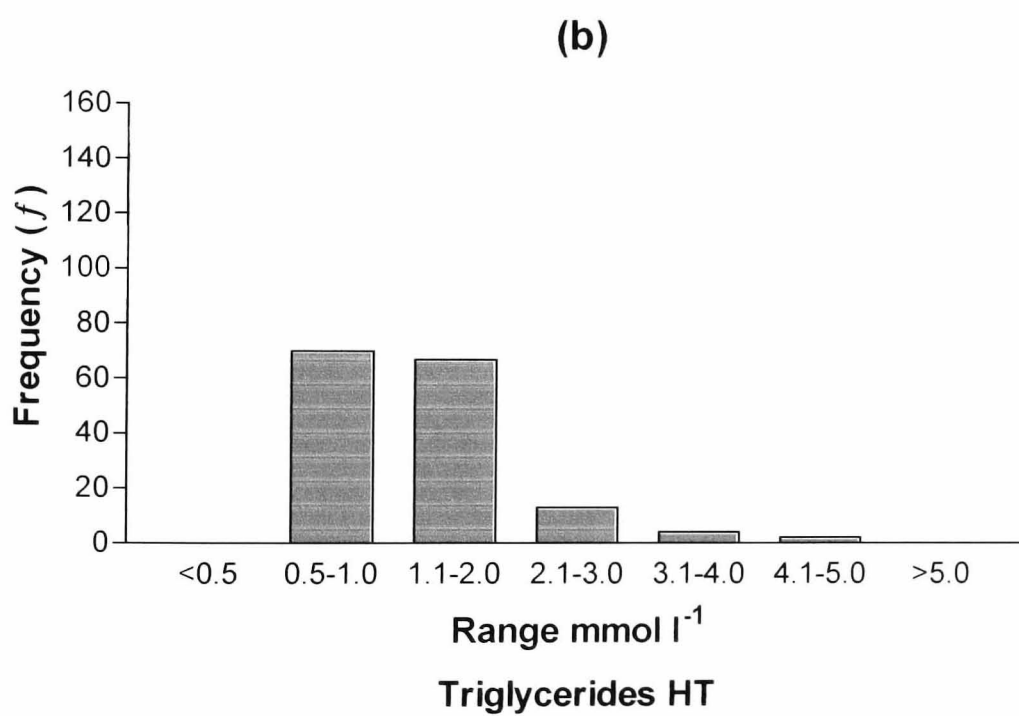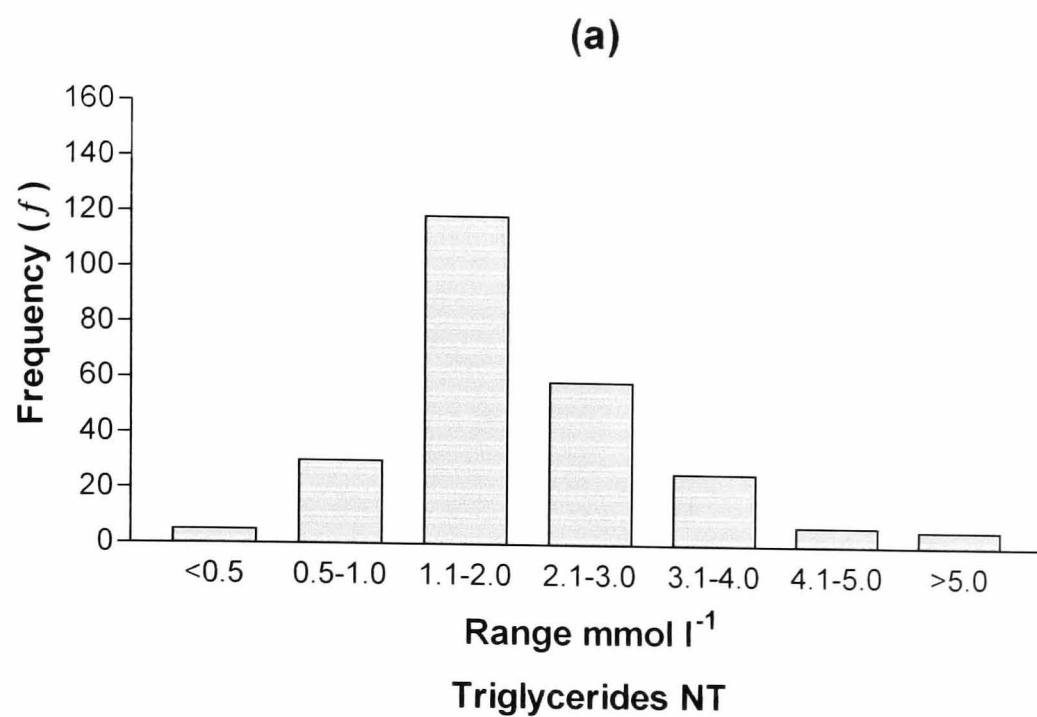
Triglycerides HT

Figure 3.10 Frequency of triglycerides of (a) normotensive (n=252; mean 2.01 ± 1.11) and (b) hypertensive (n =156; mean 1.38 ± 0.73) African Trinidadians.

(a)

HDL NT

(b)

HDL HT
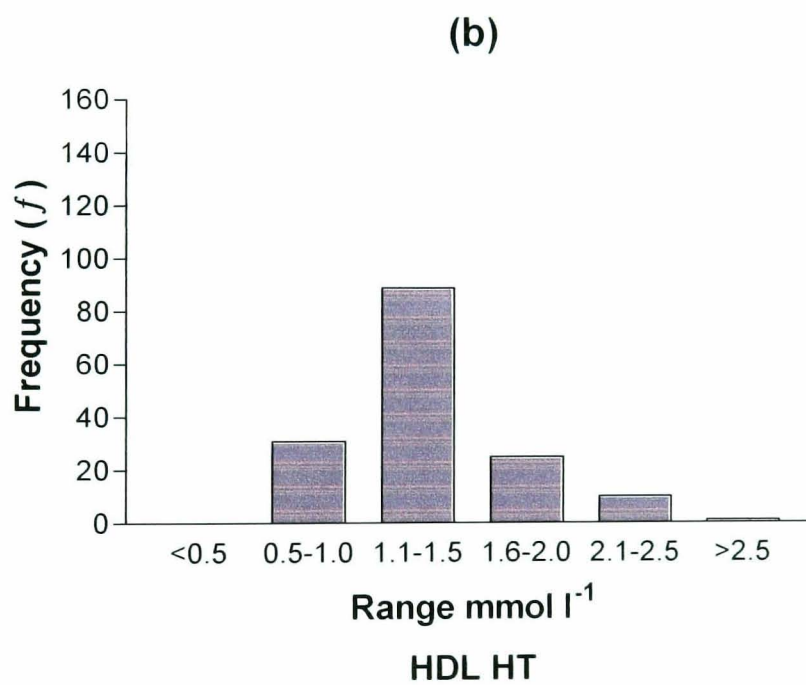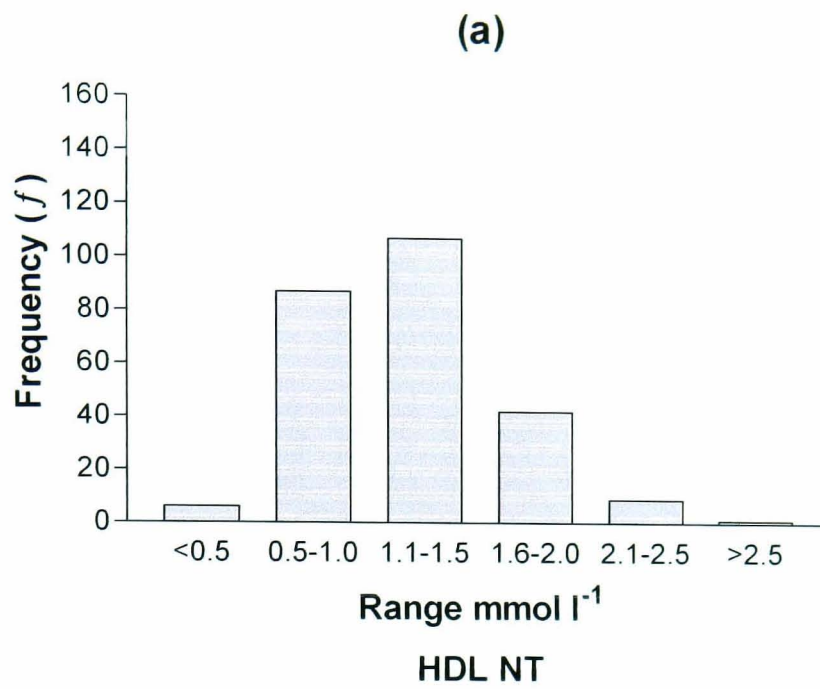
Figure 3.11 Frequency of high density lipoprotein of (a) normotensive (n= 252; mean 1.24 ± 0.39) and (b) hypertensive (n= 156; mean 1.31 ± 0.37) African Trinidadians.

(a)

LDL NT

(b)
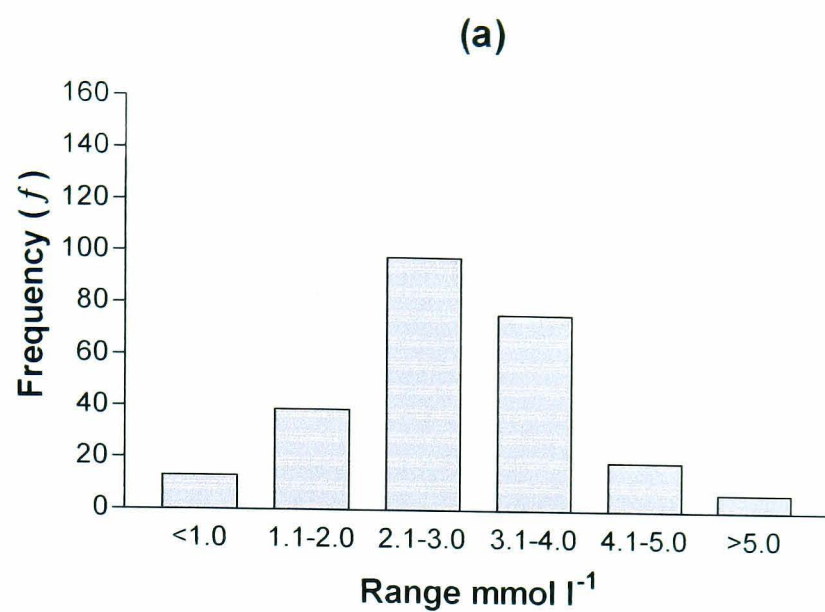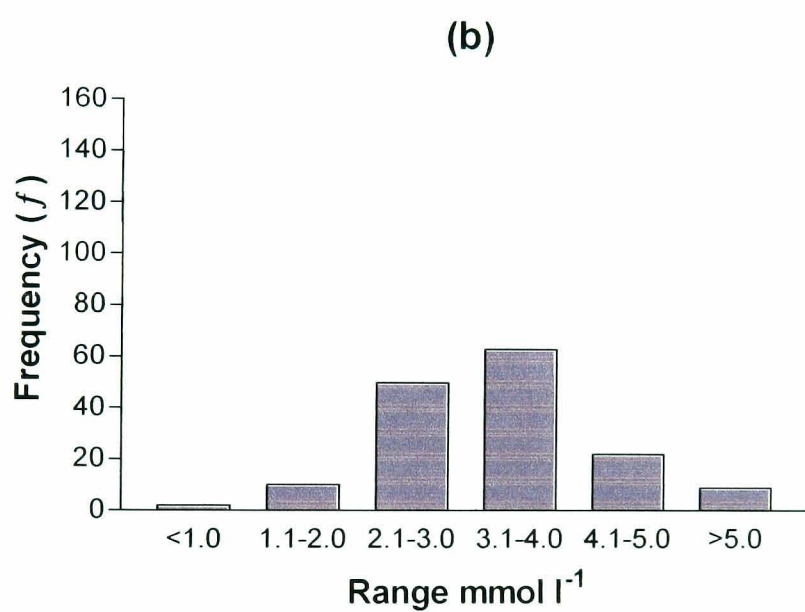
LDL HT

Figure 3.12 Frequency of low density lipoprotein of (a) normotensive (n= 252; mean 2.82 ± 1.13) and (b) hypertensive (n=156; mean 3.32 ± 0.97) African Trinidadians.

(a)

(b)
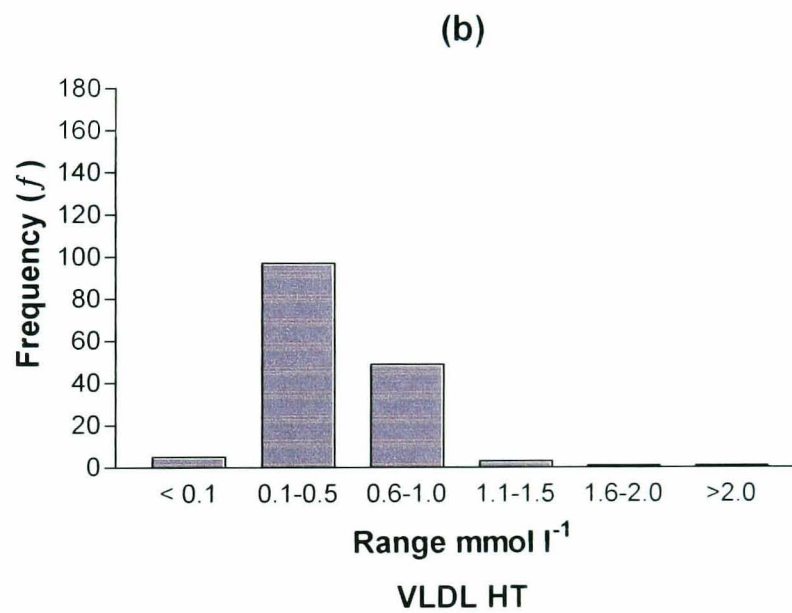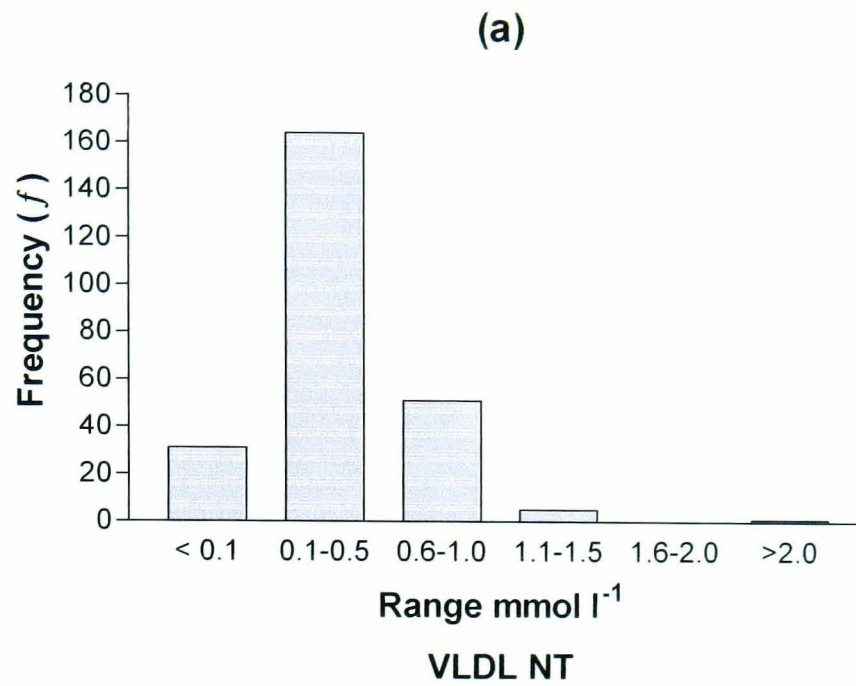
Figure 3.13 Frequency of very low density lipoprotein of (a) normotensive (n= 252; mean 0.40 ± 0.28) and (b) hypertensive (n=156; mean 0.52 ± 0.29) African Trinidadians.

(a)

(b)
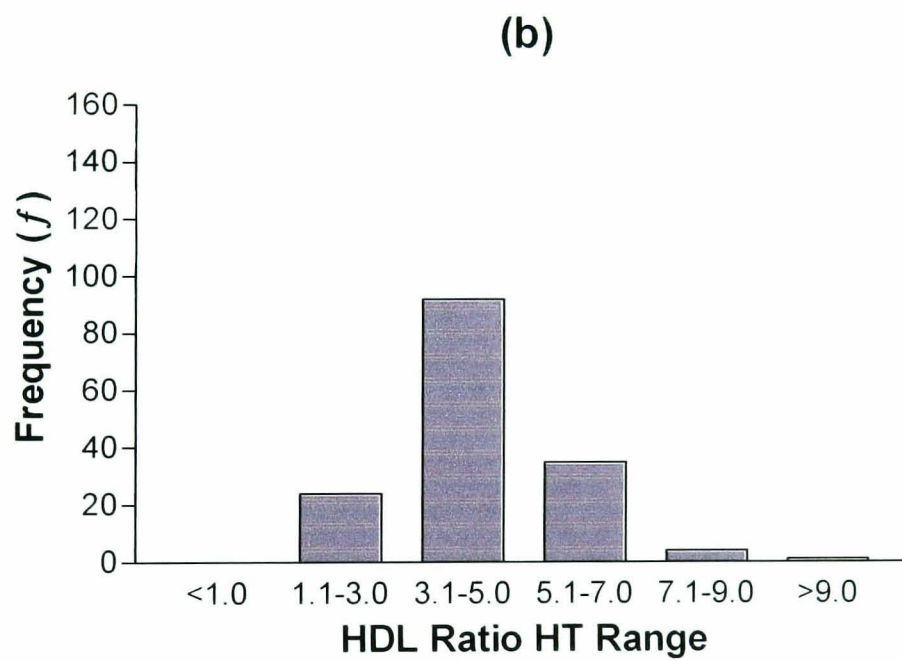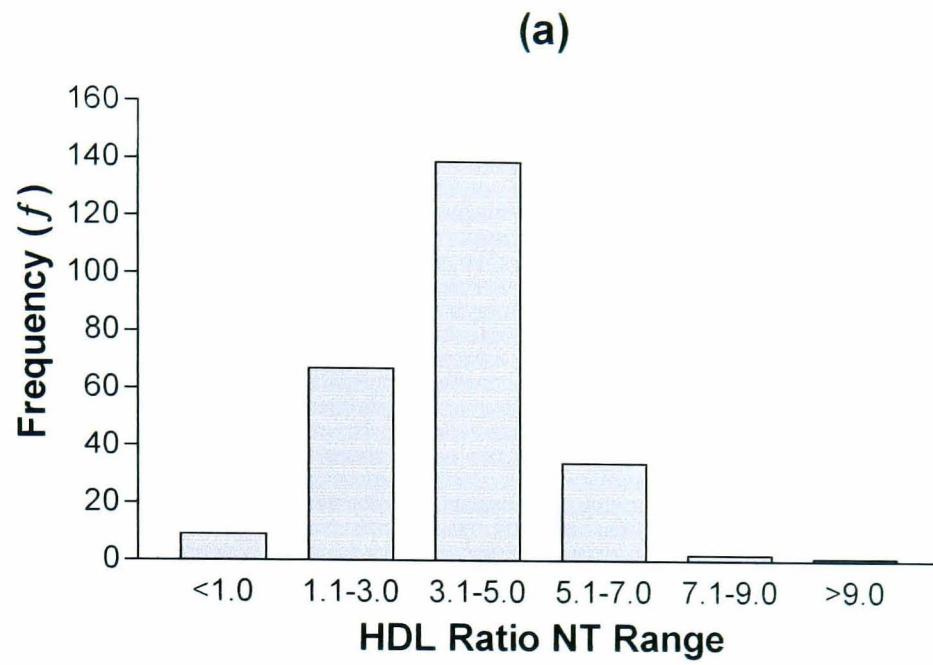
Figure 3.14 Frequency of high density lipoprotein ratio of (a) normotensive (n= 252; mean 3.84 ± 1.07) and (b) hypertensive (n= 156; mean 4.32 ± 1.28) African Trinidadians.

(a)

(b)
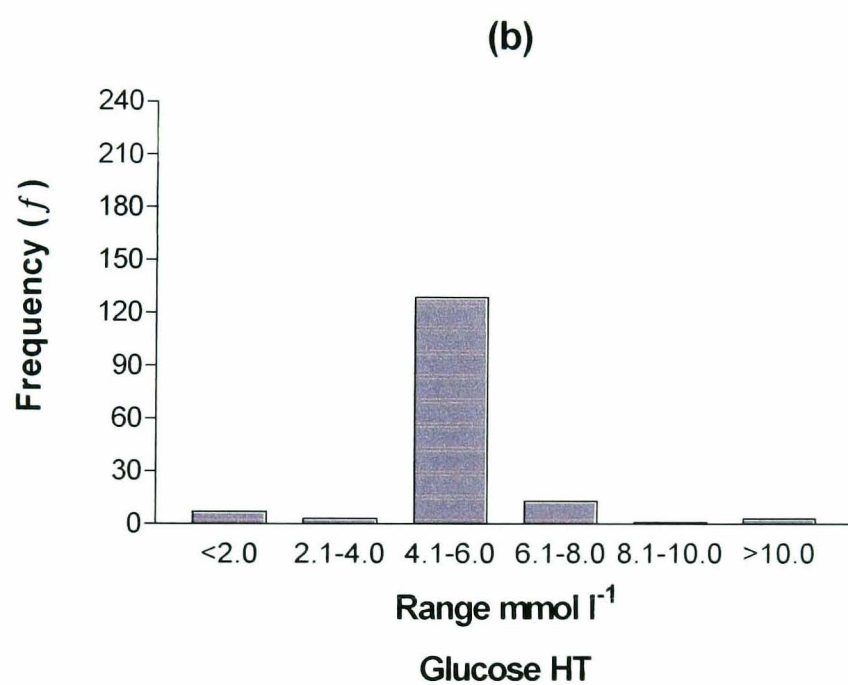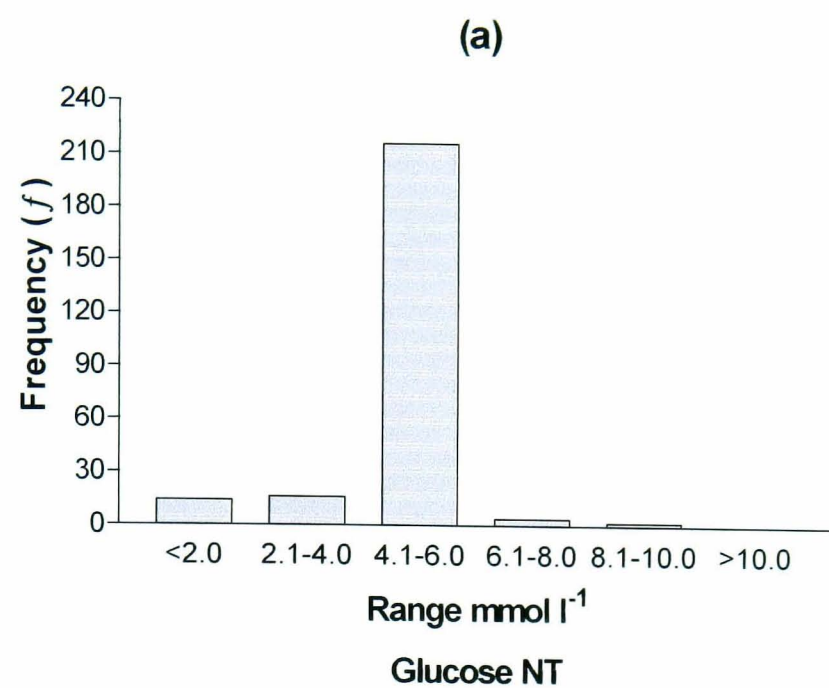
Figure 3.15 Frequency of glucose of (a) normotensive (n= 252; mean 4.55 ± 1.32) and (b) hypertensive (n=156; mean 4.98 ± 1.55) African Trinidadians.

Table 3.1 Results for age and age range, haemodynamic readings from PWA, lipid profiles and genotyping of subjects.

	NORMOTENSIVES			HYPERTENSIVES				
	Male	Female	Total	Male	Female	Total	Overall Total	P-Anova
Subjects	102	150	252	64	92	156	408 (100%)	
Age range years	20-75	20-79	20-79	34-84	25-84	25-84	20-84	
Mean age±SD (years)	44.38 ± 10.86	42.7 ± 11.99	43.41 ±11.55	51.0 ± 10.65	54.6 ± 10.16	53.15 ± 10.47	47.1 ± 12.1	< 0.001
BMI (kg m^{-2})	26.4 ± 3.97	26.7 ± 5.2	26.6 ± 4.74	28.75 ± 5.0	29.5 ± 5.83	29.5 ± 5.5	27.6 ± 5.2	< 0.001
SBP (mmHg)	121.2 ± 11.7	116.1 ± 13.32	118.2± 12.89	141.9 ± 25.21	147.4± 19.4	146.0 ± 21.96	128.8 ± 21.6	< 0.001
DBP (mmHg)	74.1 ± 7.1	72.5 ± 8.35	73.2 ± 7.87	90.5 ± 11.25	89.5 ± 8.48	89.95 ± 9.69	79.6 ± 11.9	< 0.001
PP (mmHg)	47.1 ± 10.1	43.6 ± 9.4	45.0 ± 9.8	53.5 ± 17.8	57.8 ± 16.3	56.0 ± 17.0		< 0.001
MAP (mmHg)	89.8 ± 7.5	87.1 ± 9.3	88.2 ± 8.7	108.3 ± 15.0	108.9± 10.7	108.6± 12.6	96.0 ± 14.4	< 0.001
HR (beats/min)	64.2 ± 9.43	68.9 ± 9.81	67.02± 9.91	73.1 ± 51.6	69.5 ± 11.8	71.0 ± 34.2	68.54 ± 22.6	> 0.05
AIx (AI) %	120.2± 19.37	133.5 ± 21.41	128.1± 21.57	127.86± 16.35	149.36±26.32	144.32 ± 25.0	132.9 ± 23.7	< 0.001
Lipids								
Total Cholesterol mmols^{-1}	4.61 ± 1.47	4.53 ± 1.06	4.55 ± 1.24	5.23 ± 1.02	5.33 ± 1.04	5.29 ± 1.03	4.84 ± 1.21	< 0.01
Triglycerides mmols^{-1}	2.17 ± 1.25	1.90 ± 0.99	2.01 ± 1.11	1.34 ± 0.6	1.40 ± 0.81	1.38 ± 0.73	1.77 ± 1.02	> 0.05
HDL mmols^{-1}	1.06 ± 0.34	1.36 ± 0.37	1.24± 0.39	1.30 ± 0.36	1.32 ± 0.37	1.31 ± 0.37	1.27 ± 0.38	> 0.05
LDL mmols^{-1}	2.99 ± 1.34	2.70 ± 0.95	2.82 ± 1.13	3.27 ± 0.95	3.36 ± 0.99	3.32 ± 0.97	3.01 ± 1.1	> 0.05
VLDL mmols^{-1}	0.43 ± 0.27	0.39 ± 0.28	0.40 ± 0.28	0.5 ± 0.23	0.53 ± 0.32	0.52 ± 0.29	0.45 ± 0.28	> 0.05
HDL Ratio	4.52 ± 3.19	3.38 ± 1.07	3.84 ± 2.25	4.25 ± 1.2	4.38 ± 1.34	4.32 ± 1.28	4.02 ± 1.95	< 0.05
Glucose mmols^{-1}	4.55 ± 1.48	4.55 ± 1.20	4.55 ± 1.32	5.06 ± 1.79	4.93 ± 1.36	4.98 ± 1.55	4.72 ± 1.43	< 0.01
Genotype								
Arg 16	24	32	56	16	20	36	92 (22.5%)	
Het 16	53	88	141	30	48	78	219 (53.8%)	
Gly 16	25	30	55	18	24	42	97 (23.8%)	
Gln 27	54	113	167	37	61	98	265 (64%)	
Het 27	43	33	76	23	27	50	126 (30.9%)	
Glu 27	5	4	9	4	4	8	17 (4.2%)	

3.3 Subjects Medical History

3.3.1 Smokers

Table 3.2, shows the number of smokers in the NT and HT groups. Referring to table 3.4 there were more male smokers in the NT group (n=18) with only one NT female smoker. In the HT groups, male smokers were less prominent (n=7) and only one HT female smoker. Overall, male smokers (n=25), represented the highest number of smokers from both groups and the overall percentage total from all groups was only 6.6% (n=27).

Table 3.2   Representation of smokers and non-smokers in African Trinidadians

	Smokers	Non Smokers
Normotensives (NT)	n= 19 (7.5%)	n=233 (92.5%)
Hypertensives (HT)	n= 8 (5.1%)	n= 148 (94.9%)

3.3.2 Personal Medical History

A personal medical history was taken from all subjects and this mainly focussed on cardiovascular disease. NT subjects reported no episodes of cardiovascular events, with only n=15 subjects reporting being treated for hypercholesterolaemia. This was or was being achieved through drug therapy or non-pharmacological intervention by the reduction of saturated fats in their diet. Of the hypertensives, 27 subjects had or were being treated for high plasma lipids. This overall was 10.3% (n=42) of the total population studied.

Hypertensives had reported being diagnosed with angina 1.7% (n=7), stroke 0.98% (n=4), myocardial infarction 0.49% (n=2), and renal disease (unspecified) 0.49% (n=2). Although numbers were small, and these conditions only appeared in the

hypertensive group, some members of both groups gave a family history of their condition. (See table 3.4).

3.3.3 Medication

Table 3.3 shows the anti hypertensive medication used for the treatment of hypertension. This refers solely to the HT group.

In the HT group 52.6% (n=82) were taking regular drug therapy for hypertension, females being more compliant than males. The most popular monotherapy was thiazides followed by calcium channel blockers. Few subjects were on combined therapy, which may have been due to cost rather than therapeutic value to control of blood pressure. The remaining 47.4% (n=74) were not taking any anti-hypertensive drug therapy, however some of these subjects would have been newly diagnosed and would not previously have known they had hypertension.

Drug therapy other than anti-hypertensives taken by both groups varied but had no impact on blood pressure. No subjects were entered into the study with other significant acute or chronic illnesses.

3.3.4 Education

Subjects in both NT and HT groups (n=202(49%)) had completed secondary education and 141 subjects from both groups (34.5%)) had completed university or higher education study (Refer to table 3.4).

Table 3.3 Anti-Hypertensive Drugs used to control high blood pressure in the study population.

Anti-Hypertensive monotherapy used for hypertensive African Trinidadians	Examples of drugs commonly used	n= Hypertensives on this type of medication	Percentage (%) of all hypertensives n=156
Thiazides & Loop Diuretics	Bendroflurazide; Indapamide; Brenadine; Amiloride; Frusemide	n=34	21.8%
Calcium Channel Blockers	Nifedipine; Diltizem	n= 15	9.6%
Angiotensin converting enzymes inhibitors (ACE)	Enalapril; Lisinopril	n=9	5.8%
Beta Blockers & Non Beta Blocker Sympatholytics	Atenolol; Metapropolol	n=3	1.9%
Obeah (Herbal Teas)	Unknown combination	n=2	1.3%
Combined anti-hypertensive therapy	Combination of either Thiazides; ACE; CaCBs; Beta Blockers.	n=19	12.2%
No medication	Either newly diagnosed or non-pharmalogically controlled.	n=74	47.4%

3.3.5 Family History

61.2% (n=250) gave a family history of hypertension and 41.9% (n=171) gave a family history of diabetes. 27.2% (n=111) gave a family history of morbidity and/or mortality from stroke.

Table 3.4 Data representing smoking, personal medical history, educational

background and family medical history.

Personal Medical History	NORMOTENSIVES			HYPERTENSIVES			Overall Total (%)
	Male	Female	Total	Male	Female	Total	
Smokers	18	1	19	7	1	8	27 (6.6%)
Hypertensive	0	0	0	64	92	156	156 (38.2%)
Diabetic	0	0	0	0	0	0	0 (0%)
Hypercholesterolaemia	7	8	15	14	13	27	42 (10.3%)
Angina	0	0	0	2	5	7	7 (1.7%)
Stroke	0	0	0	3	1	4	4 (0.98%)
Myocardial infarction	0	0	0	0	2	2	2 (0.49%)
Cardiac Failure	0	0	0	0	0	0	0 (0%)
Renal Disease	0	0	0	0	2	2	2 (0.49%)
Medication							
Anti-Hypertensive	0	0	0	27	55	82	82 (20%)
Other than anti-hypertensives.	13	37	50	13	18	31	81 (19.8%)
Education of subjects							
Primary	19	13	32	10	23	33	65 (15.9%)
Secondary	51	75	126	31	45	76	202 (49.5%)
Tertiary	32	62	94	23	24	47	141 (34.5%)
Family History (includes immediate family)							
Hypertension	41	97	138	41	71	112	250 (61.2%)
Diabetes	40	75	115	26	30	56	171 (41.9%)
Hypercholesterolaemia	10	23	33	6	8	14	47 (11.5%)
Angina	8	15	23	5	7	12	35 (8.5%)
Stroke	24	37	61	22	28	50	111 (27.2%)
Myocardial Infarction	13	23	36	9	14	23	59 (14.4%)
Cardiac Failure	7	10	17	7	11	18	35 (8.57%)
Renal Disease	3	14	17	4	7	11	28 (6.86%)

3.4 Discussion

3.4.1 Age and Augmentation index

Arterial compliance differences are usually observed in ethnic groups following adolescence (Gillum, 1979; Kaplan, 1998). Therefore, the target population was adult. However, there are no known studies that have investigated the augmentation of an African Caribbean population using the technique of pulse wave analysis. Many studies have described their results using augmentation as a marker for arterial stiffness and its diagnostic application, which have been previously described in chapter 1.

As age advances, vascular changes, both structural and functional occur. Blood pressure increases with diminishing arterial compliance due to the arterial walls and elastic fibres becoming hardened and fatigued, affecting endothelial function (Black et al, 1999). The absolute value of augmentation depends upon age, sex, height and a number of other variables (Cockcroft et al, 1997; Cameron et al, 1998). Augmentation will double over a 50-60 years period (Black et al, 1999) as will arterial stiffness.

These variables are taken into account and are clearly reflected in the results obtained. However, it should be noted that there is a mismatch in the NT and HT groups with the mean age for HT being significantly higher than the NT group. Age has been proven to be an independent variable of augmentation (Benetos et al, 1997; Kelly et al, 1989) and further analysis by linear and multiple regression (in chapters 5 and 6) determines whether variables of cholesterol, sex, BMI are independent or dependent of augmentation.

3.4.2 Body Mass Index and Diet

A typical Trinidadian diet is not to dissimilar to Creole, utilising the technique of caramelising meats and vegetables, well seasoned with hot spices and often cooked in copious amounts of coconut milk. This African style cuisine could have a complex effect on blood pressure and arterial compliance. A strong association with elevated blood pressure is increased body mass index with obesity increasing cardiac output with impaired peripheral vasodilatation. This was apparent in the smokers described in this chapter with hypertensives having a higher BMI 29.2 $\pm$ 5.5 (SEM) to that of the normotensive group 26.6 $\pm$ 4.74 (SEM).

This association between hypertension and obesity has been well documented in numerous clinical and epidemiological studies. Data from the Framingham study (Kannel et al, 1967) showed that subjects who were overweight at the start of the study later developed hypertension more often than subjects who were not overweight, (Kannel et al, 1969). Central obesity is much more strongly linked to hypertension, diabetes, and cardiovascular disease than peripheral fat distribution, with black Africans (Blair et al, 1989), and may explain the low levels of high density lipoprotein (HDL) levels among blacks of both sexes compared with whites (Haigh et al, 1983). Indeed, a significant inverse correlation of body mass index and high-density lipoprotein cholesterol was noted in a random sample of low socio-economic status black women in Baltimore (Haigh et al, 1983).

Insulin resistance is strongly linked to body fat distribution, and this may in part explain the increasing prevalence of Type II diabetes as well as essential hypertension in black populations. Hyperinsulinemia may contribute to hypertension via activation of the sympathetic nervous system and renal salt retention (Sowers et al, 1991) and insulin resistance appears in young black men with borderline hypertension (Falkner

et al, 1990). Such observations emphasise the importance of insulin resistance in the pathogenesis of hypertension in black individuals.

With this in mind, all subjects were screened for diabetes, with a fasting glucose as well as lipid profile. Dependent on the subjects' results, health education was offered and further investigation into the subjects' dietary intake was examined. Interestingly, subjects reported a high intake of saturated fatty acids, sugar and sodium intake, with minimal exercise. This composed of large quantities of fried chicken, heavily spiced foodstuffs and a high intake of sugar and salted assorted nuts (a popular and abundant available snack, with a strong cultural belief that they have aphrodisiac properties for men). Sweet alcoholic beverages such as rum and alcoholic lager are widely available locally, as they are distilled and produced very cheaply, and some male subjects reported to drink these products in large quantities. However, no newly diagnosed diabetics were identified when attending the clinic in this study.

This propensity for salt and the desire to add to food is still apparent despite long documentary evidence of salt sensitivity in black Africans and their descendants. (Fray and Douglas, 1993). Similarly, there is a greater desire for intense sweet tastes which maybe a factor in the elevated incidence of obesity and diabetes in African blacks (Shiffman et al, 2000). However, total cholesterol and glucose, in both groups were within normal range despite the elevated BMI.

African Caribbean's have a higher prevalence of hypertension at every level of BMI compared to Caucasians, which suggests that ethnic differences hold some strength in the association between BMI and hypertension (Schiffman et al, 2000). Interest in ethnicity-specific definitions of obesity has been hindered by the of lack of data whether or not obesity-related co morbid conditions occur at different levels of body

mass index (BMI) in different ethnic groups (Bell et al, 2000). This could warrant further investigation to identify individuals at risk from obesity.

3.4.3 Blood Pressure, Pulse pressure and Heart rate

Raised blood pressure has been long recorded as an early onset in African Caribbean's (Fray and Douglas 1993). The classification of blood pressure was overall optimal in the NT group and Stage 1 classification of hypertension in the HT group. The most significant independent variable affecting blood pressure for the NT group was age. The true levels of blood pressure in the HT may have been underestimated due to 52.5% (n=82) of the subjects being on anti-hypertensive medication. Had a 'washout' period been possible on these subjects then the blood pressure readings may have been different. The findings are consistent with previous studies (Lui et al, 1989; Lackland et al, 1992; Chatvurdi et al, 1993; Burt et al 1995; Cappuccio et al, 1997).

Pulse pressure was significantly different between the NT and HT subjects. Again, age was the most obvious independent variable to influence results. Interestingly, pulse pressure was raised with increasing augmentation and blood pressure, but this was not reflected with heart rate (> 0.05) in particular in the HT subjects. Increased pulse pressure in blacks is consistent with previous findings (Lui et al, 1989; Chatvurdi et al, 1993; Burt et al 1995). An increasing PP and SBP does reflect arterial stiffening, and age is strongly correlated to pulse pressure.

Despite there being little significant difference in HR between the NT and HT groups, it is known that a resting heart rate is a potential independent risk factor for morbidity and mortality both cardiovascular and from non cardiovascular disease, and there is a strong association between elevated heart rate, influence on augmentation and sudden death (Sa Chuna et al, 1997). Young black adults have been found to have lower

heart rates than whites, with differences disappearing in middle age (Lui et al, 1989). Interestingly, this is not reflected in this study, which HR is only marginally raised in the HT subjects compared with the NT subjects. This maybe due to biasing factors, in particular that approximately half of the HT cohort was being treated on anti-hypertensive medication in particular those subjects taking beta blockers which will slow heart rate, thus reduce arterial stiffening and aid arterial sensitivity.

3.4.4 Lipids and glucose

Racial differences rather than nutritional trends have explained why serum lipids, excluding high-density lipoprotein (HDL) have been lower in blacks than Caucasians (Mazzarolo-Cruz et al, 1994; Zoratti, 1998). The findings from this study are in keeping with previous observations, with lipid results being within acceptable levels despite a reported high intake of saturated fats, which may reflect genetic rather than dietary factors (Cappuccio et al, 1997; Khattar et al, 2000).

Previous literature shows that male blacks have lower rates of cardiovascular disease than male Caucasians, despite having higher prevalence of hypertension, cigarette and alcohol consumption and diabetes (Kaplan, 1998). No significant differences have been detected in females (Zoratti, 1998). Research suggests (Poulter, 1987; Vorster et al, 1987), a low level of oxidised stress protects against the development of cardiovascular disease despite a higher propensity to hypertensive illness.

Interestingly, serum lipids and apolipoproteins, total and fractionated bile excretion and gallbladder motility have been evaluated with large intake of mineral water with high sodium content (Capurso et al, 1999). The reduction in serum and LDL cholesterol levels observed during active treatment ran parallel to increased excretion of bile acids in the stools. This suggests that salt rich water reduces serum and LDL

cholesterol levels through a mechanism of increased excretion of faecal bile acid sterols. This would be consistent with the reported high intake of salt rich products that subjects desired to consume in this study.

Thus, blacks, may have lower levels of serum and LDL cholesterol due to the high levels of circulatory sodium, and the use of thiazides in the treatment of hypertension in this group might raise the total and LDL cholesterol, due to salt eradication.

3.4.5 Smoking

Cigarette smoking diminishes arterial compliance by impairing endothelium-dependent dilatation and predisposes to atherosclerosis (Celermajer et al, 1996). Those who have or continue to smoke have been found to have poor arterial compliance than non-smokers.

Measurement of tobacco intake was complex and difficult to acquire, as few subjects reported to smoke, despite cigarettes and tobacco products being available cheaply and widely. This was due to more females attending the clinic who considered cigarette smoking as undesirable in terms of gender image and were more aware that smoking has a detrimental effect to health. This study has reported lower smoking levels among African Trinidadians, however when compared to national data in other commonwealth areas this is the reverse and higher levels are recorded (Chatvurdi et al, 1993; Cappuccio et al, 1997; Acheson, 1998; Khattar, 2000).

3.4.6 Medication

Unlike the treatment of acute diseases, where the effect of therapy is known within a short time, treating hypertension entails a substantial investment of money, time and risk of medication side-effects for a potential benefit that may not become evident until some time later. African Trinidadians with hypertension are at greater risk of

complications, including mortality, and thus reviewing the efficacy of various therapies in lowering blood pressure and their success in preventing the complications of CVD is wise.

Due to cost of medication being a factor when treating a patient with limited financial resources, a major concern would be that cost is over-emphasised, minimising potential hazards. However, there is extensive marketing of newer, more expensive antihypertensives, which may exaggerate the side effects of popular inexpensive antihypertensives. Whilst clinical practitioners' overriding concern is to provide for the best therapy regardless of cost, to ignore cost would be particularly naïve in populations such as African Trinidadians where financial resources for drug therapy are limited. Subjects in both groups reported concerns over cost of medication, not surprisingly, those who were in a lower economic level, the greater the concern cost became and reluctance to take any drug therapy for disease processes. Also, compliance of drug therapy became an issue, as the more expensive drug therapy the less likely subjects would be compliant in taking medication (Schulman et al, 1986).

Not all this group were on monotherapy, but this figure supports the literature that over half can be controlled on monotherapy in spite of racial or ethnic resistance (Saunders et al, 1990; Wright et al, 1991).

Other than anti-hypertensive therapy, some subjects from both groups took other forms of medication. This was either complimentary to their anti-hypertensive medication such as aspirin, or medication for minor ailments that did not exclude the subject from the study.

3.4.7 Hormonal therapy

Arterial compliance diminishes quickly in women following menopause (Bulpitt et al, 1999). However, with hormone replacement therapy (HRT) this has provided a continuing protective measure, preventing acceleration of stiffening of vessels, not unlike that prior to menopause (Rajkumar et al, 1997). Only 3 female subjects were taking HRT, and were not excluded from the study, as females who were pre or postmenopausal, the same age and not taking HRT were included. This would have little significant impact on the data collected.

3.4.8 Herbal Medicine

Traditional herbal medicine is one of the most widely practised aspects of the traditional African belief system known, as *'obeah'* is herbal medicine. Trinidadians make use of herbal medicines, and most know the uses of the common plants, barks, roots and herbs that make up the material medica of what is called *'Bush'*. Concoctions are usually brewed into a tea and drunk or infused into the skin through *'bush bathing'*.

Elements of these traditions remain strong in Trinidadian attitudes towards health, with blood pressure a custom of cooling and purging are used to lower, purify and cleanse the circulating blood in the system. This is achieved by using *'cooling herbs'*, such as wild senna, mauby or pawpaw bark. The *'purging'* is induced by the ingesting aloes or castor oil. There are no data or verification that such potions are effective or conclusive with their application. However, due to the poor public health system, this type of medicine is the only the option for treatment for some Trinidadians. No subjects who participated in the study took *'obeah'* solely for their ailments, this

includes essential hypertension. It was as a complimentary medication, sometimes alongside conventional drug therapy.

There is no comparison data on alternative medication in the treatment of hypertension to suggest they are effective in the treatment of hypertension in African Trinidadians.

3.4.9 Education and socio-economic status

Disease patterns are frequently confounded by socio-economic differences especially when related to ethnicity (Bhopal, 1997). Socio-economic status examines the underlying causes or risk factors that relate to health. With hypertension, smoking, excessive alcohol, obesity, physical inactivity, physical (isometric) and psychological stress, high sodium and low potassium and calcium intake will affect arterial compliance.

As shown in Table 3.4, information on education of subjects was acquired at interview to assist in the classification of occupations into social classes and whether social inequalities existed due to education and demographic position. However, despite its power in revealing social disparity the basis for clarifying occupations into social classes has been criticised for lacking explanatory power (Chandola, 2000). Therefore, by measuring the level of education that was obtained by the subjects and employment gained, this gave a greater indication of the principles of structure of a modern society, which is viewed as being more stable and generic (Rose and Prevalin, 2000). This classification was based on the National Statistics of Socio-Economic Classification (NS-SEC) (Chandola, 2000), which has been previously applied to occupational work samples and can measure social classification through occupational position and educational background. It was aimed at uncovering whether subjects who had gained a higher level of education were more aware of the impact and

consequences of essential hypertension. There has been a significant inverse association between relative weight, hypertension, and duration of formal education as previously reported (Hypertension Detection and follow-up Program Co-operative Group, 1977). In the United States, a disproportionate number of blacks have limited education and financial resources (Task Force, 1986). Among black African women, the presence of obesity clearly varies inversely with socio-economic status (Rimm et al, 1974; Garn et al, 1977; Oken et al, 1977). However, most subjects expressed concern of family medical history of CVD and sought participation as a result. This is shown by the data obtained, with 61.2% (n=250) of the total population studied having a family history of hypertension, 41.9% (n=171) diabetes, and 27.2% (n=111) stroke, which may have been more reflective of why subjects accessed the cardiovascular risk assessment clinic.

3.4.10 Exercise

Data suggested that exercise among African Trinidadians was not a strong feature and undertaken infrequently and sporadically. This is consistent with data found with other ethnic groups (Acheson, 1998).

Most subjects reported that strenuous exercise could be physically draining due to the tropical climate of heat and humidity. Most public and private dwellings do not have sophisticated air conditioning due to expensive installation and maintenance. Therefore, daily life has to be conducted at a slower pace to compensate for heat exhaustion. Most subjects did little exercise due to the climate and that public transport is accessible, reducing physical walking. Trinidad's bus and taxi system is effective and operates cheaply due to the island's petroleum production, keeping oil prices low.

3.4.11 Family history

Having a family history of cardiovascular disease (CVD) may increase the risk of arterial stiffness. Several studies have shown lower blood cholesterol levels in African Caribbean's, that appear to reflect an inherited cause of essential hypertension rather than purely dietary factors or environmental factors, (Cappuccio et al, 1997; Khattar et al, 2000). During interview with subjects, they were asked what mode of referral brought them to the cardiovascular risk assessment clinic. Most subjects expressed that they had an immediate family member who had or has a history of vascular disease. This suggests that African Trinidadians have a strong awareness of their mortality and propensity to hypertension, stroke and diabetes. Interestingly, subjects who were from a poorer socio-economic background travelled the furthest to access the clinic and due to family history were acutely aware of the detrimental impact that coronary heart disease (CHD) and hypertension could have emotionally and financially on not only themselves but their families.

Self-referral was mainly evident with hypertensive subjects expressing further insight into the nature of their disease and if hereditary factors had played a significant part in the development of hypertension.

As both groups had a strong immediate family history of hypertension, CHD, and stroke this may be a likely reason why subjects attended and volunteered for the research study. Since this applies to both groups, data collected are unlikely to affect comparison.

3.5 Summary

❖ Blood pressure may be influenced by the African Trinidadian's environment and socio-economic and cultural factors.

❖ Age is an independent variable that affects arterial stiffness in African Trinidadians.

❖ Heart rate does not appear to contribute to aortic stiffening, despite raised systolic and diastolic pressures.

❖ Genetic factors may play a role, for example, in the degree of obesity or renal insufficiency and sodium handling and lipid levels.

Chapter 4

Allele Frequencies of codon 16 and 27 β2-Adrenoceptor Polymorphisms and Genetic Association Data

4.1 Introduction and Genetics Results

4.1.1 β_2-Adrenoceptor

The β_2-adrenergic receptor gene (β_2-AR) is a major target for the treatment of pulmonary disease. In addition, the β_2-AR are important in the regulation of vascular tone (Johnson, 1999). The aim of the study described in this chapter was to determine whether genetic variation in the human β_2 AR might contribute to altered vascular reactivity.

The β_2-AR is situated on the endothelial cell, and when stimulated alters the vascular tone with the release of nitric oxide (NO). There is evidence that the beta-adrenergic receptors are down regulated in white hypertensives, whilst blacks possess a higher beta-receptor density (Lifton, 1996). A number of studies have also studied the possibility that polymorphism in the β_2-AR may contribute to the development of hypertension (Jindra et al, 2002; Tomaszewski et al, 2002; Meirhaeghe et al, 2001; Kato et al, 2001; Xie et al, 2000; Candy et al 2000).

The gene encoding the β_2-adrenoceptor contains nine single nucleotide polymorphism (SNP) (Reihaus et al, 1993). Five of these SNPs are degenerate and are not thought to cause a detrimental effect on the adrenoceptor, as the amino acid sequence remains unaltered from the wild type receptor. However, the remaining four SNPs do cause amino acid changes within the receptor. The work described in this chapter concentrates on the two common coding region polymorphisms, where SNPs at 46 (A-G) and 79 (C-G) cause amino acid changes from arginine 16 to glycine (Arg16→Gly) and glutamine 27 to glutamate (Gln27→Glu).

The specific aim of the work presented in this chapter were:

i) To determine allele frequencies for the polymorphisms Arg16→Gly and Gln27→Glu in normotensive and hypertensive African Trinidadians.

ii) To determine the extent of linkage disequilibrium between these polymorphism in this population.

iii) To examine for association between these polymorphisms and (a) hypertension and (b) markers of vascular reactivity in these populations.

4.2 Results

4.2.1 Genotype Distribution

An initial analysis was performed on the combined data set of normotensive and hypertensives subjects in order to define the allele frequency of the β_2-adrenoceptor polymorphisms in the African Trinidadian population. This is shown in table 4.1.

Table 4.1 Allele Frequencies for polymorphisms of Gln27→Glu, and Arg16→Gly in normotensive and hypertensive African -Trinidadians, represented in percentages and then in decimal fractions.

Polymorphism	Normotensives	Hypertensives	Total Population
Gln 27 frequency	81.3% (0.81)	78.8% (0.788)	80.4% (0.8)
Glu 27 frequency	18.7% (0.19)	21.1% (0.211)	19.6% (0.2)
Arg 16 frequency	50.1% (0.501)	48% (0.480)	49% (0.49)
Gly 16 frequency	49.8% (0.498)	52% (0.520)	51% (0.51)

Table 4.2 shows the distribution of genotypes within the subjects studied.

Table 4.2 Distribution of Gln27→Glu and Arg16→Gly genotypes in normotensive and hypertensive African-Trinidadians.

Genotype	Normotensive	Hypertensive
Homozygous Gln 27	167(66.3%)	98(62.8%)
Heterozygous Gln/Glu 27	76(30.2%)	50(32.0%)
Homozygous Glu 27	9(3.6%)	8 (5.1%)
Homozygous Arg 16	56(22.2%)	36(23.0%)
Heterozygous Arg/Gly 16	141(55.9%)	78(50.0%)
Homozygous Gly 16	55(21.8%)	42(26.9%)

Numbers of individuals are shown, followed by percentages in brackets.

There was no significant difference in either allele or the genotype distribution in the NT or HT groups for either the codon 16 or 27 polymorphism in the African Trinidadian population for either Gln27→Glu and Arg16→Gly (p > 0.05).

4.2.2 Hardy-Weinberg equilibrium analysis

The Hardy-Weinberg test is used to check that the observed genotype frequencies do not deviate from those expected allele frequencies seen in the population. Such deviation can occur due to population heterogeneity, error in genotyping, or when one allele confers a survival advantage.

Expected genotype frequencies were calculated from the observed allele frequencies using the Hardy-Weinberg equation.

i.e.

Equation 4

$P^2 + q^2 + 2pq = 1$

p= **observed** frequency of the **wild type** allele.

q= **observed** frequency of the **mutant** allele.

As there was no significant difference in genotype distribution between the two groups, the combined data from the normotensive and hypertensive population were used to test for Hardy-Weinberg equilibrium.

The predicted number of individuals of each genotype can be calculated from the allele frequencies observed and compared with the observed genotype frequencies. The data are shown in Tables 4.3 and 4.4.

Tables 4.3 and 4.4 describe the **expected** and **observed** frequencies of genotypes using the Hardy Weinberg equation.

Table 4.3 Comparison between observed frequencies of genotypes for the Gln27→Glu locus using the Hardy -Weinberg equation.

Genotype	Allele Frequency	Expected	Observed	$\dfrac{(O-E)^2}{E}$
Gln 27 (p^2) (0.8 ^{2})	O.64	261.1	265	0.06
Het 27 (2pq) 2(0.8 x 0.2)	0.32	130.6	126	0.1
Glu 27 (q^2) (0.2 ^{2})	0.04	16.3	17	0.03
Total	1	408	408	0.19

Gln27→Glu 27 Hardy-Weinberg

$0.8^2 + 0.2^2 + 2(0.8 \times 0.2) = 1$ χ^2 (1df.) = 0.19   P= > 0.05

Table 4.4 Comparison between observed frequencies of genotypes for the Arg16→Gly locus using Hardy -Weinberg equation.

Genotype	Allele Frequency	Expected	Observed	$\frac{(O-E)^2}{E}$
Arg 16 $(0.49)^2$	0.24	98	92	0.36
Het 16 (2pq) 2(0.49 x 0.51)	0.5	204	219	1.1
Gly 16 $(0.51)^2$	0.26	106	97	0.76
Total	1	408	408	2.22

Arg 16→Gly16 Hardy Weinberg

$0.49^2 + 0.51^2 + 2(0.49 \times 0.51) = 1$ χ^2 (1df.) = 2.22   P= > 0.05

There were no significant differences between the observed and expected values for either polymorphism, therefore the population was shown to be in Hardy-Weinberg equilibrium with respect to polymorphisms at codon Arg/Gly16 and Gln/Glu27

4.2.3 Linkage disequilibrium

In order to examine potential linkage disequilibrium between two polymorphisms, (which has been previously described in Caucasian populations, Dewar et al, 1997), haplotypes were constricted where possible. Whilst it is possible to estimate haplotypes for double heterozygotes using the Estimated Haplotype Frequency

(EH program) (Terwillinger and Ott 1992 and 1994) in practice this does not provide a marked increase in information in a large data set.

The distribution of the haplotypes in the normotensive and hypertensive groups was estimated using the data shown in Tables 4.5, 4.6 and 4.7.

Table 4.5 Genotyped Normotensives at both loci (NT, n=252).

	Arg/Arg	Het	Gly/Gly
Gln/Gln	49	96	22
Het	4	45	27
Glu/Glu	1	3	5
Total			252

Table 4.6 Genotyped Hypertensives at both loci (HT, n=156).

	Arg/Arg	Het	Gly/Gly
Gln/Gln	33	42	23
Het	3	31	16
Glu/Glu	0	4	4
Total			156

Table 4.7 Results for each pair of loci in the combined NT and HT groups.

27	16 Arg/Arg	Arg/Gly	Gly/Gly
Gln/Gln	82	138	45
Gln/Glu	7	76	43
Glu/Glu	1	7	9
Total			**408**

This data was then used to calculate haplotype frequencies in the total population represented in Table 4.8.

Table 4.8 Represents the number of haplotypes for each pair of loci in combined NT and HT groups, ignoring double heterozygotes.

Arg/Gln = (82 x 2) + 138 + 7 = **309**
Arg/Glu = 7 + (1x 2) + 7 = **16**
Gly/Gln = 138 + (45 x 2) + 43 = **271**
Gly/Glu = 7 + (9 x 2) + 43 = **68**

It can be seen that a great number of individuals' predominantly carry the Gly/Glu haplotype than the Arg/Glu haplotype (and similarly the Arg/Gln is greater than Gly/Gln) indicating the presence of linkage disequilibrium.

Data taken from table 4.8 can be expressed as in table 4.9 to show haplotype numbers and frequencies. Double heterozygotes have been omitted.

Table 4.9 Haplotype numbers and the frequencies determined using the data in Table 4. 8 for Levin's δ calculation. Double heterozygotes have been omitted.

16 v 27

	Arg 16	Gly 16
Gln 27	309/0.47	271/0.41
Glu 27	16/0.02	68/0.10

4.2.4 Genotypic data for analysis

Linkage disequilibrium analysis was performed on 408 individuals. Table 4.7 shows the distribution of genotypes for each codon. Table 4.9 shows the frequencies of haplotypes without double heterozygotes using Levin's δ calculation of linkage disequilibrium.

4.3 Genetic Association Data

4.3.1 Statistical Analysis

GraphPad Prism® (GraphPad Software Inc., 1999) was used to combine analyse biostatistics, and produce the scientific graphing. All data are summarised in tables 4.10 and 4.11, with means and standard deviations (SD ±) correlated to genotype.

4.4 Results

4.4.1 Genotype vs. Age

There is no significant difference between genotype groups when assessed by age. However, the Glu/Glu, homozygotes in codon 27 show the youngest mean age in the NT group and the oldest mean age in the HT group. Refer to figure 4.1

4.4.2 Genotype vs. BMI

There is no significant difference between genotype groups when assessed by BMI in either the NT or HT groups. The NT and HT groups were classified as being '*overweight*'. Refer to figure 4.2

4.4.3 Genotype vs. SBP

There was no significant difference between genotype groups when assessed by SBP in the NT group. However, the Arg/Arg homozygotes in codon 16 of the HT group did have the lowest SBP with differences of 4.1-7.3 mmHg and the mutant genotype of Gly/Gly homozygotes in codon 16 show the highest SBP in the HT group. Refer to figure 4.3
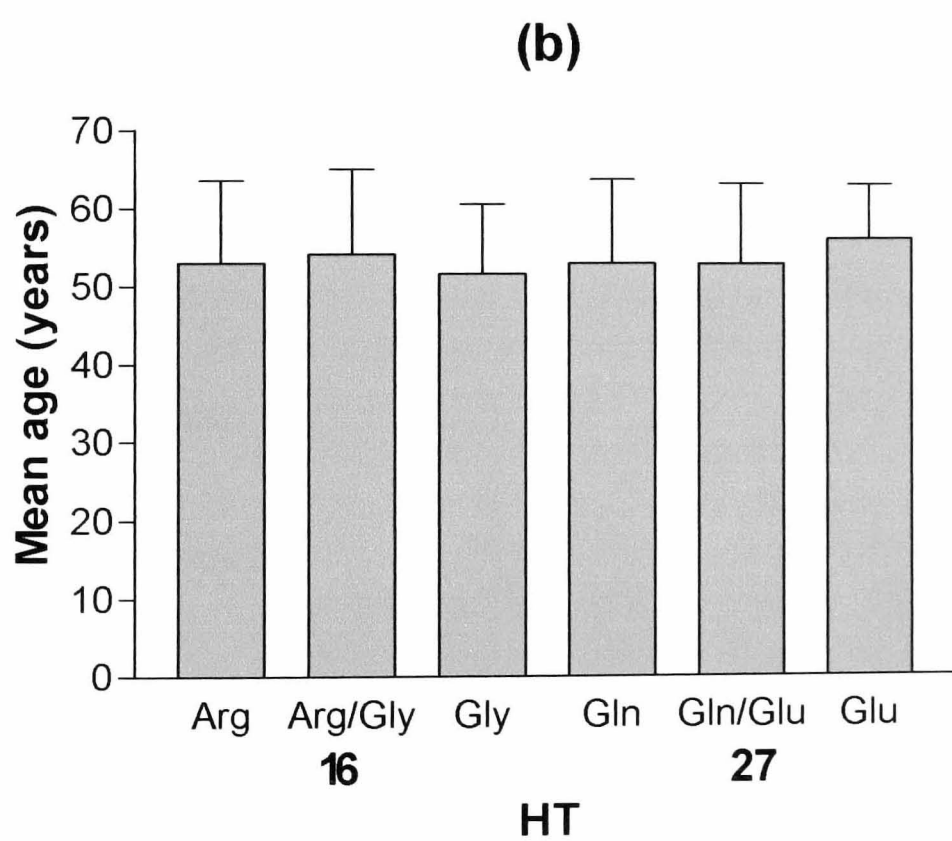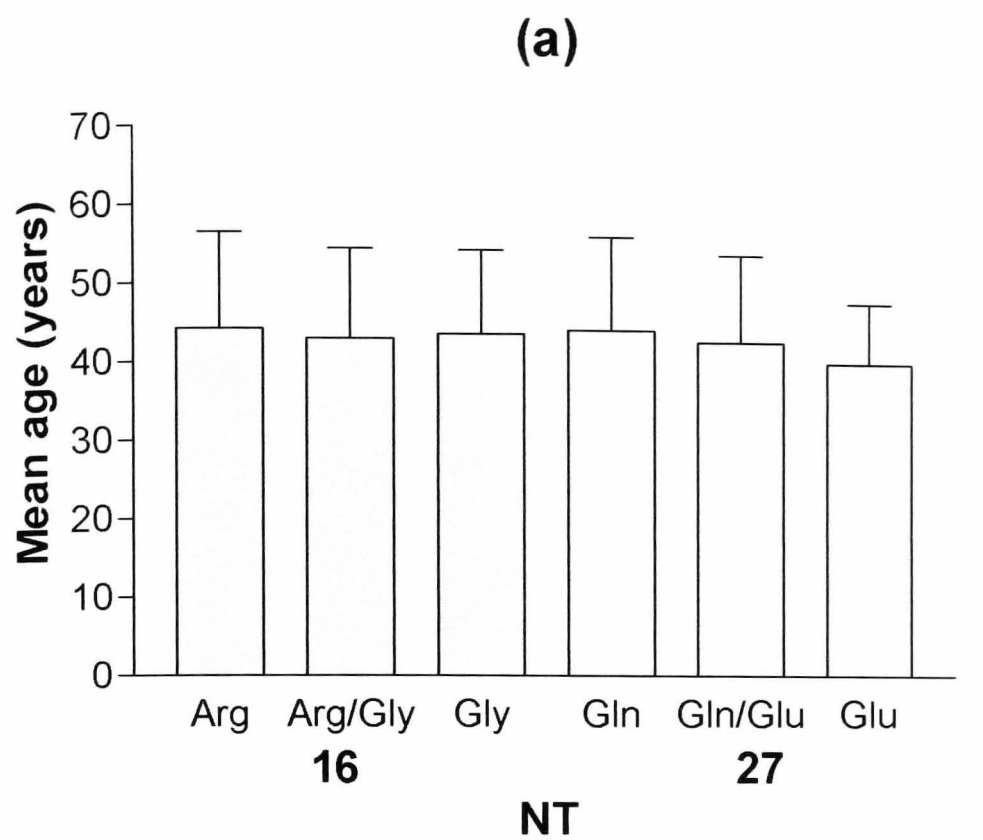
Figure 4.1 Genetic association of mean age in (a) normotensive and (b) hypertensive against genotype.
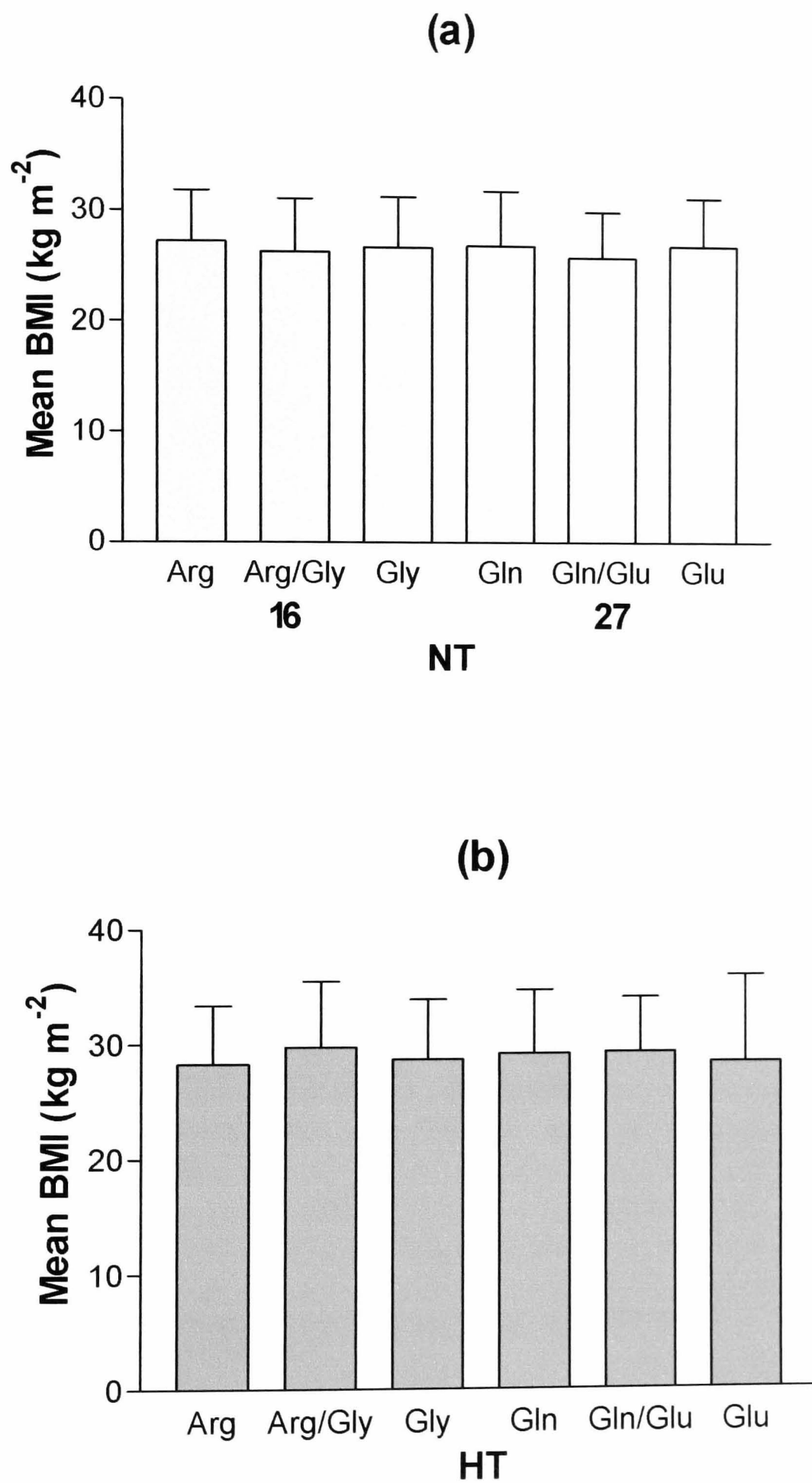
Figure 4.2 Genetic association of mean BMI in (a) normotensive and (b) hypertensive against genotype.

(a)

(b)
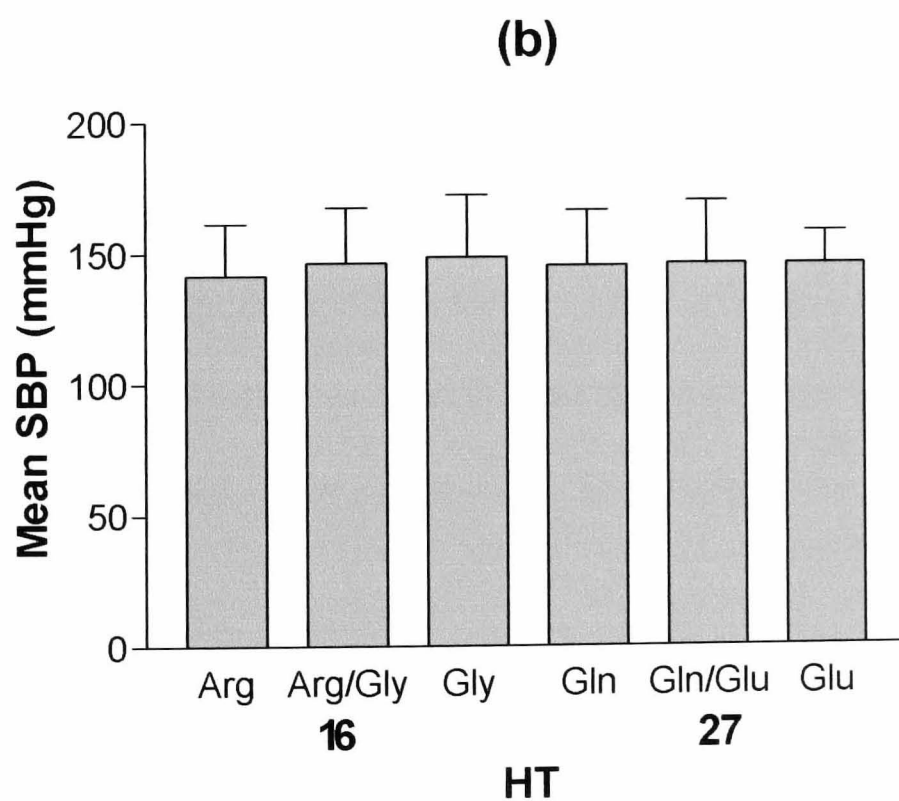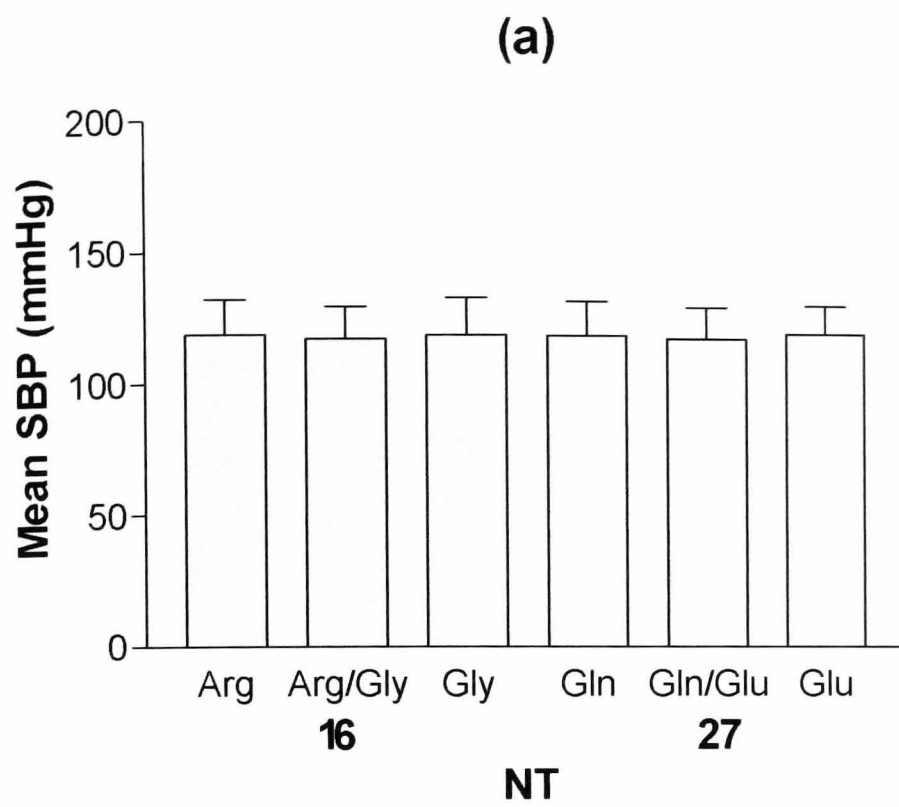
Figure 4.3 Genetic association of mean SBP in (a) normotensive and (b) hypertensive against genotype.

4.4.4 Genotype vs. DBP

There was no significant difference between genotype groups when assessed by DBP in either the NT or HT groups. The NT group had the highest DBP (75.0 ± 8.1) in the Glu/Glu homozygote group in codon 27 and the HT group had the highest DBP (92.1 ± 9.7) in the Gly/Gly homozygote group in codon 16. Refer to figure 4.4

4.4.5 Genotype vs. PP

There was no significant difference between genotype groups when assessed by PP in the NT and HT groups. As expected the PP was higher in the HT group, due to the population in this group being older. Refer to figure 4.5

4.4.6 Genotype vs. MAP

There was no significant difference between genotype groups when assessed by MAP in the NT and HT groups. The HT group had the highest MAP (111.1 ± 13.5) in the Gly/Gly homozygote group in codon 16. Refer to figure 4.6
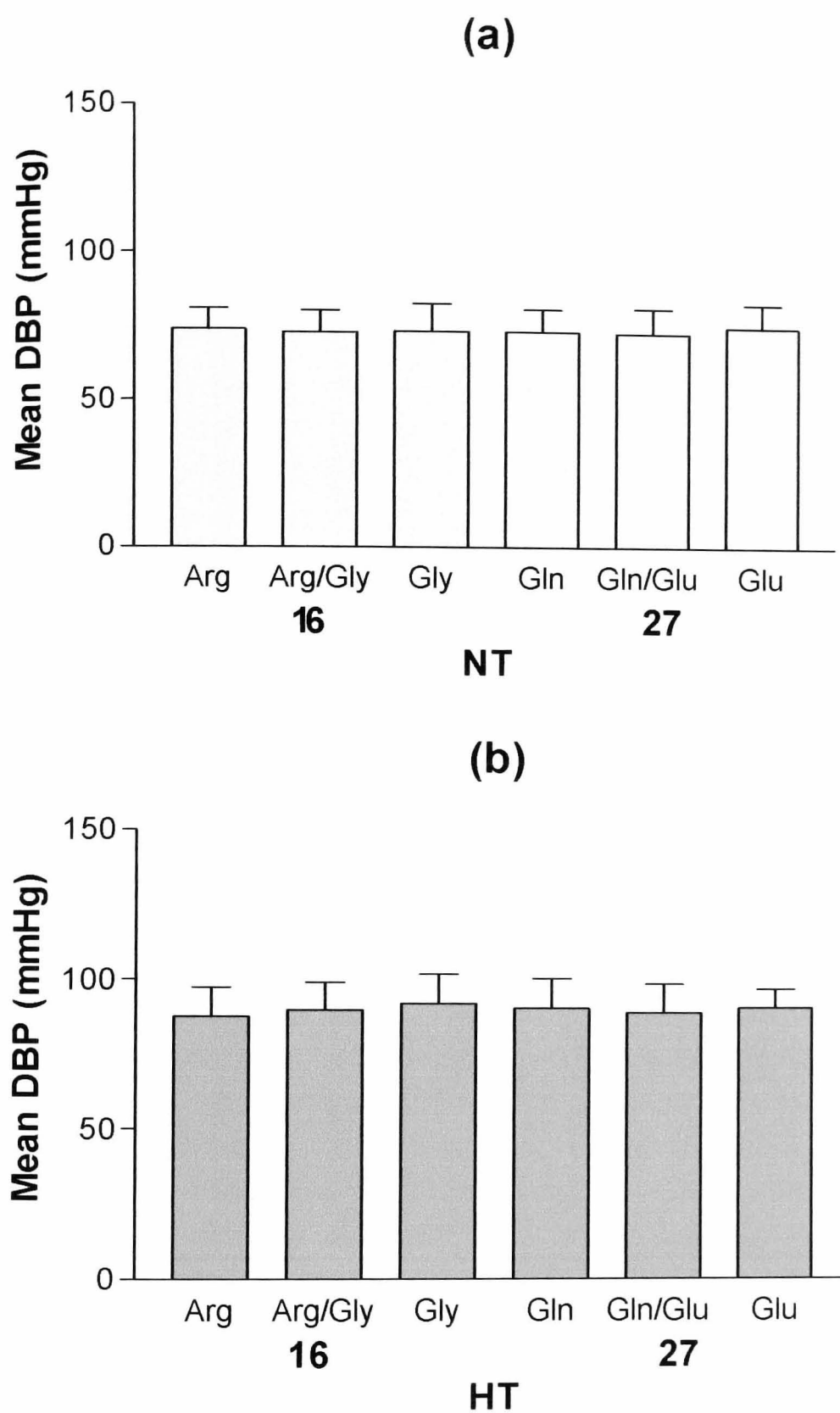
Figure 4.4 Genetic association of mean DBP in (a) normotensive and (b) hypertensive against genotype.

(a)

(b)
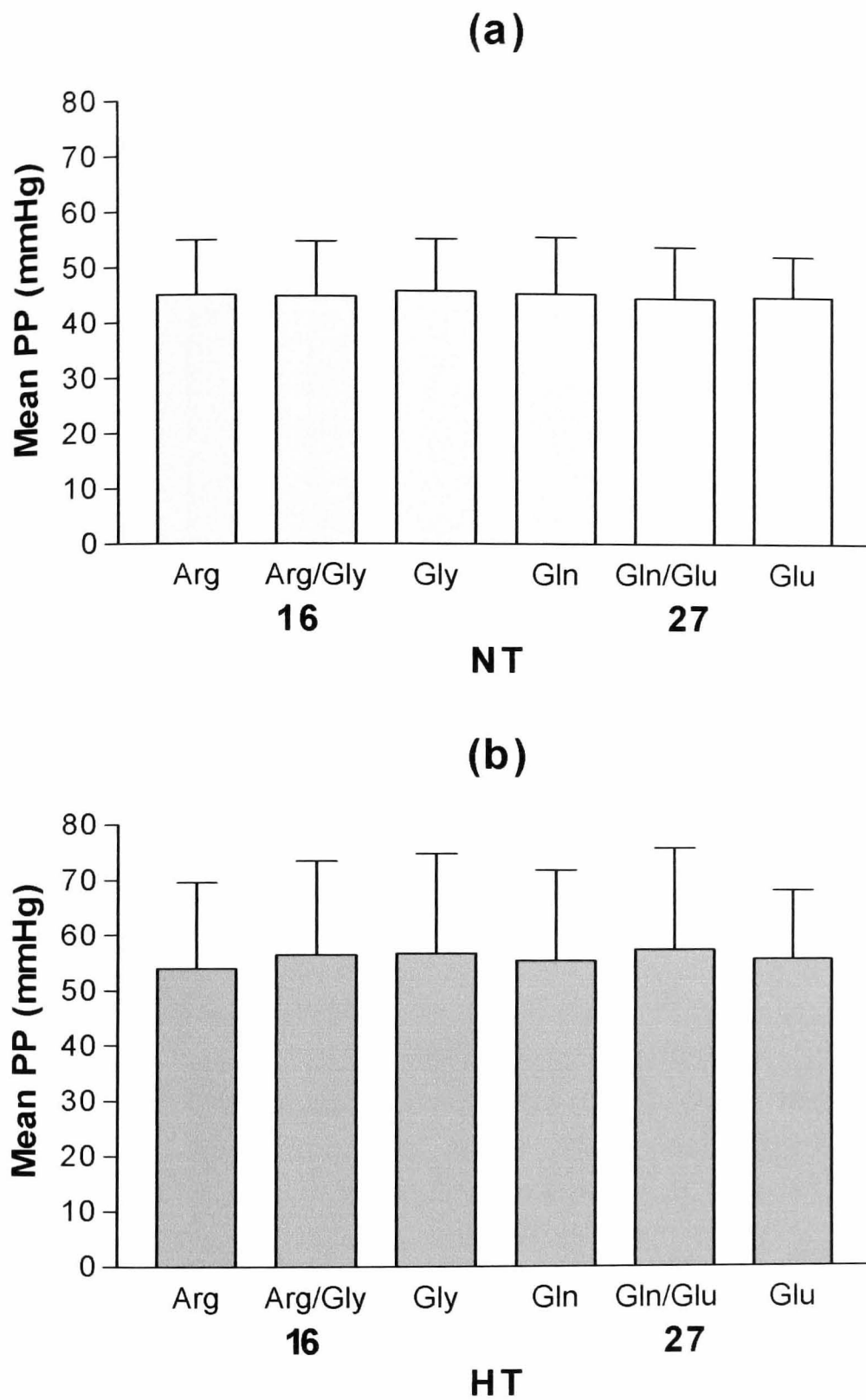
Figure 4.5 Genetic association of mean PP in (a) normotensive and (b) hypertensive against genotype.

(a)

(b)
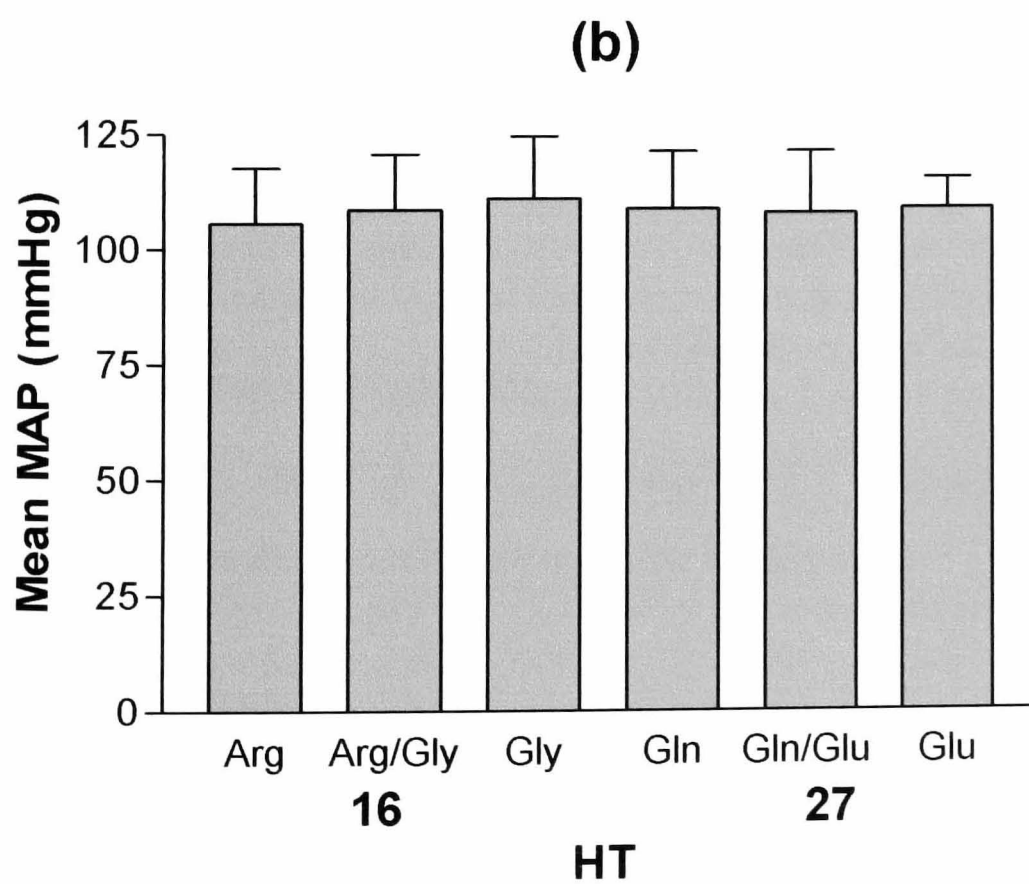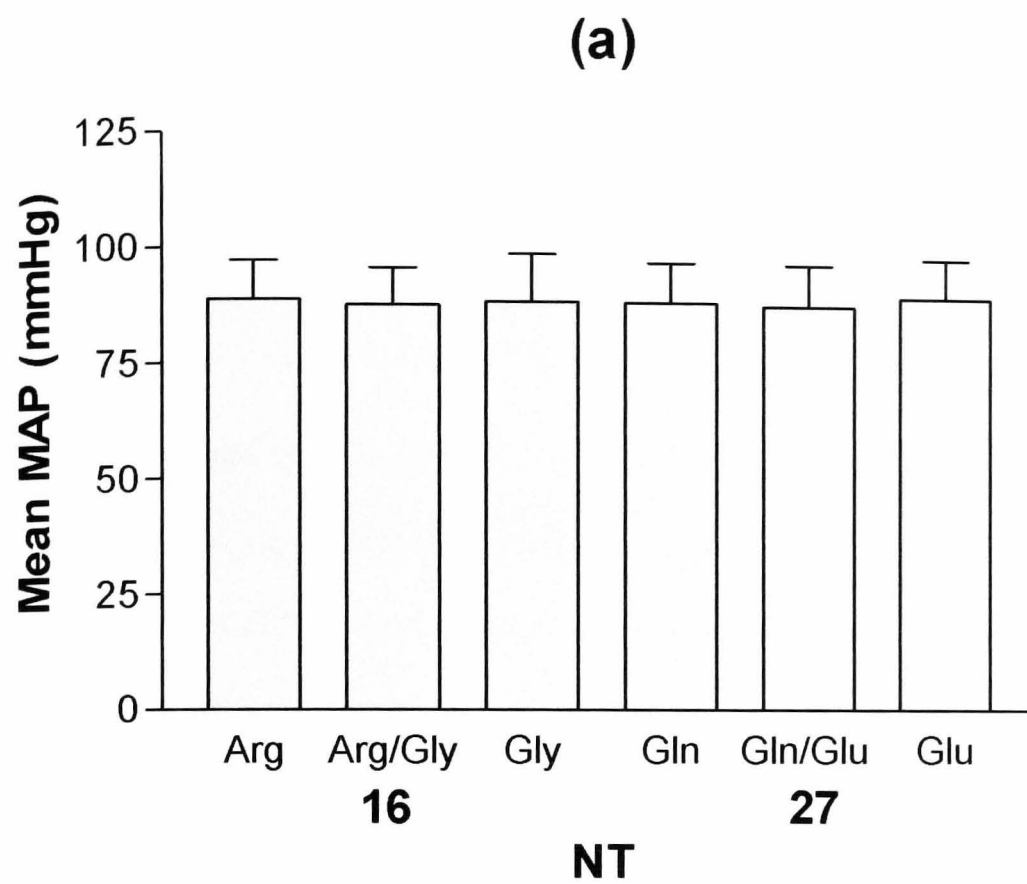
Figure 4.6 Genetic association of mean MAP in (a) normotensive and (b) hypertensive against genotype.

4.4.7 Genotype vs. HR

There was no significant difference between genotype groups when assessed by HR in the NT and HT groups. This also applied to HR comparison between the NT and HT groups. Refer to figure 4.7

4.4.8 Genotype vs. AIx

There was no significant difference between genotype groups when assessed by AIx in the NT and HT groups. As expected the AIx was higher in the HT group. Refer to Figure 4.8

4.4.9 Genotype vs. Total Cholesterol

There was no significant difference between genotype groups when assessed by total cholesterol in the NT and HT groups. However, the HT group did have higher means in all genotypes compared to the NT group. Refer to figure 4.9

4.4.10 Genotype vs. Triglycerides

There was no significant difference between genotype groups when assessed by triglycerides in the NT and HT groups. Refer to Figure 4.10
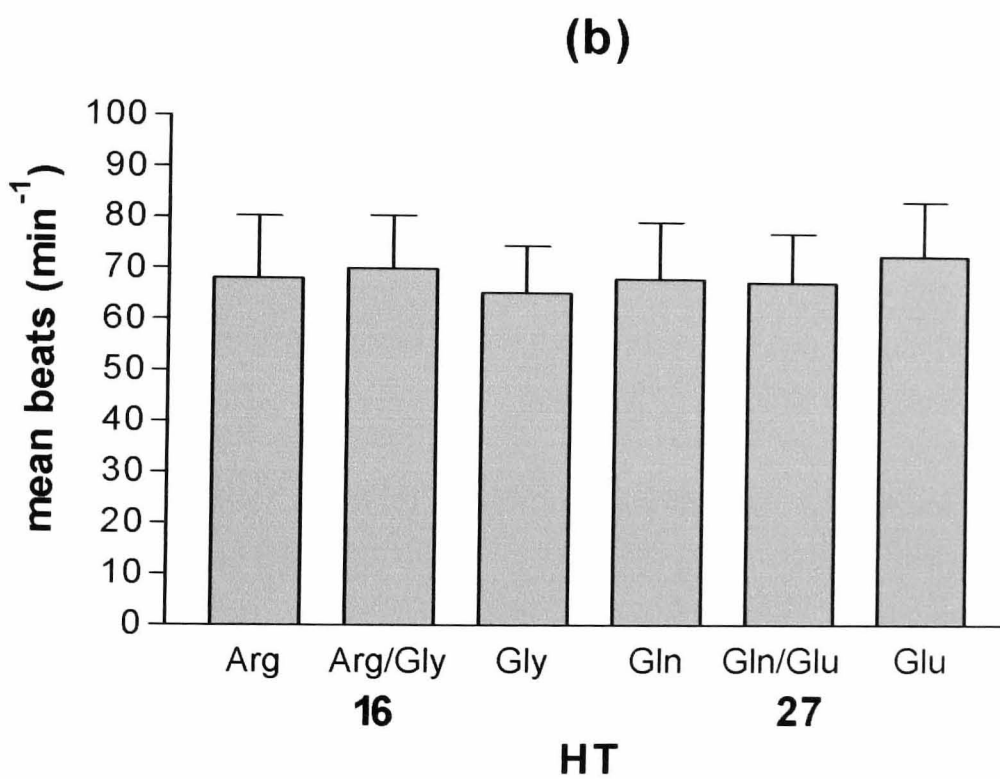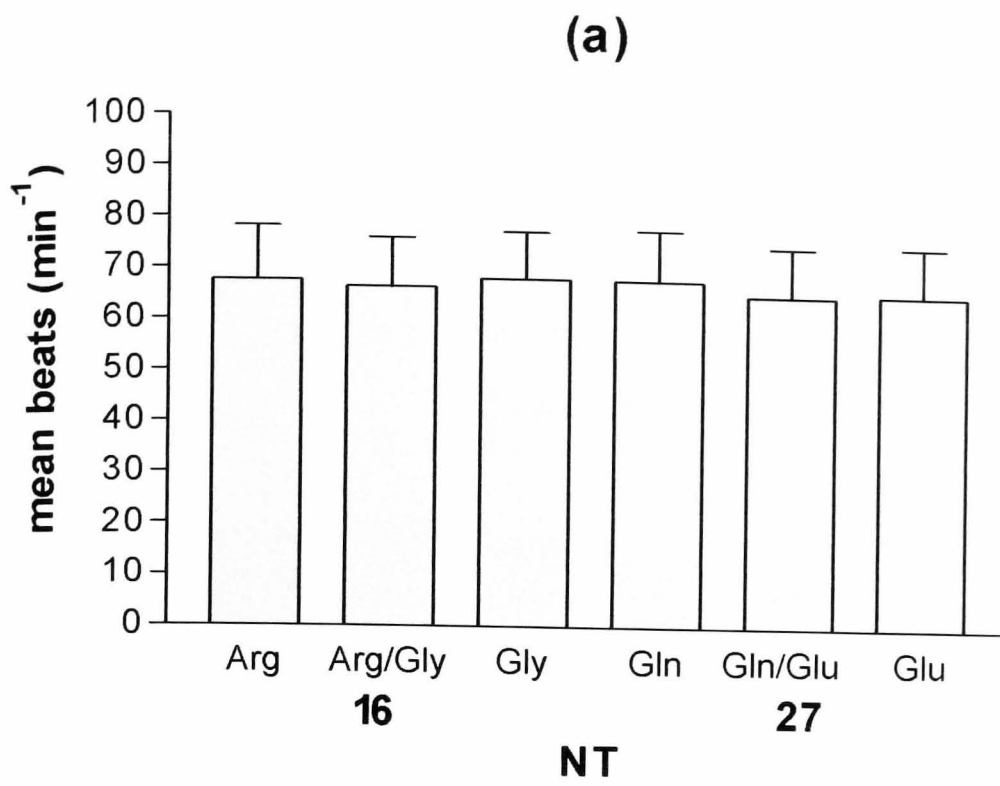
Figure 4.7 Genetic association of mean HR in (a) normotensive and (b) hypertensive against genotype.

(a)

(b)
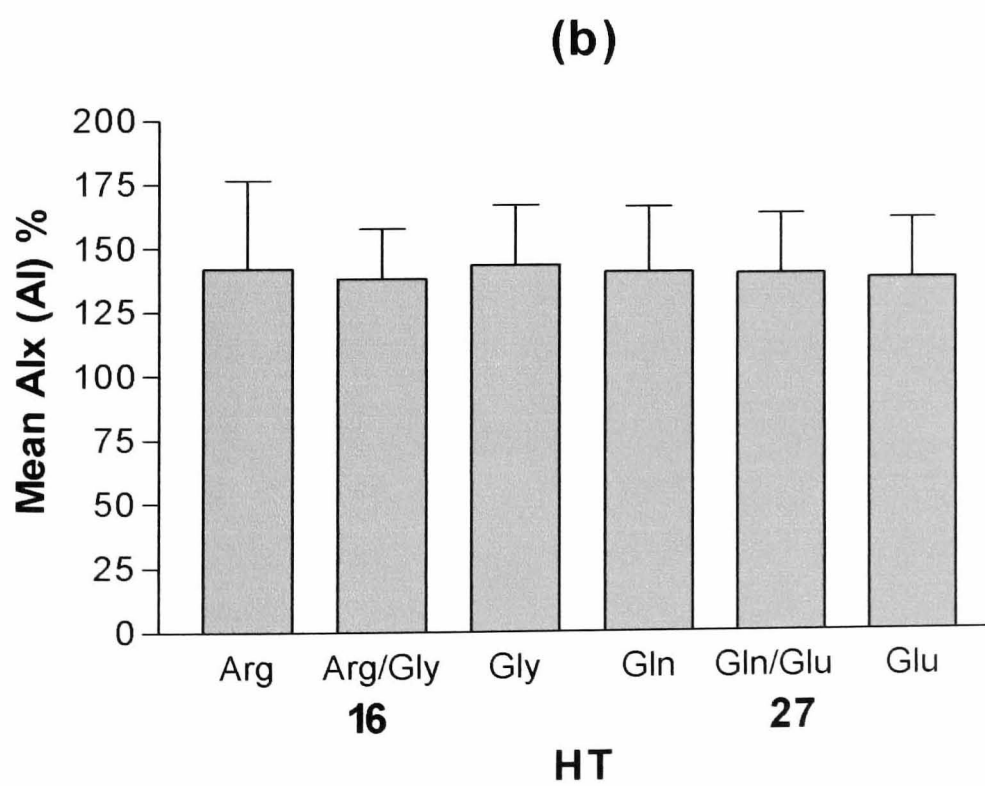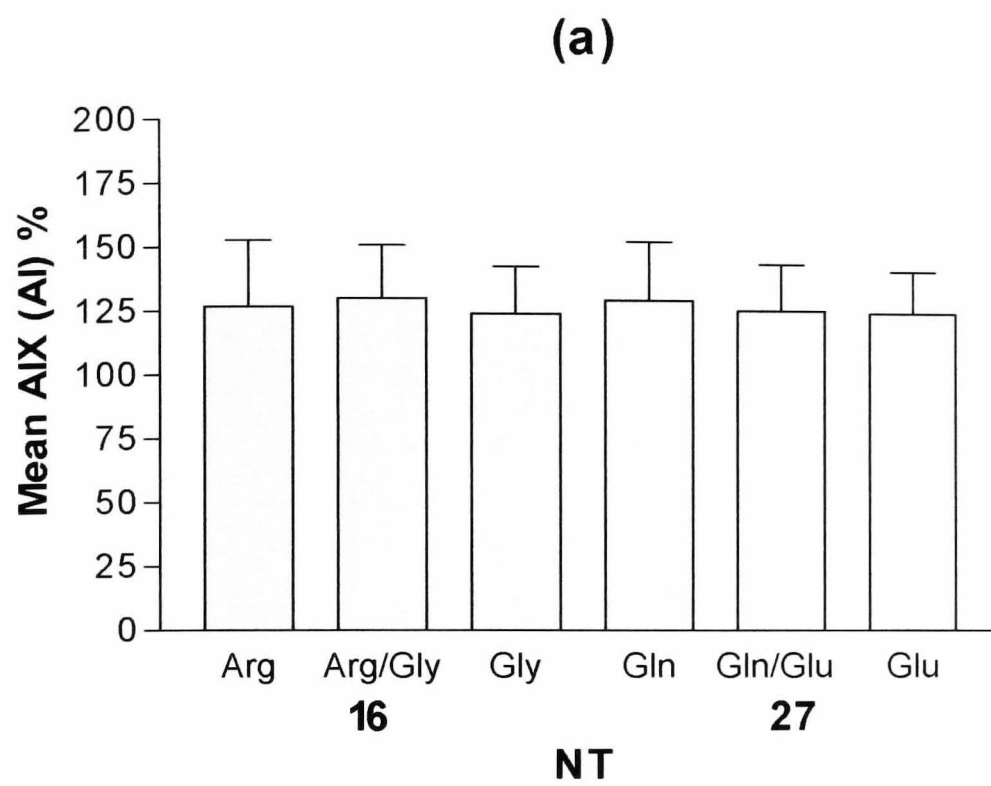
Figure 4.8 Genetic association of augmentation index in (a) normotensive and (b) hypertensive against genotype.

(a)

(b)
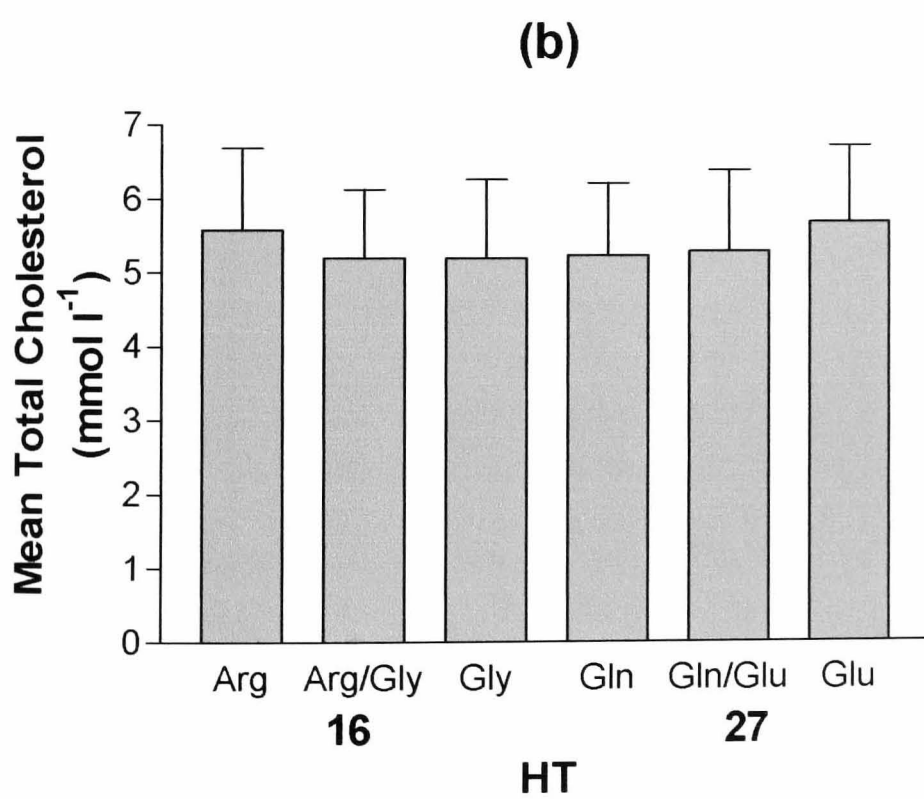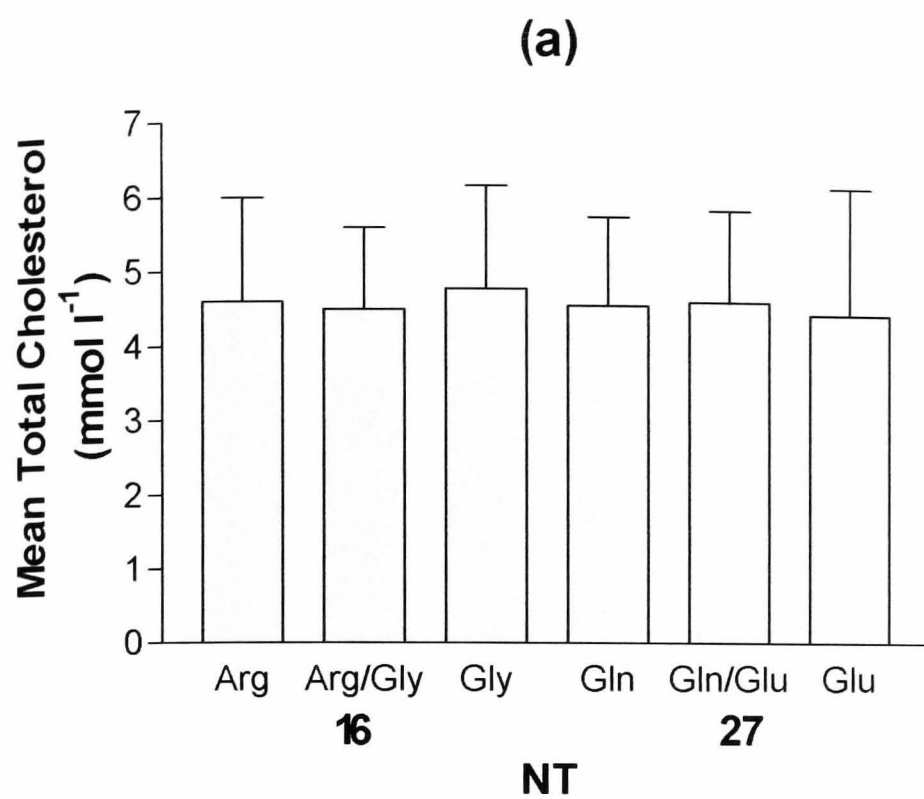
Figure 4.9 Genetic association of mean total cholesterol in (a) normotensive and (b) hypertensive against genotype.

(a)

(b)
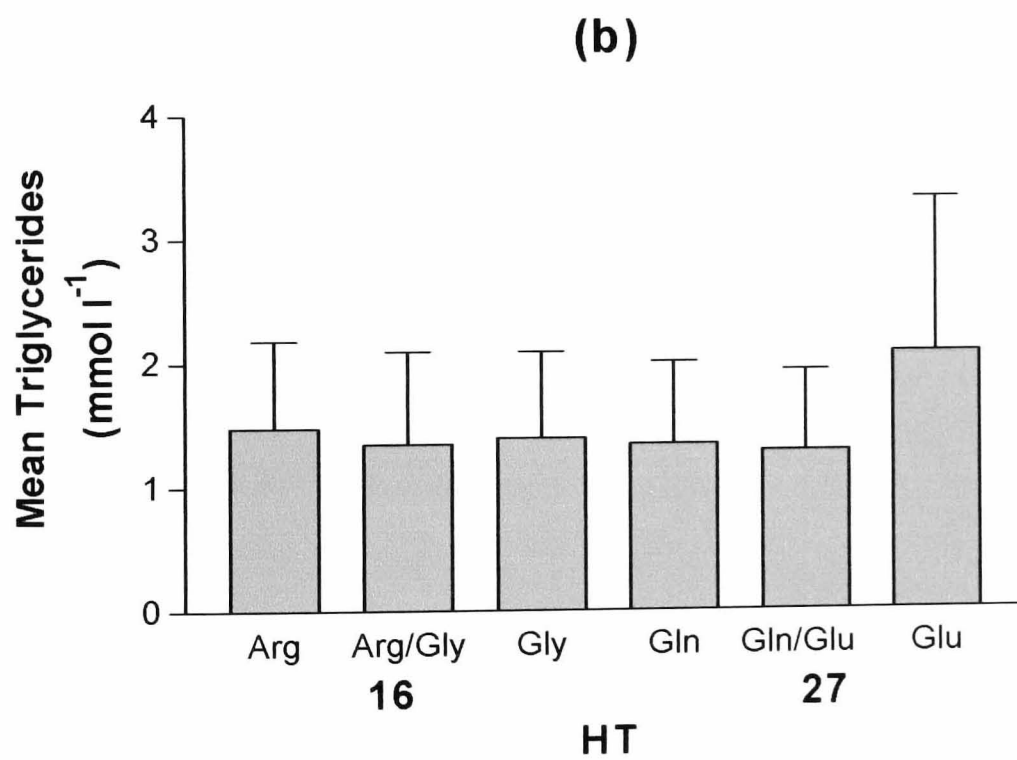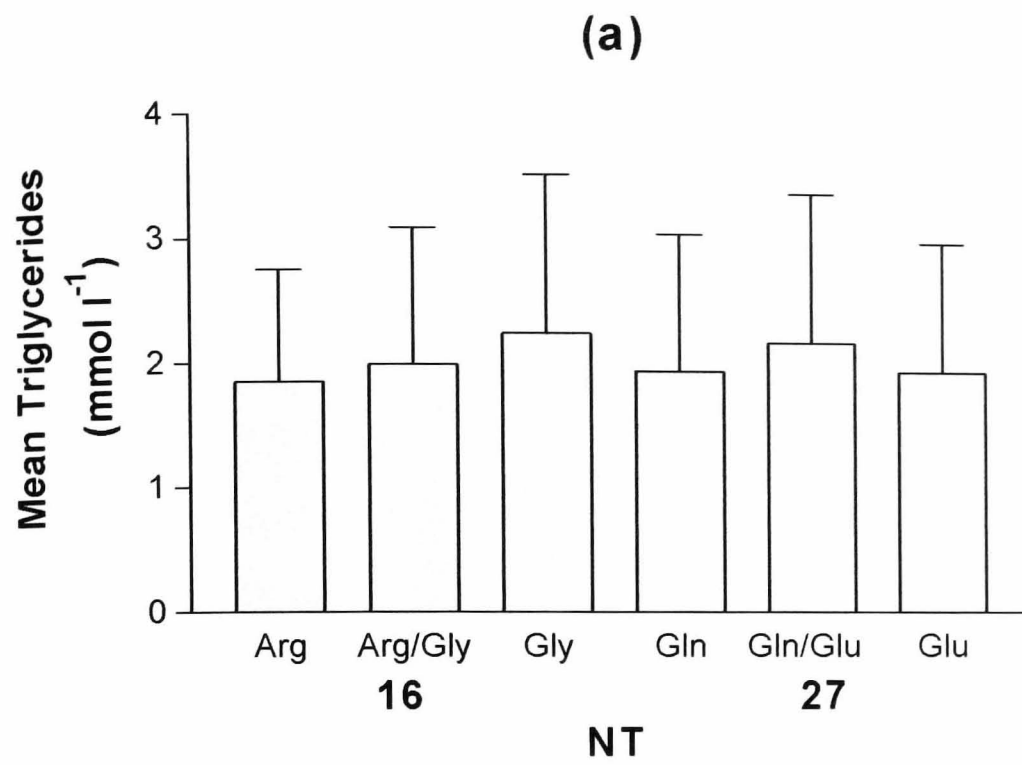
Figure 4.10 Genetic association of mean triglycerides in (a) normotensive and (b) hypertensive against genotype.

4.4.11 Genotype vs. HDL

There was no significant difference between genotype groups when assessed by HDL in the NT and HT groups. Refer to Figure 4.11

4.4.12 Genotype vs. LDL

There was no significant difference between genotype groups when assessed by LDL in the NT and HT groups. However, the HT group did express higher LDL levels in all genotype groups compared to the NT group. Refer to Figure 4.12

4.4.13 Genotype vs. VLDL

There was no significant difference between genotype groups when assessed by VLDL in the NT and HT groups. However, the HT group did express higher VLDL levels in all genotype groups compared to the NT group. Refer to Figure 4.13

4.4.14 Genotype vs. HDL Ratio

There was no significant difference between genotype groups when assessed by HDL ratio in the NT and HT groups. However, the HT group did express higher HDL ratio levels in all genotype groups compared to the NT group. Refer to Figure 4.14

4.4.15 Genotype vs. Glucose

There was no significant difference between genotype groups when assessed by glucose in the NT and HT groups. Refer to Figure 4.15
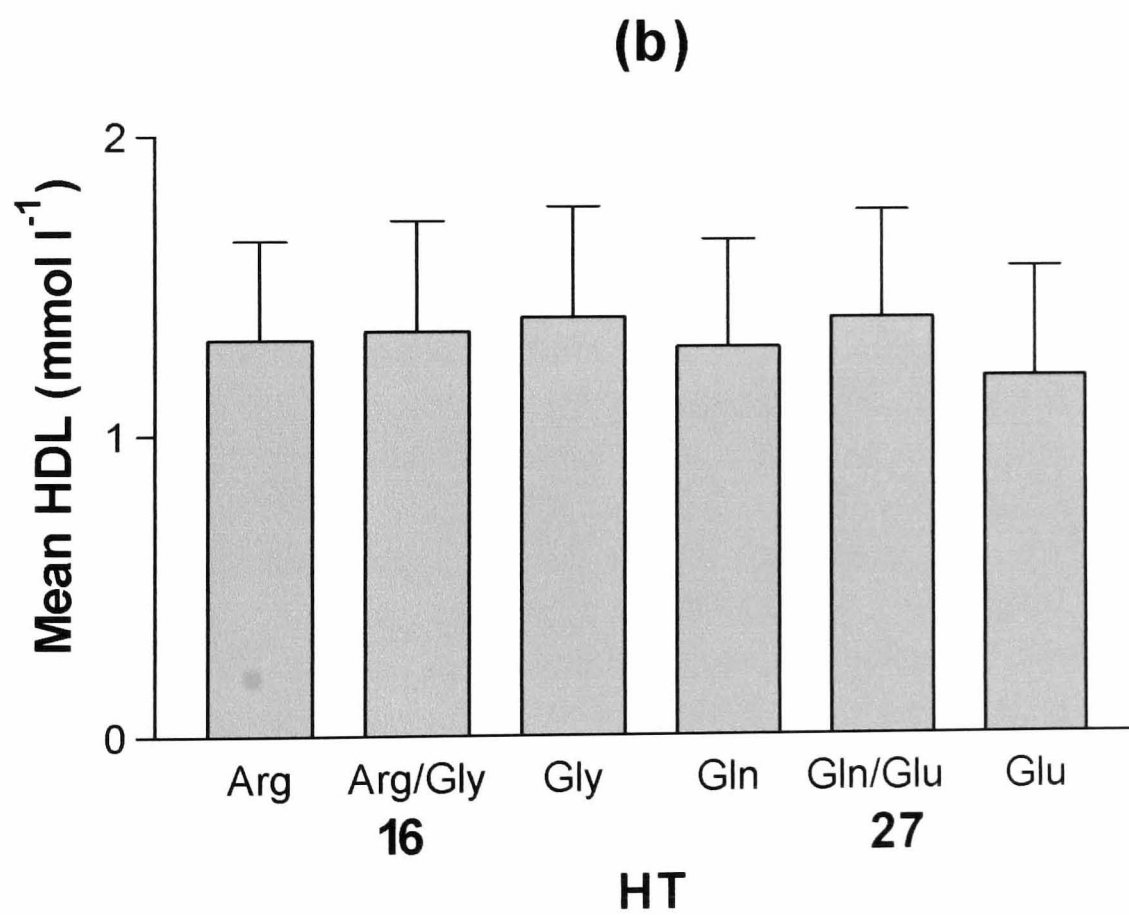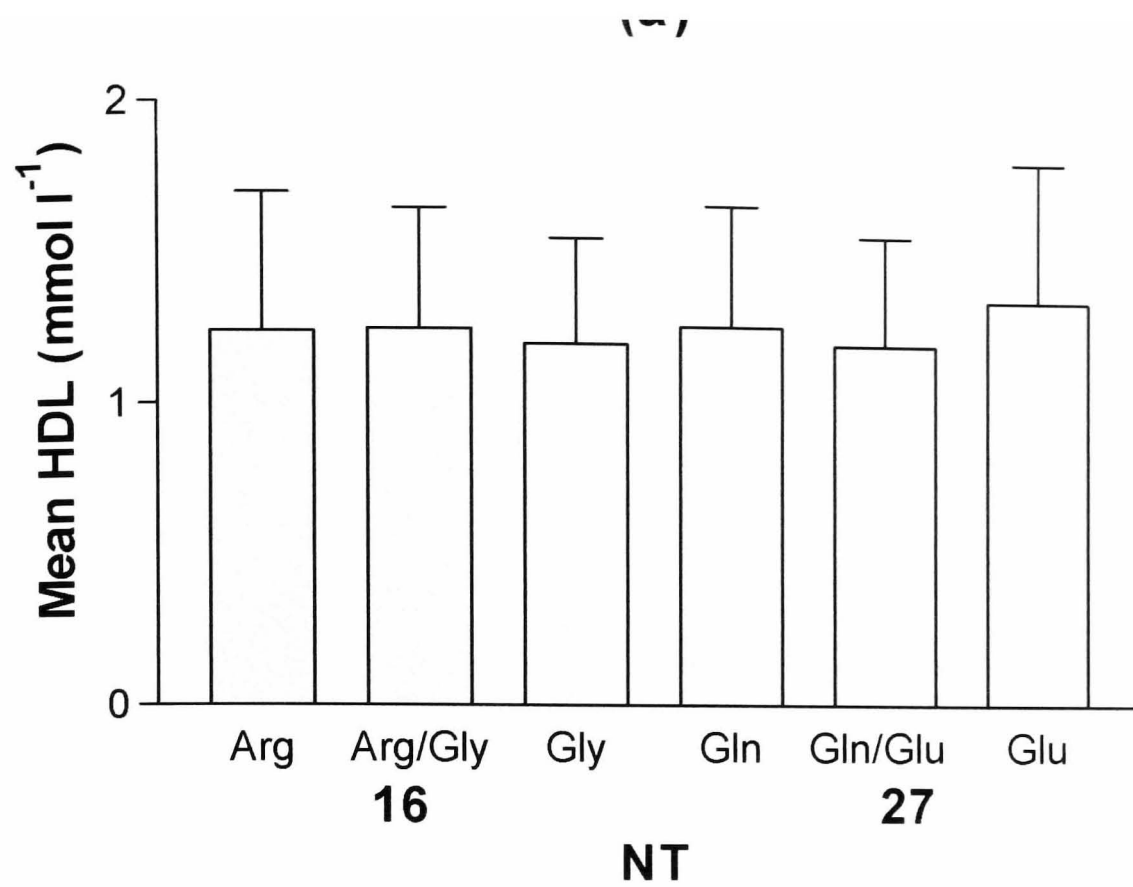
Figure 4.11 Genetic association of mean HDL in (a) normotensive and (b) hypertensive against genotype
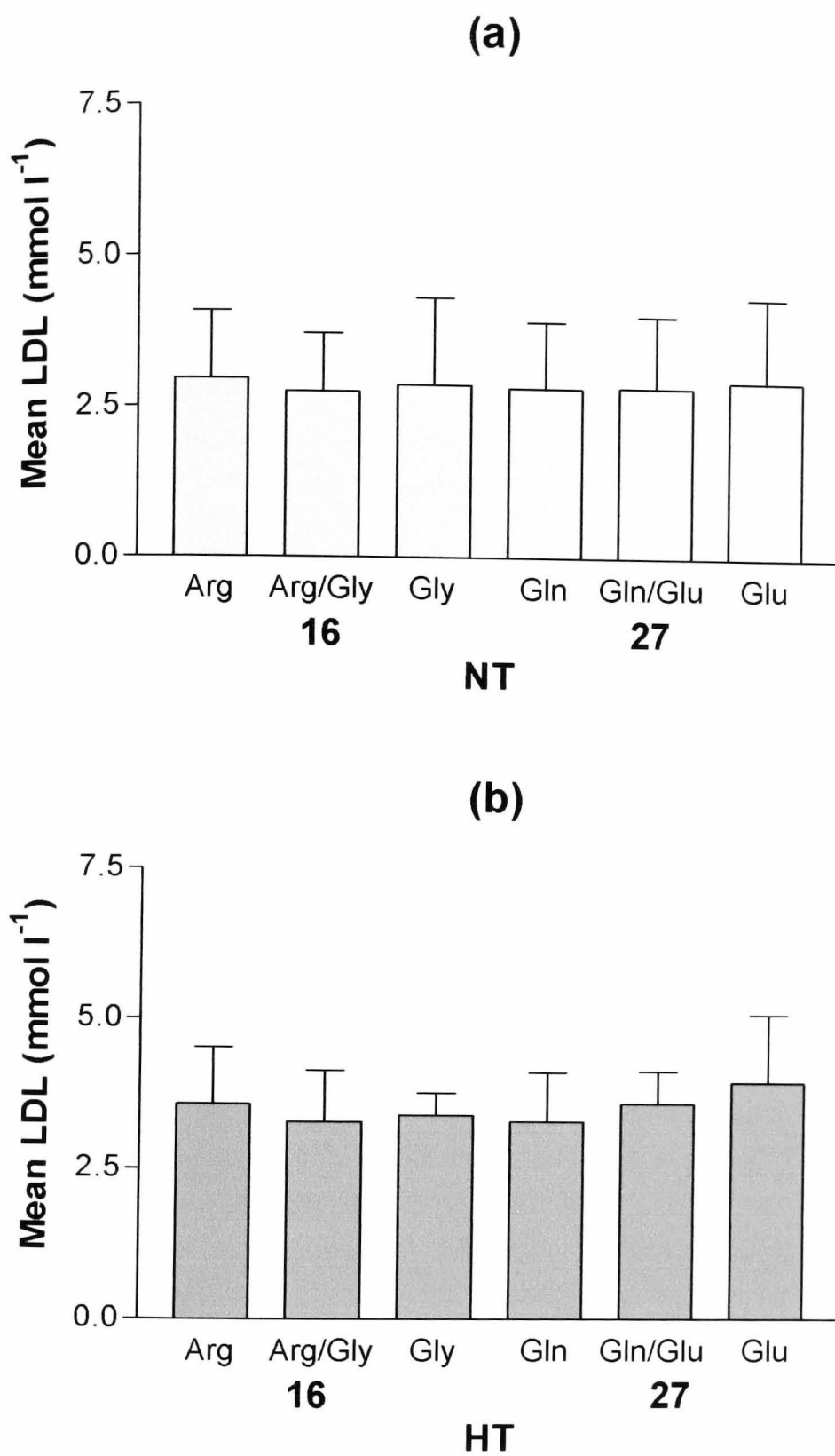
Figure 4.12 Genetic association of mean LDL in (a) normotensive and (b) hypertensive against genotype.
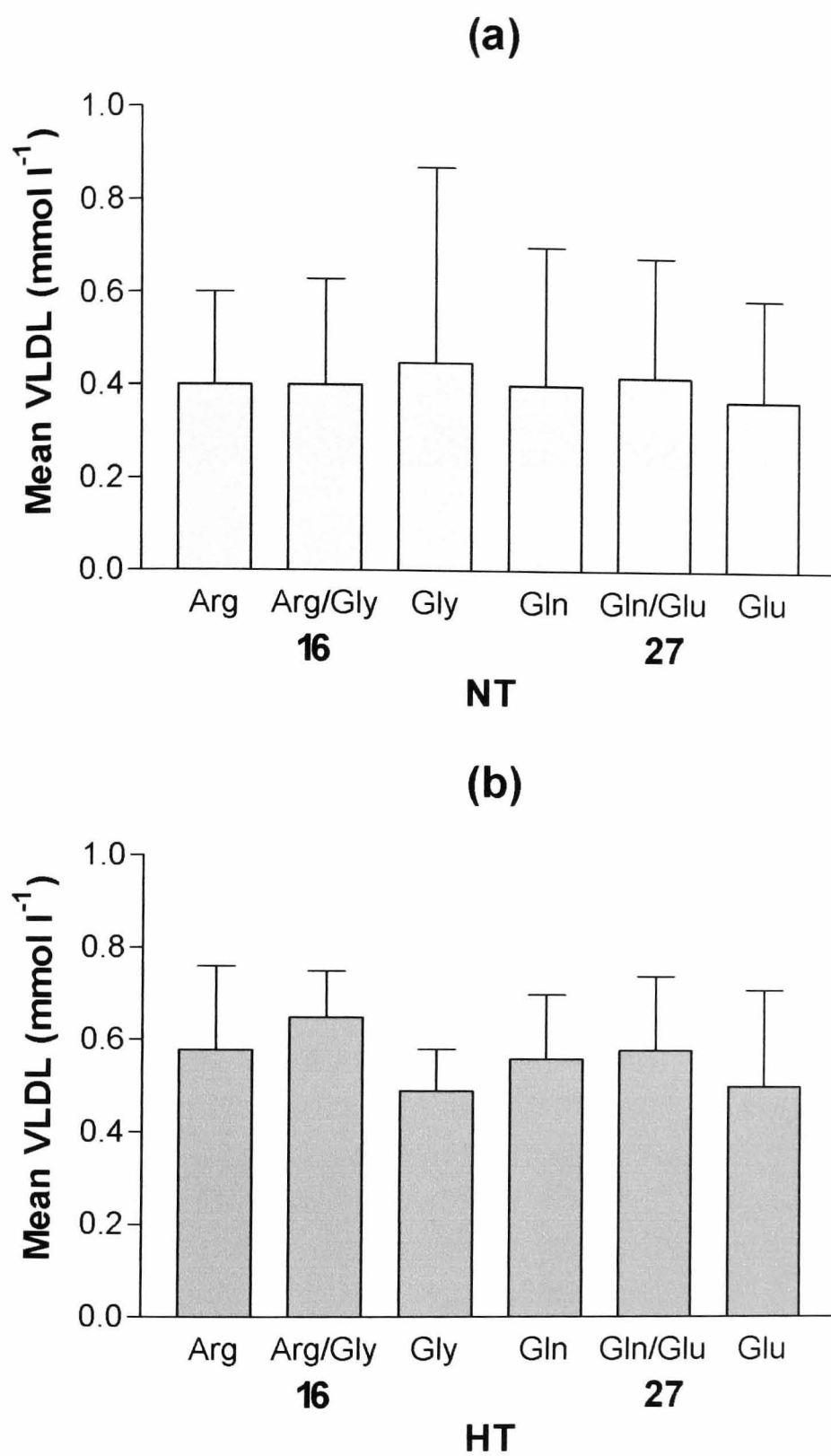
Figure 4.13 Genetic association of mean VLDL in (a) normotensive and (b) hypertensive against genotype.

(a)

(b)
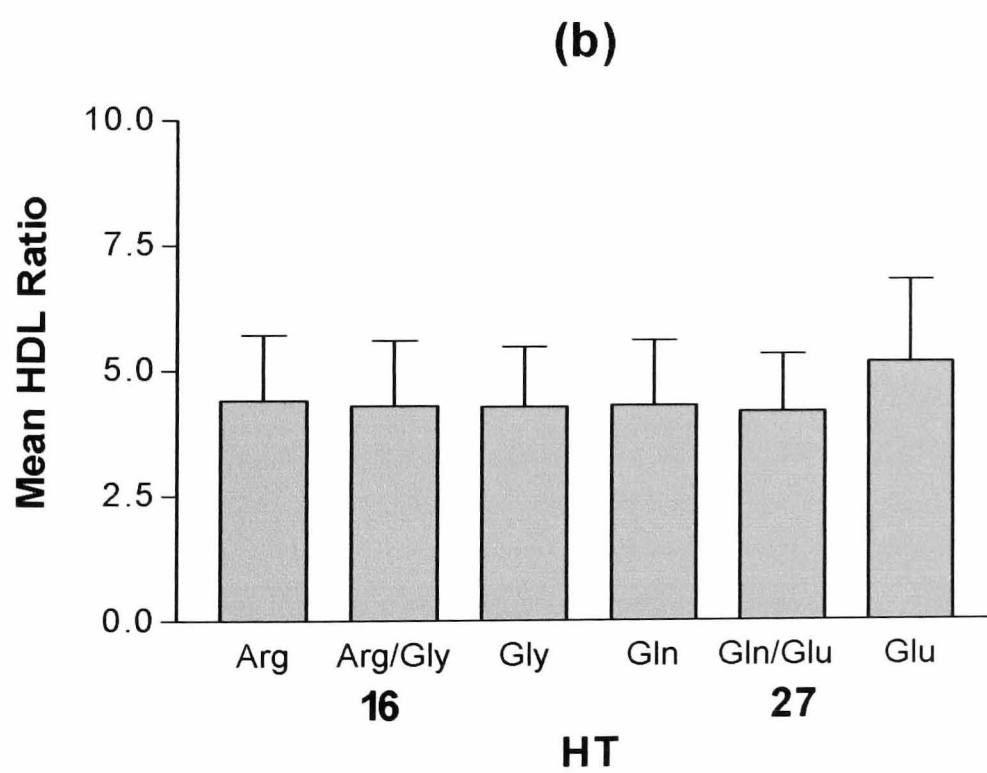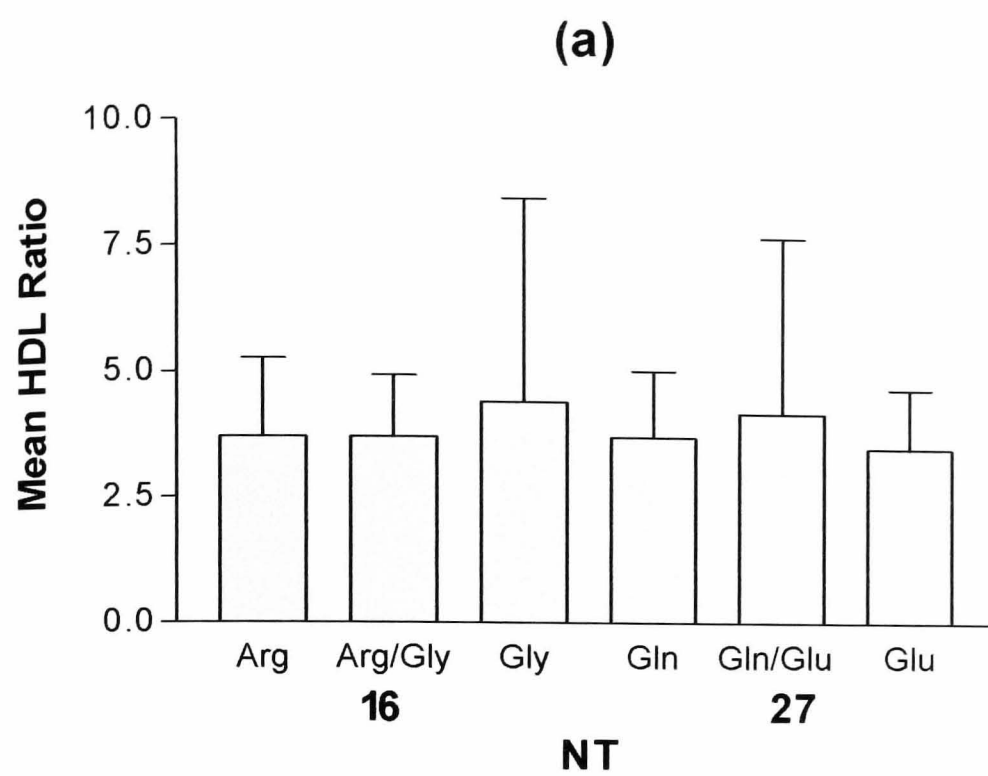
Figure 4.14 Genetic association of mean HDL Ratio in (a) normotensive and (b) hypertensive against genotype.

(a)

(b)
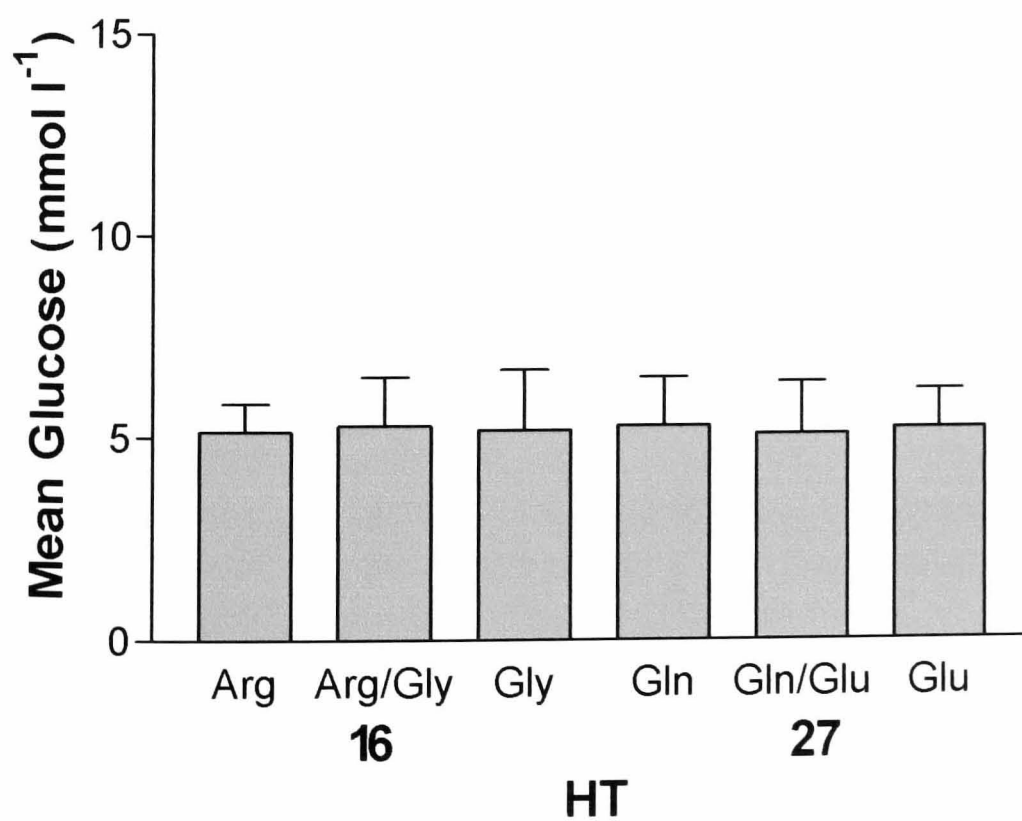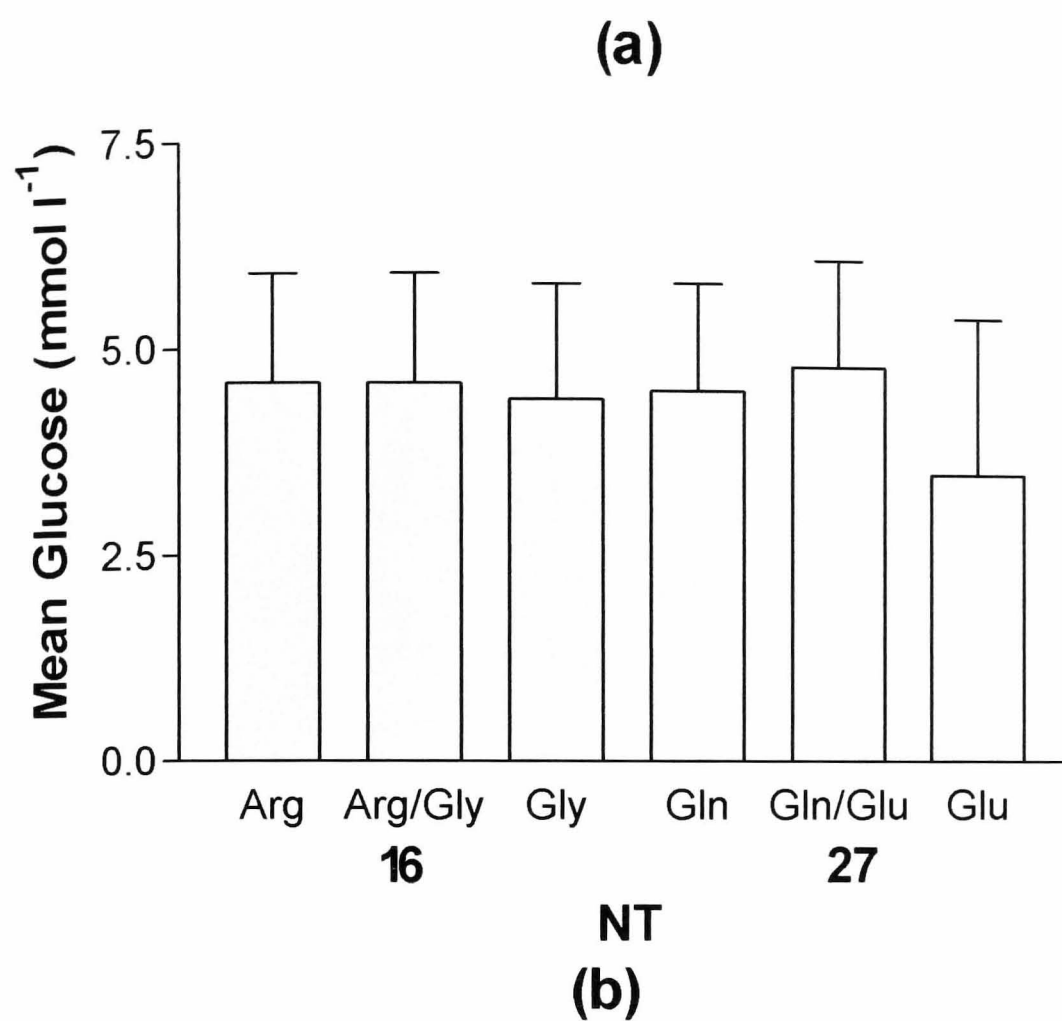
Figure 4.15 Genetic association of mean Glucose in (a) normotensive and (b) hypertensive against genotype.

Table 4.10 Genotype association data in Normotensives for codon Arg/Gly16 and codon Gln/Glu 27

|	Codon 16				Codon 27			
	Arg/Arg	**Arg/Gly**	**Gly/Gly**	**P**	**Gln/Gln**	**Gln/Glu**	**Glu/Glu**	**P**
Subjects	56	141	55		167	76	9	
Mean age±SD (years)	44.3±12.2	42.97 ± 11.5	43.6 ± 10.8	>0.05	44.1 ± 12.1	42.6 ± 11.1	40.0 ± 7.7	>0.05
BMI (kg m^{-2})	27.2 ± 4.6	26.3 ± 4.8	26.7 ± 4.5	>0.05	26.9 ± 5.0	25.9 ± 4.2	27.0 ± 9.6	>0.05
SBP (mmHg)	118.9 ± 13.3	117.6 ± 12.1	119 ± 14.2	>0.05	118.5 ± 13.2	117.4 ± 12.2	119.5 ± 10.7	>0.05
DBP (mmHg)	73.9 ± 7.1	72.8 ± 7.46	73.3 ± 9.42	>0.05	73.2 ± 7.6	72.9 ± 8.4	75.0 ± 8.1	>0.05
PP (mmHg)	45.0 ± 9.95	44.8 ± 9.9	45.7 ± 9.4	>0.05	45.3 ± 10.1	44.5 ± 9.4	44.9 ± 7.3	>0.05
MAP (mmHg)	88.9 ± 8.4	87.8 ± 8.0	88.5 ± 10.3	>0.05	88.3 ± 8.62	87.7 ± 8.8	89.6 ± 8.4	>0.05
HR (beats/min)	67.7 ± 10.5	66.4 ± 9.9	68.1 ± 9.2	>0.05	67.9 ± 1.0	65.3 ± 9.5	65.7 ± 9.6	>0.05
AG/P^2/P^1	126.8 ± 25.85	130.2 ± 20.5	124.3 ± 18.3	>0.05	129.5 ± 22.9	125.5 ± 18.3	124.5 ± 16.5	>0.05
Lipids								
Total Cholesterol mmols^{-1}	4.6 ± 1.36	4.5 ± 1.1	4.78 ± 1.4	>0.05	4.55 ± 1.2	4.6 ± 1.23	4.44 ± 1.7	>0.05
Triglycerides mmols^{-1}	1.85 ± 0.94	1.99 ± 0.4	2.24 ± 1.3	>0.05	1.94 ± 1.1	2.17 ± 1.18	1.94 ± 1.05	>0.05
HDL mmols^{-1}	1.24 ± 0.46	1.25 ± 0.4	1.2 ± 0.35	>0.05	1.26 ± 0.4	1.9 ± 0.4	1.35 ± 0.46	>0.05
LDLmmols^{-1}	2.96 ± 1.12	2.74 ± 0.97	2.86 ± 1.45	>0.05	2.81 ± 1.1	2.83 ± 1.2	2.94 ± 1.43	>0.05
VLDL mmols^{-1}	0.4 ± 0.19	0.4 ± 0.23	0.45 ± 0.42	>0.05	0.4 ± 0.3	0.42 ± 1.2	0.37 ± 0.22	>0.05
HDL Ratio	3.7 ± 1.6	3.7 ± 1.23	4.4 ± 4.1	>0.05	3.7 ± 1.3	4.2 ± 3.53	3.5 ± 1.2	>0.05
Glucose mmols^{-1}	4.6 ± 1.32	4.6 ± 1.3	4.4 ± 1.4	>0.05	4.5 ± 1.31	4.8 ± 1.3	3.5 ± 1.9	>0.05

Table 4.11 Genotype association data in Hypertensives for codon Arg/Gly16 and codon Gln/Glu27

<table>
<tr><th colspan="4" style="text-align:center">Codon 16</th><th colspan="4" style="text-align:center">Codon 27</th></tr>
<tr><th></th><th>Arg/Arg</th><th>Arg/Gly</th><th>Gly/Gly</th><th>P</th><th>Gln/Gln</th><th>Gln/Glu</th><th>Glu/Glu</th><th>P</th></tr>
<tr><td>*Subjects*</td><td>36</td><td>78</td><td>92</td><td></td><td>98</td><td>50</td><td>8</td><td></td></tr>
<tr><td>Mean age±SD (years)</td><td>53.0 ± 10.5</td><td>54.1 ± 10.98</td><td>51.6 ± 9.1</td><td>>0.05</td><td>53.0 ± 10.7</td><td>52.9 ± 10.3</td><td>56.1 ± 7.0</td><td>>0.05</td></tr>
<tr><td>BMI (kg m^{-2})</td><td>28.3 ± 5.1</td><td>29.8 ± 5.7</td><td>28.7± 5.28</td><td>>0.05</td><td>29.2 ± 5.6</td><td>29.4 ± 4.74</td><td>28.5 ± 7.6</td><td>>0.05</td></tr>
<tr><td>SBP (mmHg)</td><td>141.6 ± 19.8</td><td>146.4 ± 21.3</td><td>148.9 ± 23.9</td><td>>0.05</td><td>145.7 ± 21.2</td><td>146.4 ± 24.2</td><td>146.4 ± 12.6</td><td>>0.05</td></tr>
<tr><td>DBP (mmHg)</td><td>87.6 ± 9.8</td><td>89.85 ± 9.2</td><td>92.1 ± 9.7</td><td>>0.05</td><td>90.4 ± 9.9</td><td>89.0 ± 9.5</td><td>90.6 ± 6.3</td><td>>0.05</td></tr>
<tr><td>PP (mmHg)</td><td>54.0 ± 15.6</td><td>56.6 ± 16.8</td><td>56.8 ± 18.1</td><td>>0.05</td><td>55.4 ± 16.5</td><td>57.5 ± 18.4</td><td>55.8 ± 12.5</td><td>>0.05</td></tr>
<tr><td>MAP (mmHg)</td><td>105.6 ± 11.9</td><td>108.7 ± 12.0</td><td>111.1 ± 13.5</td><td>>0.05</td><td>108.9 ± 12.5</td><td>108.1 ± 13.4</td><td>109.2 ± 6.7</td><td>>0.05</td></tr>
<tr><td>HR (beats/min)</td><td>68.2 ± 12.1</td><td>70.13 ± 10.42</td><td>65.5 ± 9.21</td><td>>0.05</td><td>68.3 ± 11.0</td><td>67.8 ± 9.65</td><td>73.1 ± 10.7</td><td>>0.05</td></tr>
<tr><td>AG/P^{2}/P^{1}</td><td>142.0 ± 34.7</td><td>138.2 ± 19.5</td><td>143.6 ± 23.5</td><td>>0.05</td><td>140.8 ± 25.8</td><td>140.3 ± 23.5</td><td>138.6 ± 23.3</td><td>>0.05</td></tr>
<tr><td>*Lipids*</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Total Cholesterol mmols^{-1}</td><td>5.58 ± 1.14</td><td>5.2 ± 0.93</td><td>5.2 ± 1.1</td><td>>0.05</td><td>5.24 ± 0.98</td><td>5.3 ± 1.1</td><td>5.7 ± 1.0</td><td>>0.05</td></tr>
<tr><td>Triglycerides mmols^{-1}</td><td>1.48 ± 0.74</td><td>1.35 ± 0.75</td><td>1.4 ± 0.7</td><td>>0.05</td><td>1.35 ± 0.67</td><td>1.3 ± 0.65</td><td>2.1 ± 1.2</td><td>>0.05</td></tr>
<tr><td>HDL mmols^{-1}</td><td>1.32 ± 0.34</td><td>1.35 ± 0.38</td><td>1.25 ± 0.4</td><td>>0.05</td><td>1.3 ± 0.36</td><td>1.4 ± 0.66</td><td>1.2 ± 0.37</td><td>>0.05</td></tr>
<tr><td>LDLmmols^{-1}</td><td>3.57 ± 0.95</td><td>3.29 ± 0.85</td><td>3.4 ± 0.37</td><td>>0.05</td><td>3.3 ± 0.82</td><td>3.6 ± 0.54</td><td>3.95 ± 1.1</td><td>>0.05</td></tr>
<tr><td>VLDL mmols^{-1}</td><td>0.58 ± 0.18</td><td>0.65 ± 0.1</td><td>0.49 ± 0.09</td><td>>0.05</td><td>0.56 ± 0.14</td><td>0.58 ± 0.2</td><td>0.5 ± 0.21</td><td>>0.05</td></tr>
<tr><td>HDL Ratio</td><td>4.41 ± 1.28</td><td>4.3 ± 1.33</td><td>4.29 ± 1.15</td><td>>0.05</td><td>4.32 ± 1.3</td><td>4.2 ± 1.2</td><td>5.2 ± 1.65</td><td>>0.05</td></tr>
<tr><td>Glucose mmols^{-1}</td><td>5.14 ± 0.67</td><td>5.3 ± 1.24</td><td>5.2 ± 1.5</td><td>>0.05</td><td>5.3 ± 1.2</td><td>5.1 ± 1.3</td><td>5.25 ± 0.96</td><td>>0.05</td></tr>
</table>

4.5 Discussion

4.5.1 β-2 Adrenoceptor polymorphism and hypertension

One of the aims of the study was to determine if the β2-AR polymorphism contributed to the early development of essential hypertension in African Trinidadians. The main findings showed that the frequencies of both polymorphisms (Arg16→Gly and Gln27→Glu) were similar in both normotensive and hypertensive subjects (Table 4.2). There are two possible explanations for these data. Firstly, there may be no contribution of the β-2 AR locus to hypertension risk in this population. Secondly, it is possible the study-lacked power because of sample size and this maybe why the difference in frequency of alleles was not significant. To determine clearly if a specific gene is having an effect on a phenotype or ethnic group, larger cohorts are required to rule out a small contribution of a given polymorphism (Jindra et al, 2002; Tomaszewski et al, 2002; Meirhaeghe et al, 2001; Kato et al, 2001; Xie et al, 2000; Candy et al, 2000).

4.5.2 Hardy-Weinberg equilibrium

Hardy-Weinberg equilibrium was maintained in both NT and HT group as well as the total population. This was in keeping with previous studies on these polymorphisms (Jindra et al, 2002; Tomaszewski et al, 2002; Meirhaeghe et al, 2001; Kato et al, 2001; Xie et al, 2000; Candy et al, 2000).

The normotensive and hypertensive groups were combined showing that allele frequencies were similar and therefore combined for Hardy-Weinberg analysis. The observed and expected in the combined population and for both polymorphisms were not significantly different. This is due to random mating in the absence of mutation,

migration, random drift or natural selection. Although the cohort was not large it is noted that linkage disequilibrium did exist between codon 16 and codon 27.

4.5.3 Linkage disequilibrium

Linkage disequilibrium was seen in this population, although the relative rarity of the Gln 27 allele makes this less obvious than in Caucasian populations (Dewar et al, 1997). However, this was in keeping with previous studies (Jindra et al, 2002; Tomaszewski et al, 2002; Meirhaeghe et al, 2001; Kato et al, 2001; Xie et al, 2000; Candy et al, 2000).

4.5.4 Allele Frequencies

The main findings showed that the frequencies of both polymorphisms (Arg16→Gly and Gln27→Glu) were similar in both normotensive and hypertensive subjects (Table 4.2). The main conclusion of this preliminary study is the lack of a difference with the allele frequencies and this could be due to lack of size of the sample (n=252, NT, and n=156, HT). The potential contribution of a given polymorphism requires significant numbers to determine an overall effect on a phenotype. The allele frequencies described in this study are not dissimilar to those results and outcomes found in Xie et al, 2000 and Candy et al, 2000. However, it is only Xie et al, 2000 that has allele frequency and genotype data of both black and Caucasian individuals are compared. This study does have greater power in terms of individuals and specific ethnic definition, of which was not accurately determined in the other two studies. However, the resulting outcomes of the distribution of allele and genotypes of the tested β_2-adrenergic receptor variants were similar and no significant differences were found between normotensive and hypertensive groups.

4.5.5 β₂-AR polymorphism in the African Trinidadian population and hypertension

This study investigated the possibility that β₂-adrenoceptor (Arg/Gly 16 and Gln/Glu 27) polymorphism contributes to the prevalence of hypertension and arterial stiffness in a black African Caribbean (Trinidadian) population. This population was chosen for specific reasons.

Firstly, preliminary data using a restrictive fragment length polymorphism (RFLP) close to the β₂-adrenoceptor locus has suggested an association between this chromosomal region and hypertension in black populations (Svetkey et al, 1995; Kotanko et al, 1997).

Secondly, recent data has shown an association between the Gly16 form of the β₂ adrenoceptor and essential hypertension in African Caribbean individuals (Kotanko et al, 1997).

Lastly, black African populations have a blunted vasodilatory response to isoprenaline suggesting a primary defect in the β₂-adrenoceptor function (Lang et al, 1995).

There appears to be no clear association between β₂-adrenoceptor genotype at either codon Arg/Gly16 or Gln/Glu27 and the relationship to hypertension. This is in contrast to the findings of Kontanko et al, 1997, who observed a higher risk of hypertension in African Caribbean's carrying the Gly16 allele. Despite the current study having more power due to a larger cohort, this effect was not observed. The results are similar to those seen by Candy et al, 2000, despite a reversed proportion of hypertensives being represented ((n=192) with a lower number of normotensives (n=123)). Also, in keeping with Candy et al, 2000, was found that the Glu27 allele was much rarer in both the black South African (n=17 (5.4%)) and African Trinidadian (n=17 (4.2%)) population. This is further supported by data from Xie et

al, 2000, which identified that the Glu27 homozygous genotype was more common in Caucasians than in black subjects.

4.6 Summary

❖ There is no difference in the frequency of β_2-polymorphisms between normotensive and hypertensive African Trinidadians, and therefore, these are unlikely to be a contributing factor for essential hypertension.

Chapter 5

Augmentation association data using

Linear regression

5.1 Introduction

The data shown in Chapters 3 and 4 give an indication of the variability in the phenotypic and genotype parameters studied in this population. However, the time contribution of individual factors to augmentation status can only be estimated by taking account of possible confounding variables. Therefore, the data described in this chapter make use of regression analysis approaches to examine this issue.

Linear regression analyses the relationship between two or more variables. In this case the following figures and results determine the relationship between augmentation index (AIx) and the variables as expressed in table 3.1 in chapter 3. The following figures (graphs) will use the best-fit linear regression line with a 95% confidence interval, to establish relationships related to arterial stiffness in African Trinidadians. All statistical data is summarised in Tables 5.1, 5.2 and 5.3. using Bonferroni's multiple comparison test.

GraphPad Prism® (GraphPad Software Inc., 1999) was used to combine linear regression (curve fitting), analyse biostatistics, and produce the scientific graphing.

5.2 Results

5.2.1 Augmentation index and Age

In figure 5.1 the relationship of age and AIx is shown. It can be seen that AIx increases with age in both the NT and HT group and therefore have a positive correlation. The relationship is clearer in the NT group because of the wide age range.

5.2.2 Augmentation index and BMI

In figure 5.2, the relationship of BMI and AIx is shown. It can be seen that AIx increases with BMI in the NT and HT group, and therefore have a positive correlation.

5.2.3 Augmentation index and SBP

In figure 5.3, the relationship of SBP and AIx is shown. It can bee seen that AIx increases with SBP in both the NT and HT groups and therefore have a positive correlation.

5.2.4 Augmentation index and DBP

In figure 5.4, the relationship of DBP and AIx is shown. It can be seen that AIx increases with DBP in both the NT and HT group. Therefore, there is a positive correlation between DBP and AIx in both NT and HT groups.

5.2.5 Augmentation index and PP

In figure 5.5, the relationship of PP and AIx is shown. It can bee seen that AIx increases with PP in both the NT and HT groups and therefore have a positive correlation.

5.2.6 Augmentation index and MAP

In figure 5.6, the relationship of MAP and AIx is shown. It can be seen that AIx increases with MAP in both the NT and HT groups and therefore have a positive correlation.

5.2.7 Augmentation index and HR

With reference to figure 5.7, the NT group there is a negative correlation between HR and AIx. The explanation for this remains unclear. It may be due to the wide age range of the NT group. This may suggest that normotensive African Trinidadians present with stiffer arteries as age advances but do not elevate heart rate.

Interestingly, this is reversed in the HT group and a positive correlation is evident. This contradictory result may in part be due to a proportion of the HT cohort taking anti-hypertensive medication. Therefore, the HT group was further investigated and linear regression was performed on HT individuals on medication for high blood pressure and those who were not. This is demonstrated in Figure 5.15. Those individuals not taking anti-hypertensive medication show a positive correlation in HR as AIx increases. Those individuals taking anti-hypertensives show a negative correlation in HR as AIx increases. This suggests that anti-hypertensive medication in African Trinidadians may decrease HR and reduce cardiac load but does not necessarily have a protective effect on arterial stiffness. However, the confidence intervals are large in figures 5.7 and 5.15 and therefore the significance of the impact on arterial stiffness may require further investigation.

(a)

(b)
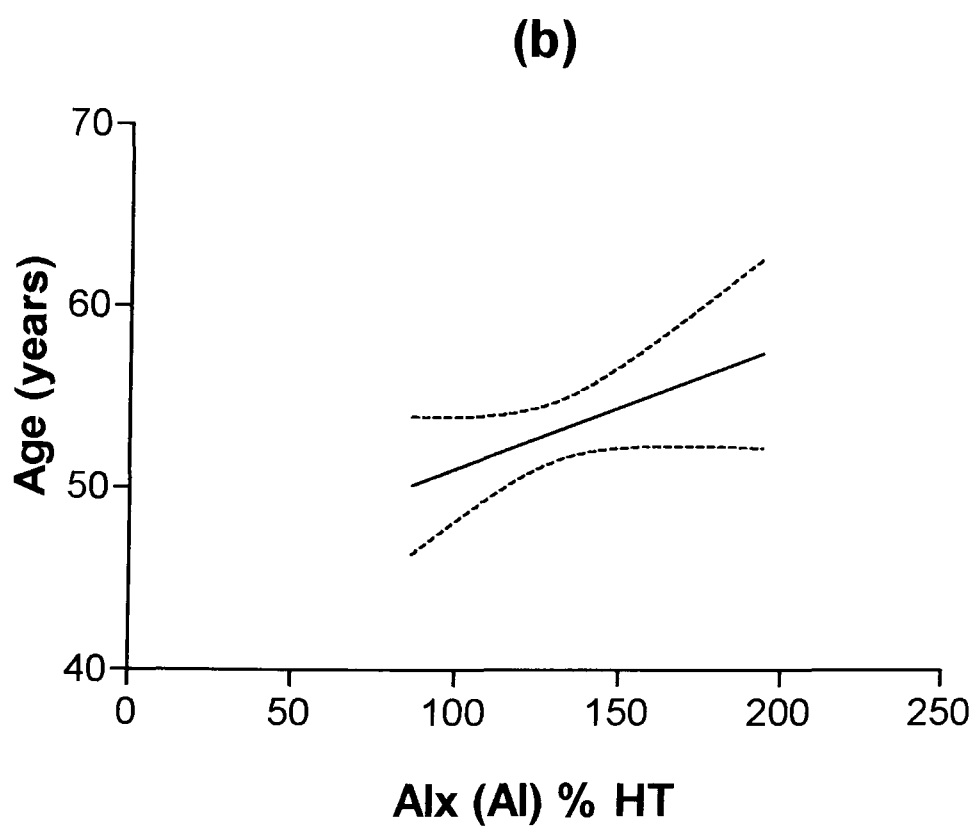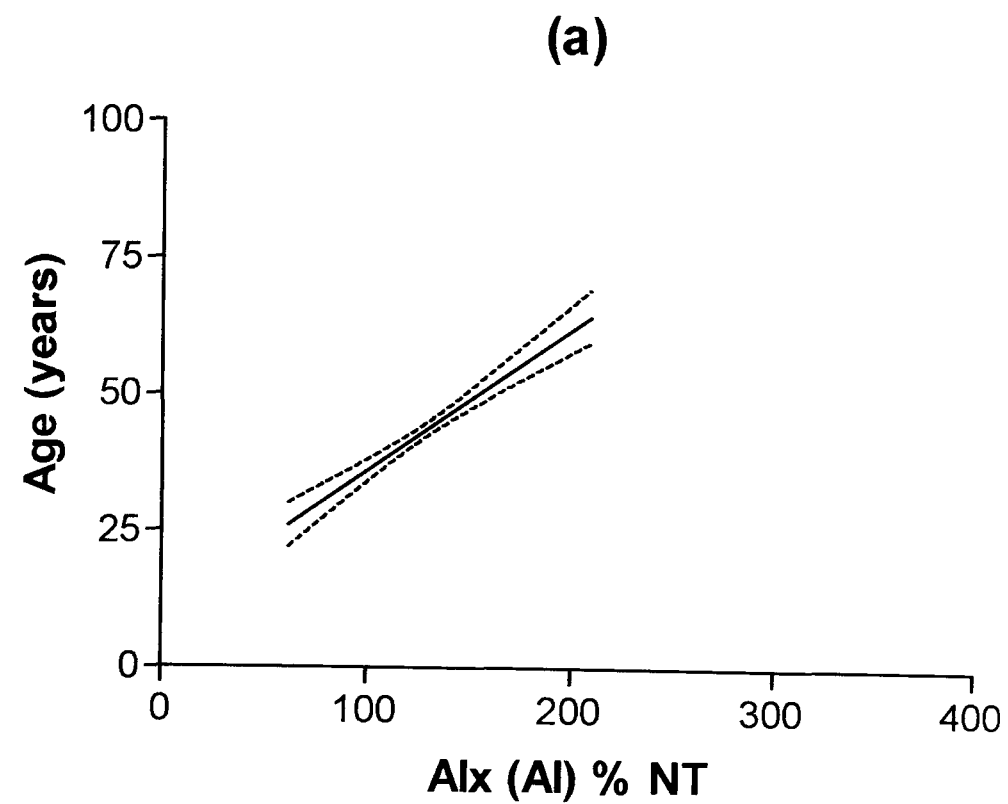
Figure 5.1 Linear regression of age against augmentation in (a) normotensive and (b) hypertensive African Trinidadians.
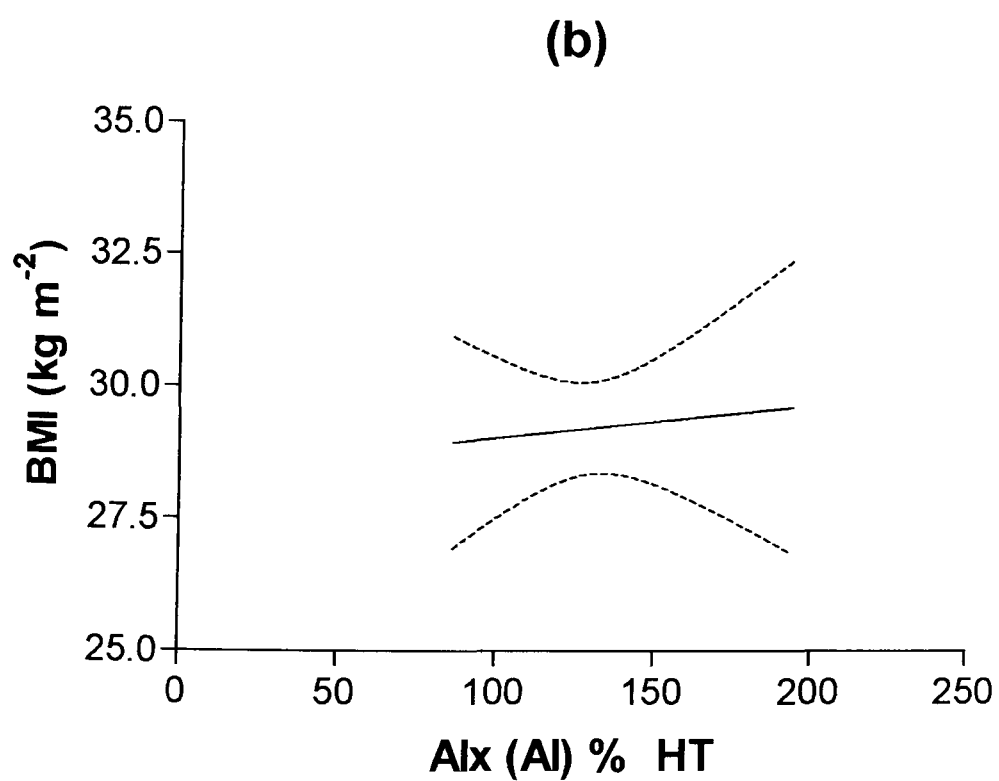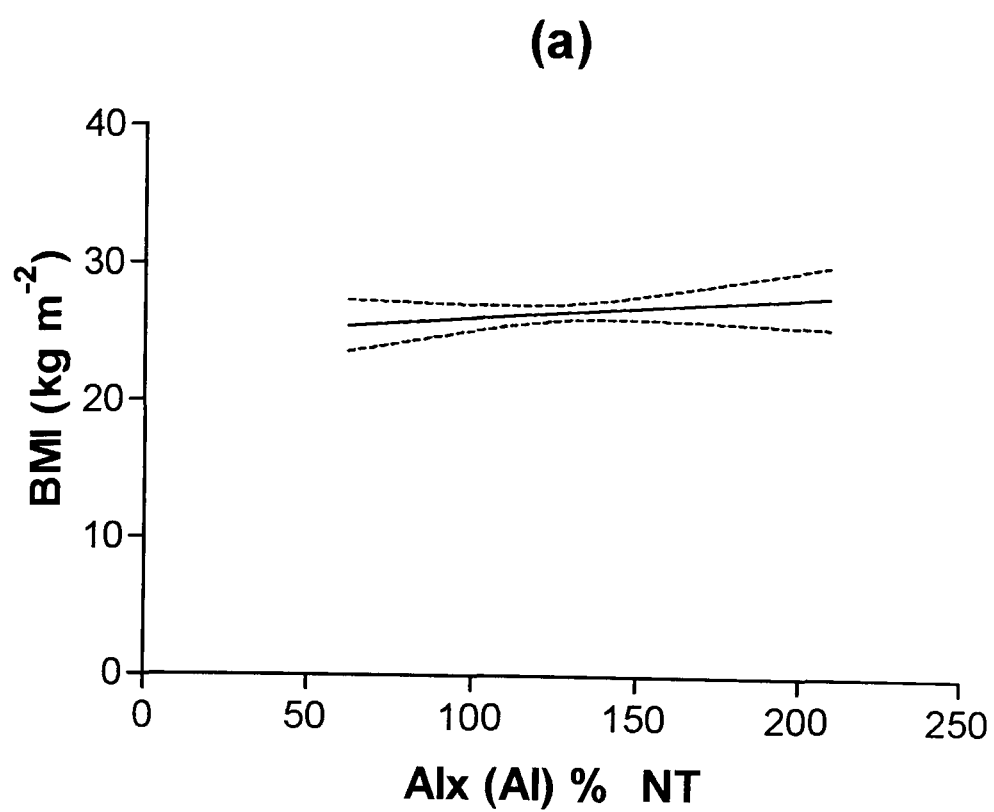
Figure 5.2 Linear regression of body mass index against augmentation in (a) normotensive and (b) hypertensive African Trinidadians.
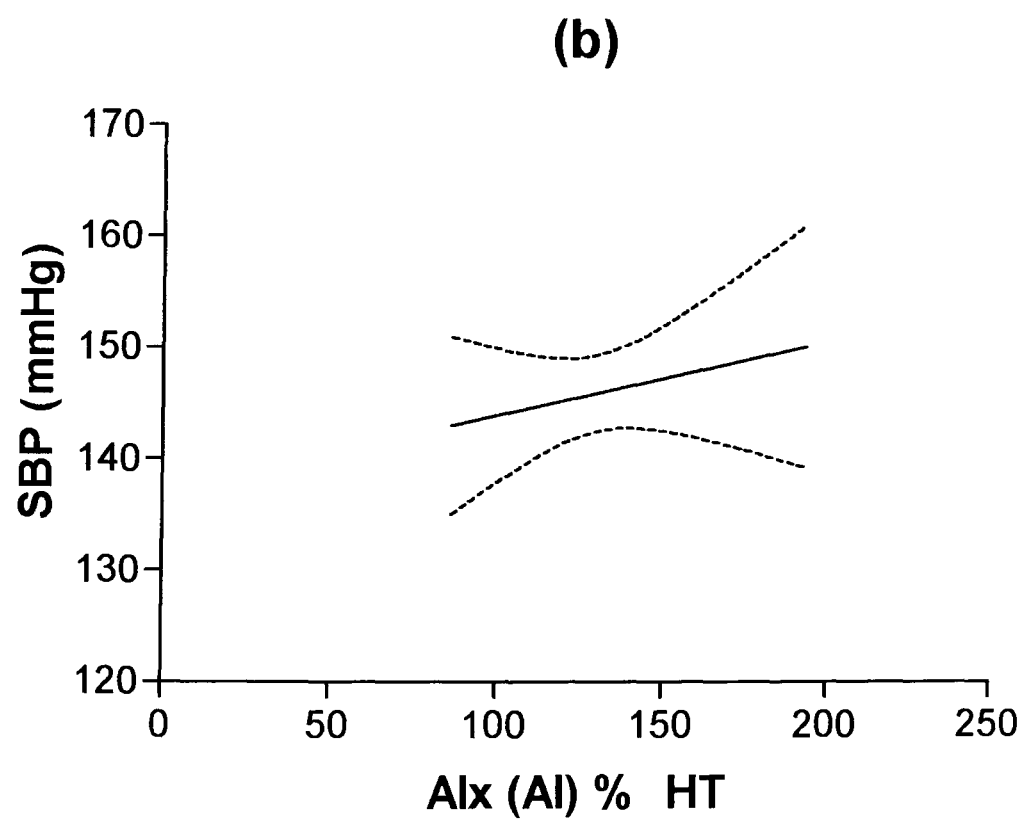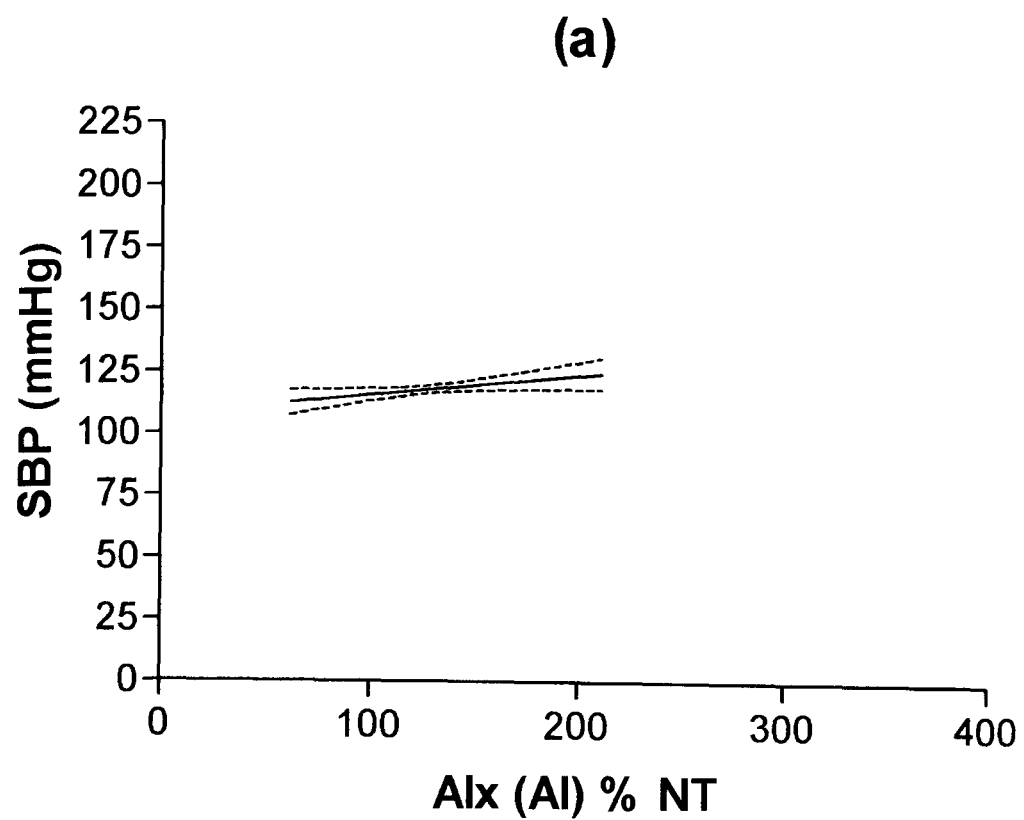
Figure 5.3 Linear regression of systolic blood pressure against augmentation in (a) normotensive and (b) hypertensive African Trinidadians.
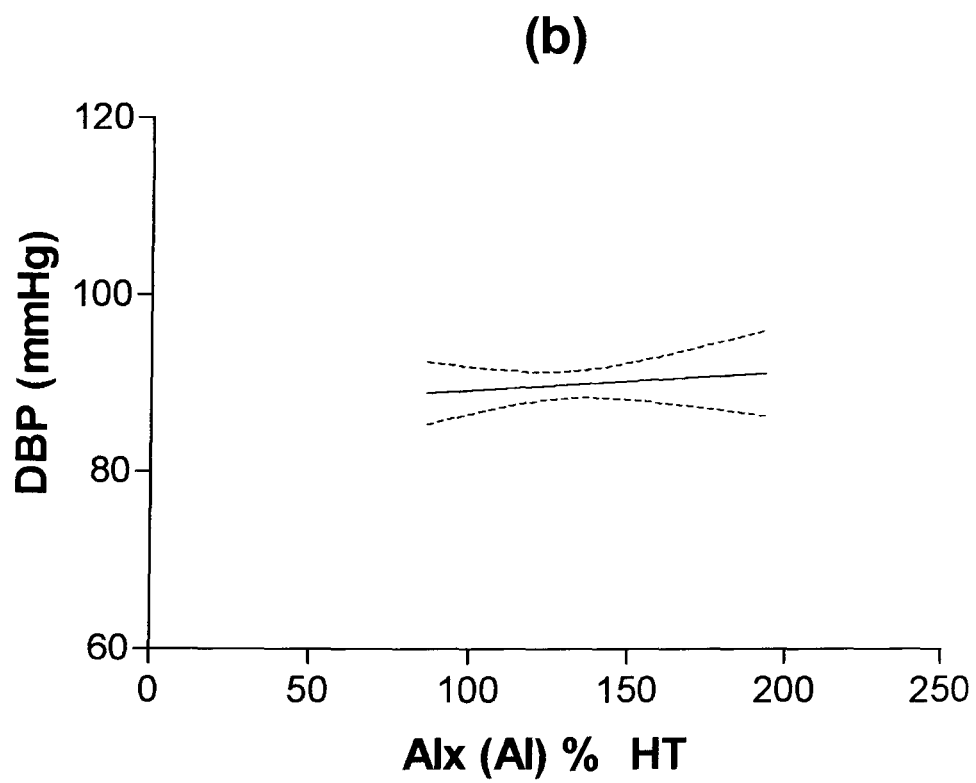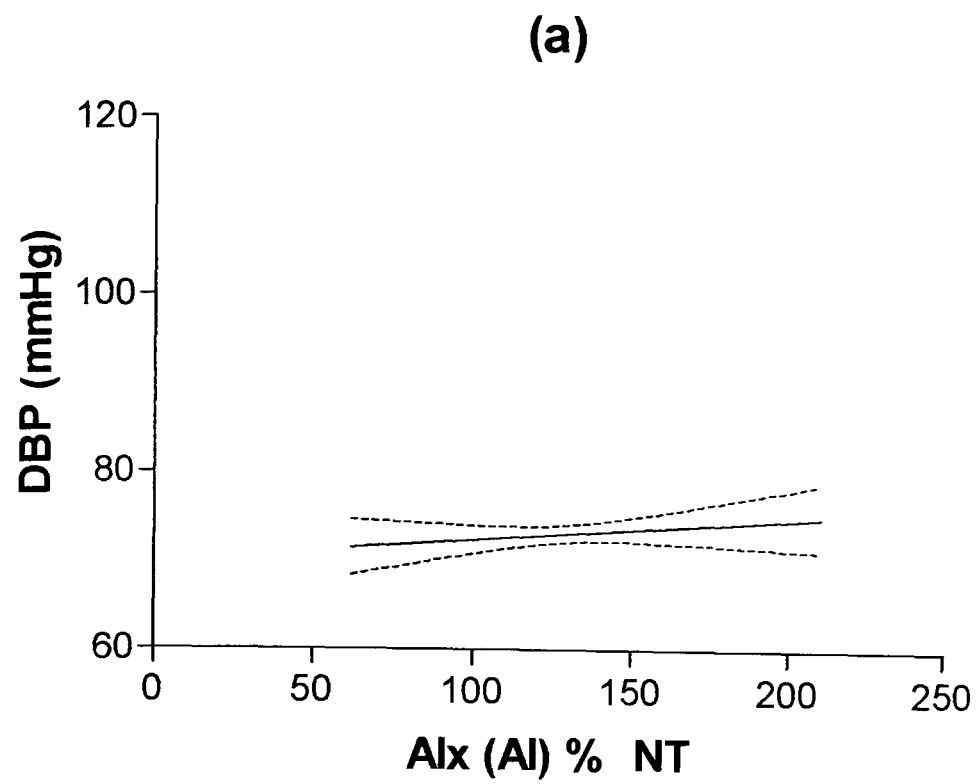
Figure 5.4 Linear regression of diastolic blood pressure against augmentation in (a) normotensive and (b) hypertensive African Trinidadians.
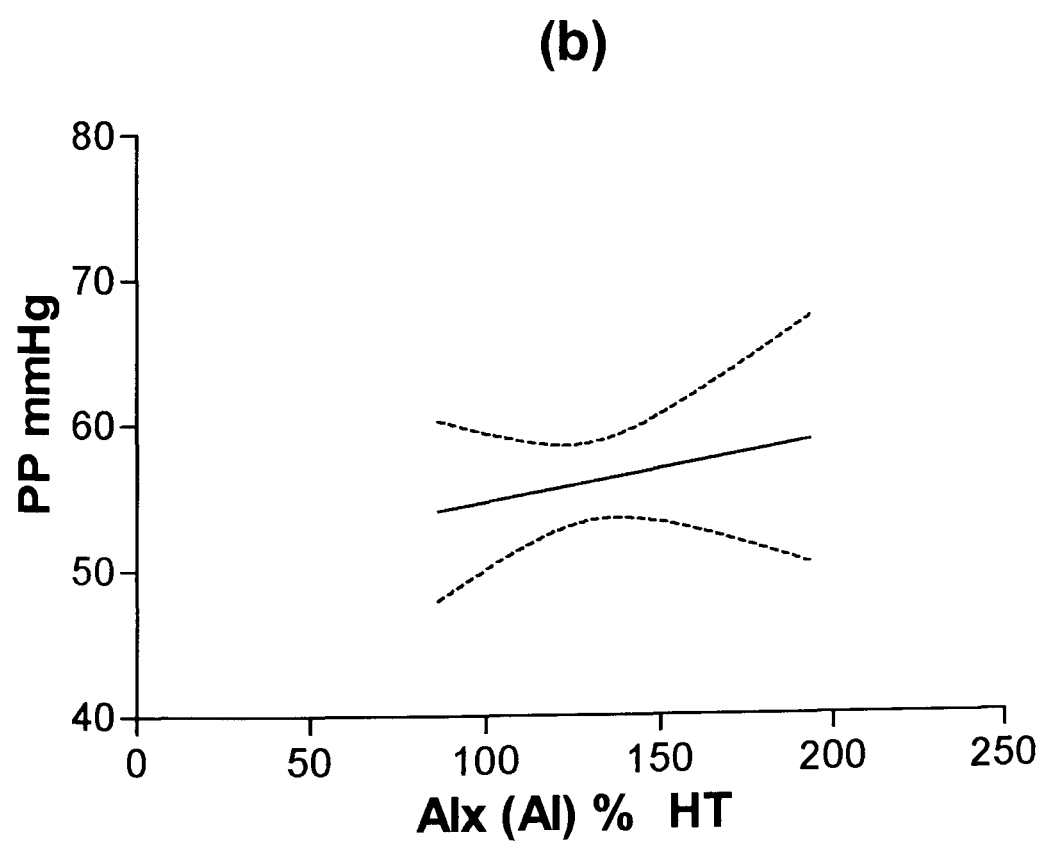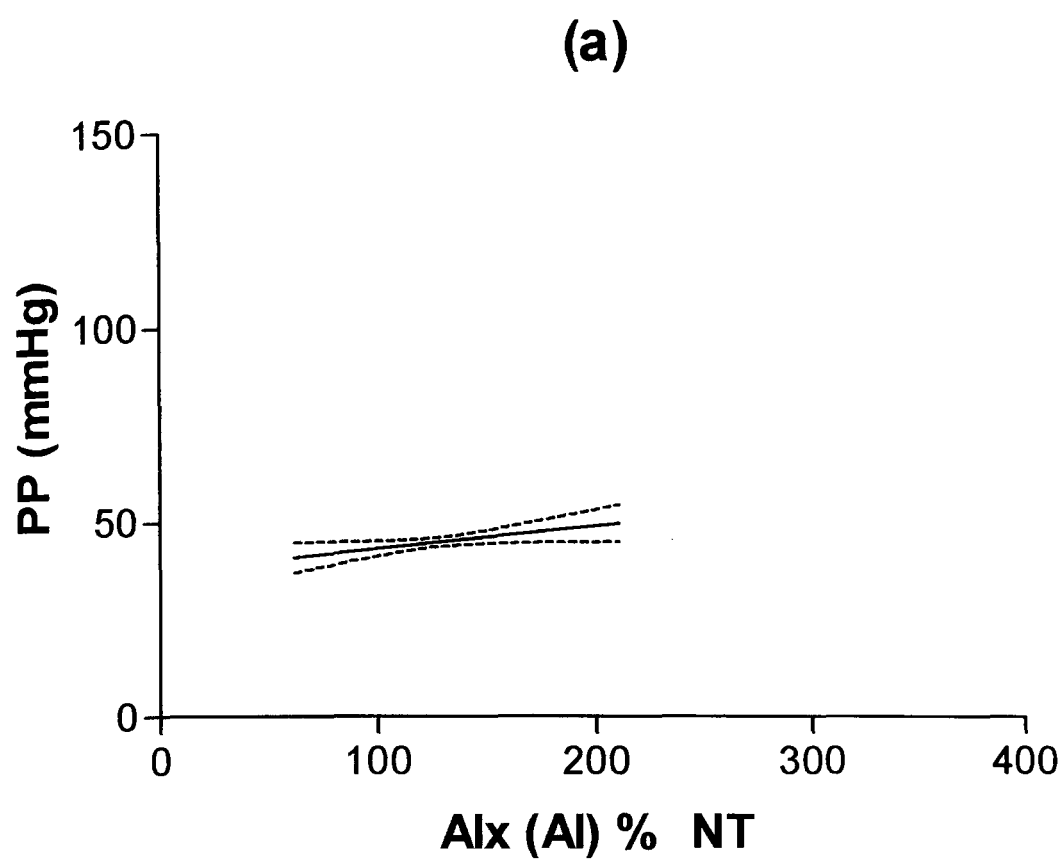
Figure 5.5 Linear regression of pulse pressure against augmentation in (a) normotensive and (b) hypertensive African Trinidadians.

171

(a)

(b)
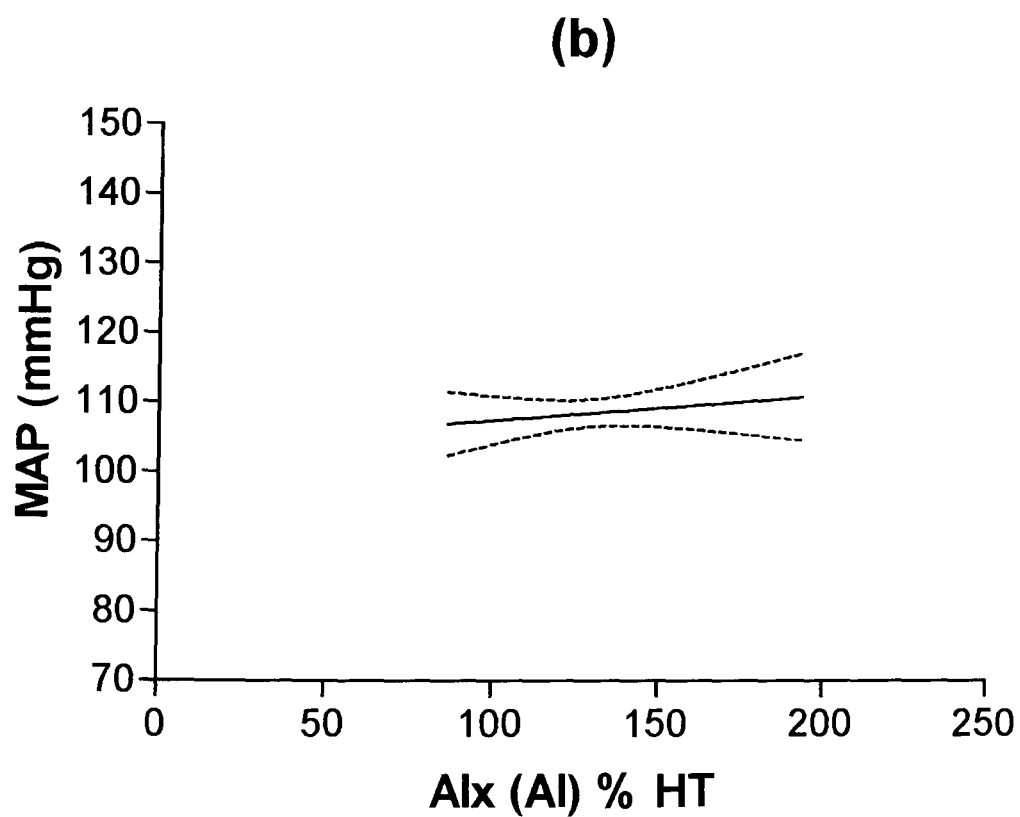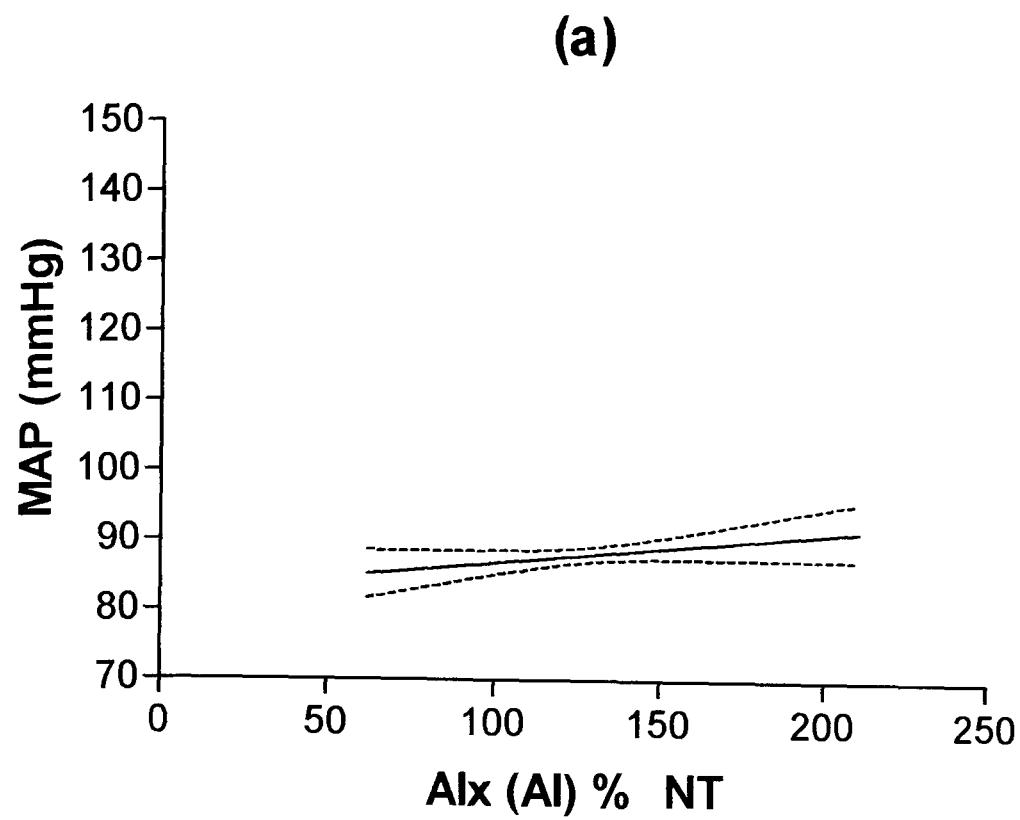
Figure 5.6 Linear regression of mean arterial pressure against augmentation in (a) normotensive and (b) hypertensive African Trinidadians.

(a)

(beats min^{-1})

100
90
80
70
60
50
40
30
20
10
0

0   50   100   150   200   250

Alx (AI) %   NT

(b)

(beats min^{-1})

100
90
80
70
60
50
40
30
20
10
0

0   50   100   150   200   250

Alx (AI) %   HT
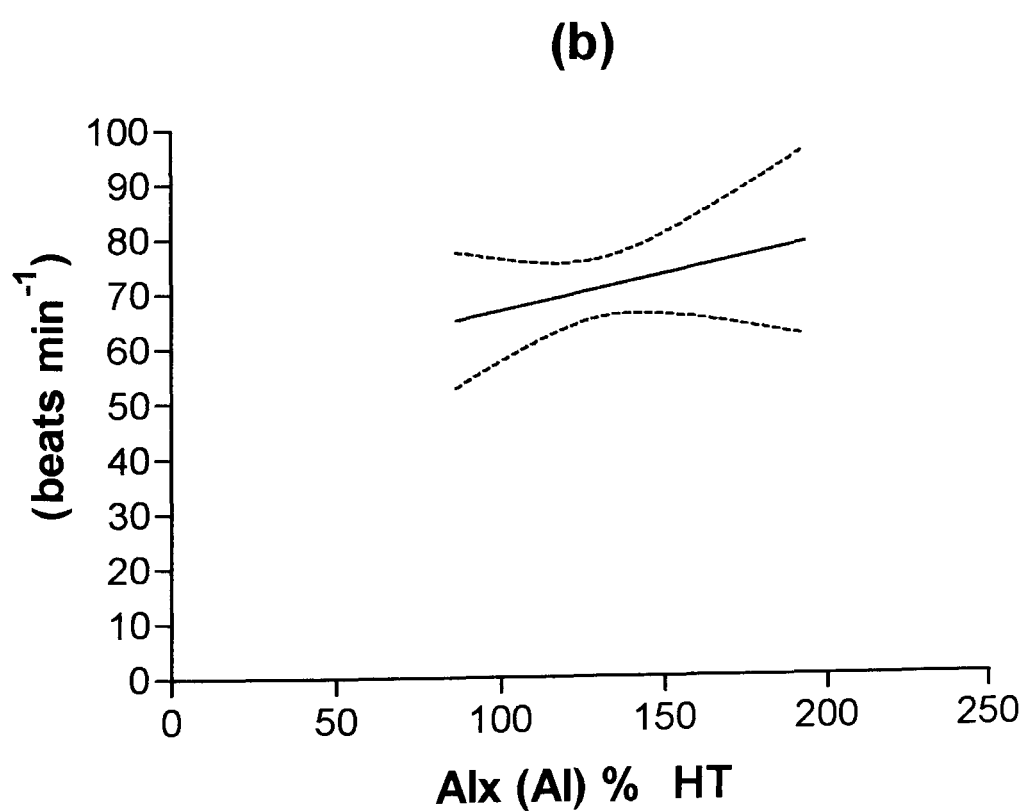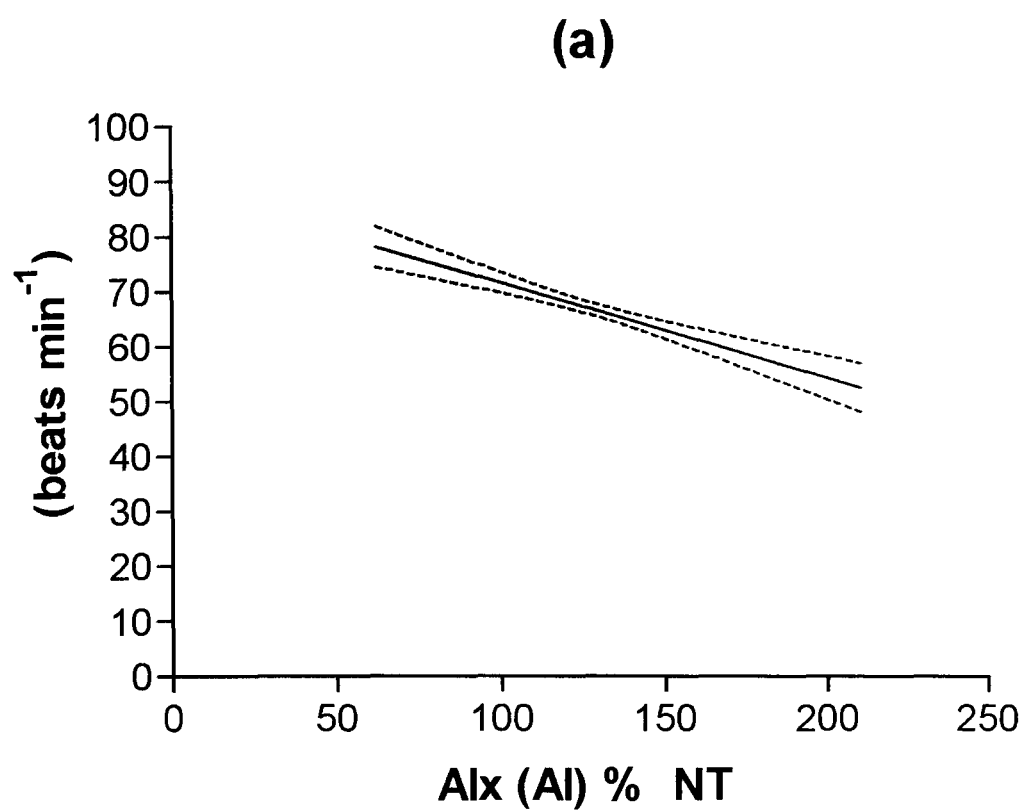
Figure 5.7 Linear regression of heart rate against augmentation in (a)normotensive and (b) hypertensive African Trinidadians.

5.2.8 Augmentation index and Total Cholesterol

In figure 5.8, the relationship of total cholesterol and AIx is shown. It can be seen that AIx increases with total cholesterol in both the NT and HT groups and therefore have a positive correlation.

5.2.9 Augmentation index and Triglycerides

In figure 5.9, the relationship of triglycerides and AIx is shown. It can bee seen that AIx increases with triglycerides in both the NT and HT groups and therefore have a positive correlation.

5.2.10 Augmentation index and High Density Lipoprotein (HDL)

In figure 5.10, the relationship of HDL and AIx is shown. The NT group demonstrates a positive correlation as AIx increases. In the HT group, a negative correlation is seen in HDL as AIx increases.

5.2.11 Augmentation index and Low Density Lipoprotein (LDL)

In figure 5.11, the relationship of LDL and AIx is shown. It can be seen that AIx increases with LDL in both the NT and HT groups and therefore have a positive correlation.

5.2.12 Augmentation index and Very Low Density Lipoprotein (VLDL)

In figure 5.12, the relationship of VLDL and AIx is shown. It can be seen that AIx increases with VLDL in both the NT and HT groups and therefore have a positive correlation.

5.2.13 Augmentation index and High Density Lipoprotein Ratio (HDL Ratio)

In figure 5.13, the relationship of HDL ratio and AIx is shown. It can be seen that AIx increases with HDL ratio in both the NT and HT groups and therefore have a positive correlation.

5.2.14 Augmentation index and Glucose

In figure 5.14, the relationship of glucose and AIx is shown. It can be seen that AIx increases with glucose in the HT and NT group and therefore has a positive correlation.
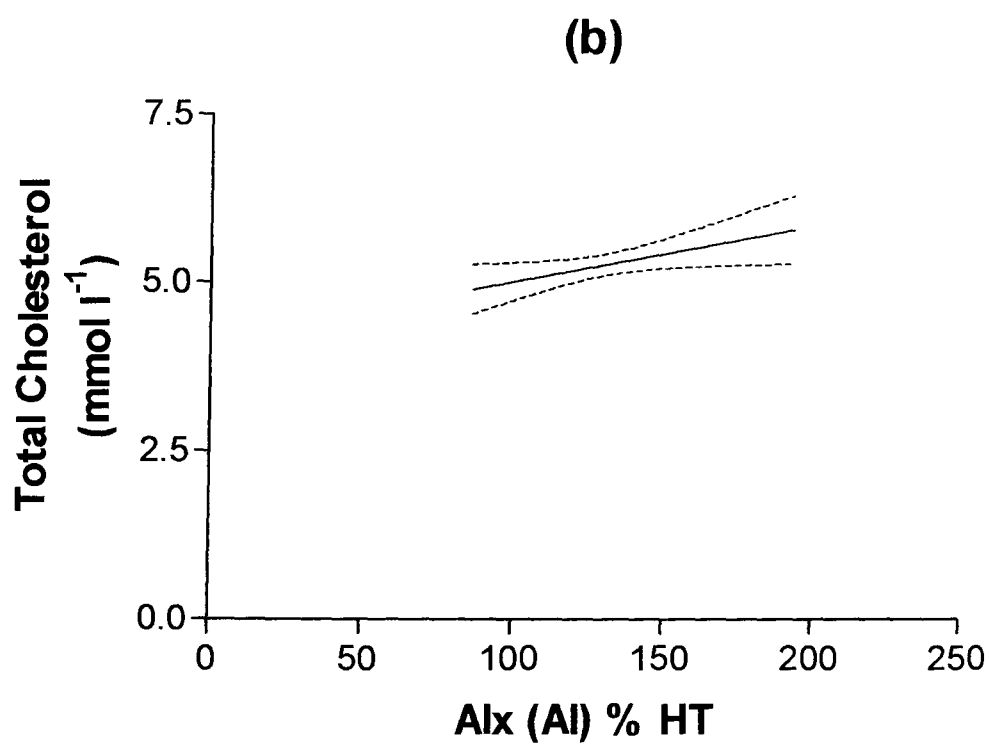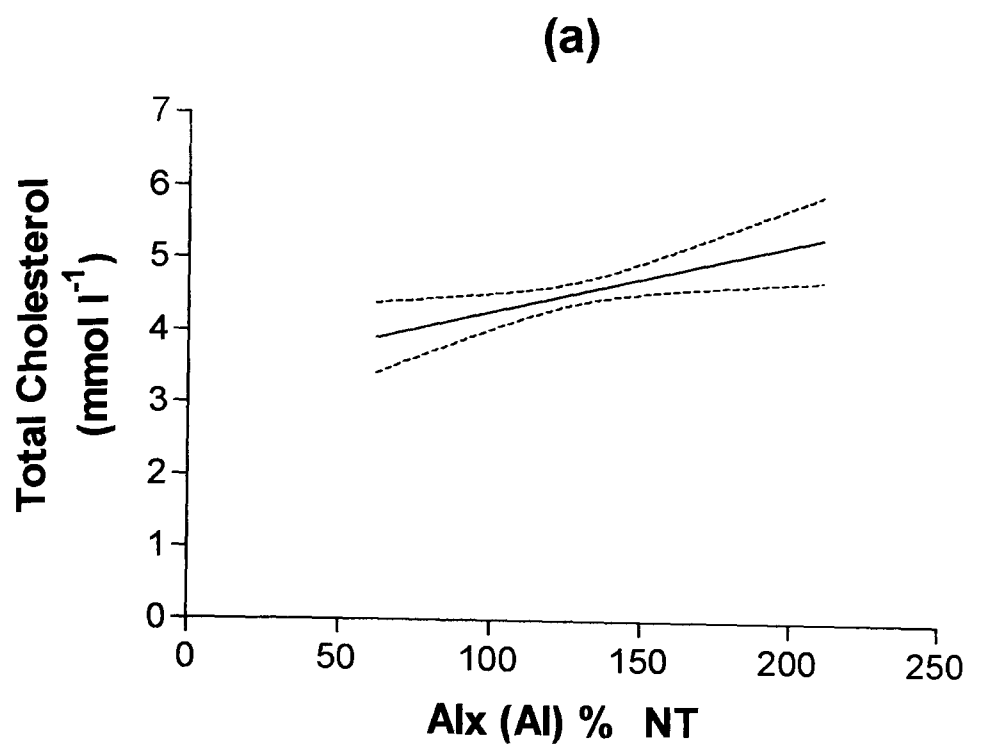
Figure 5.8 Linear regression of total cholesterol against augmentation in (a)normotensive and (b) hypertensive African Trinidadians.
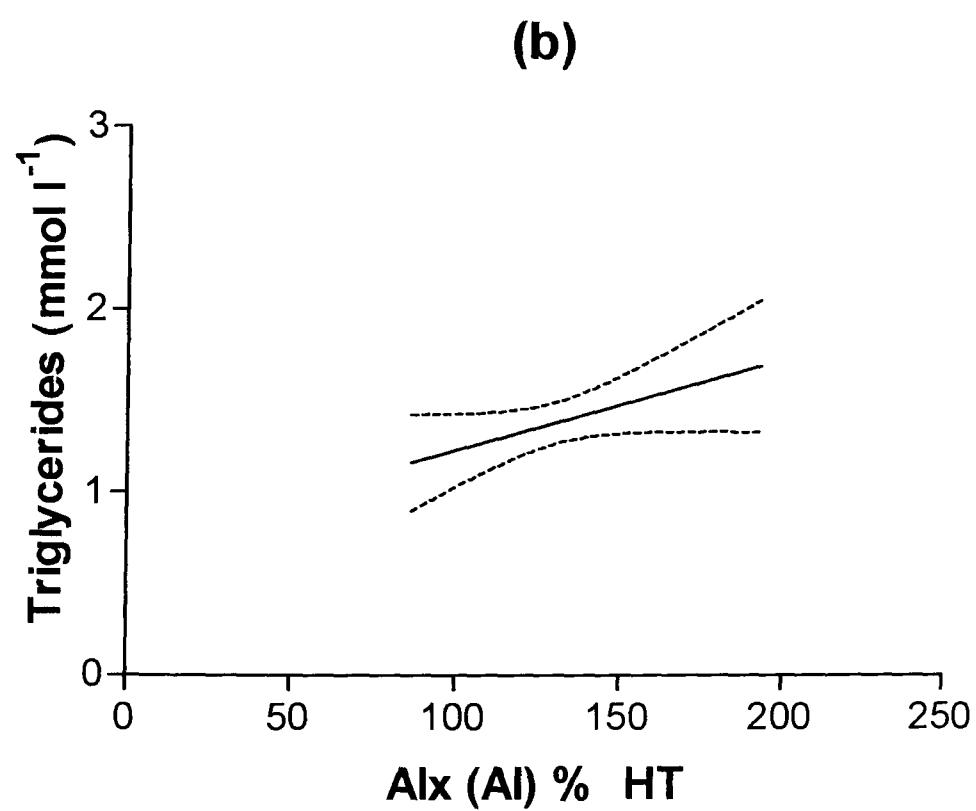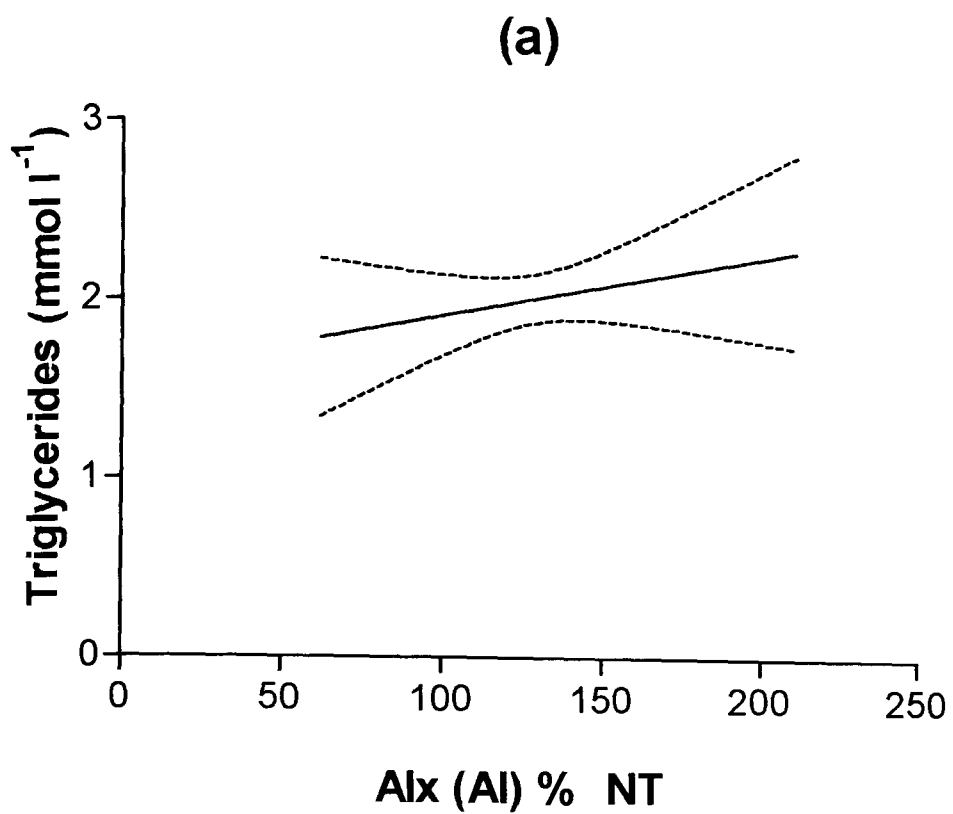
Figure 5.9 Linear regression of triglycerides against augmentation in (a)normotensive and (b) hypertensive African Trinidadians.

(a)

(b)
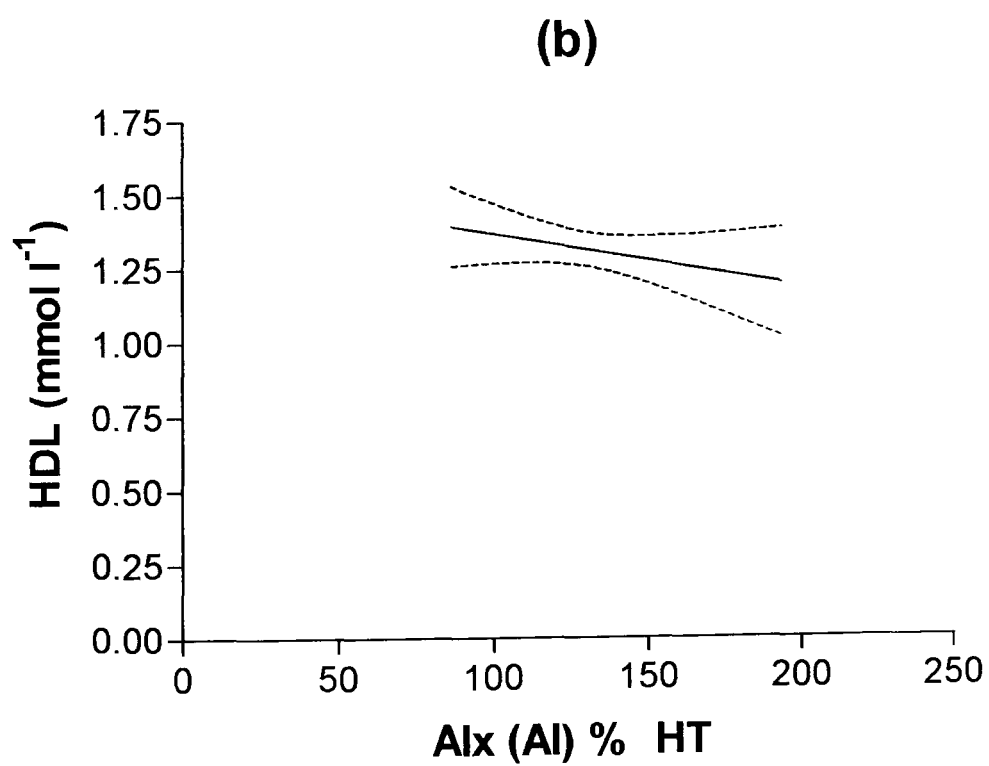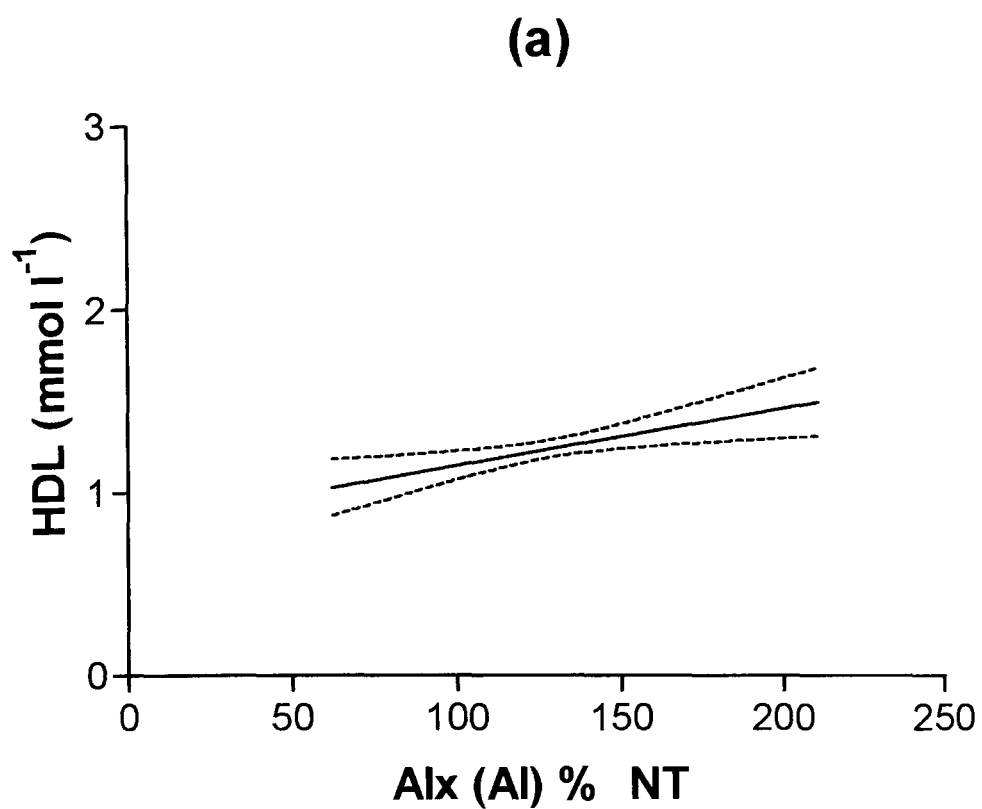
Figure 5.10 Linear regression of HDL against augmentation in (a)normotensive and (b) hypertensive African Trinidadians.
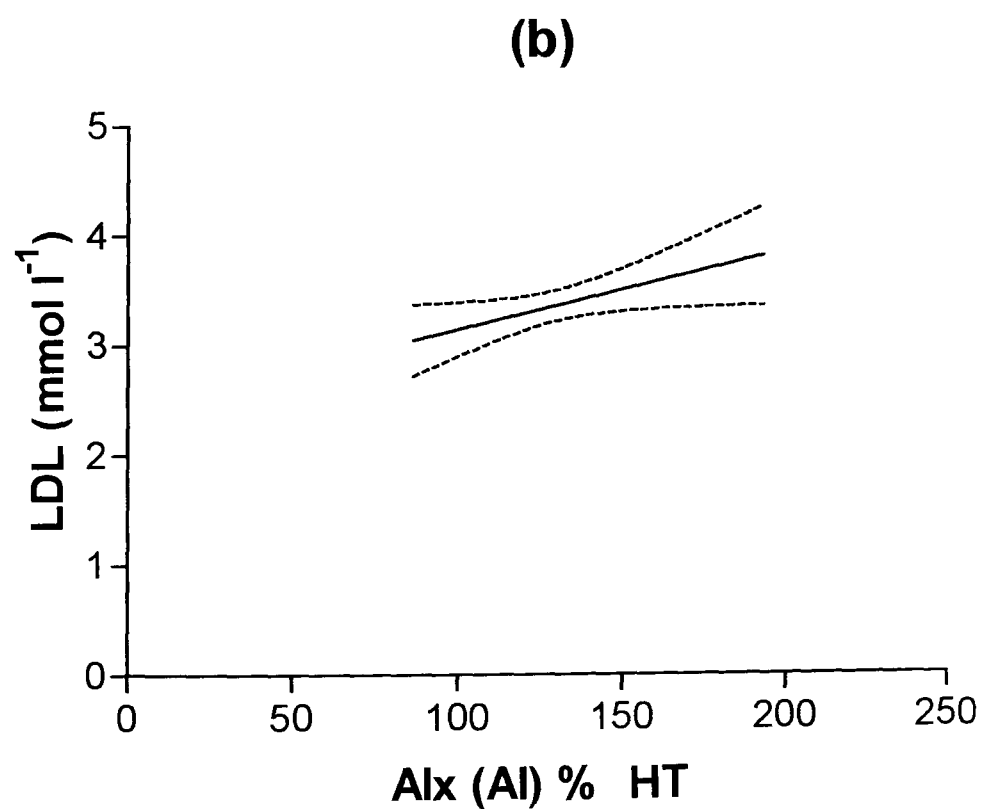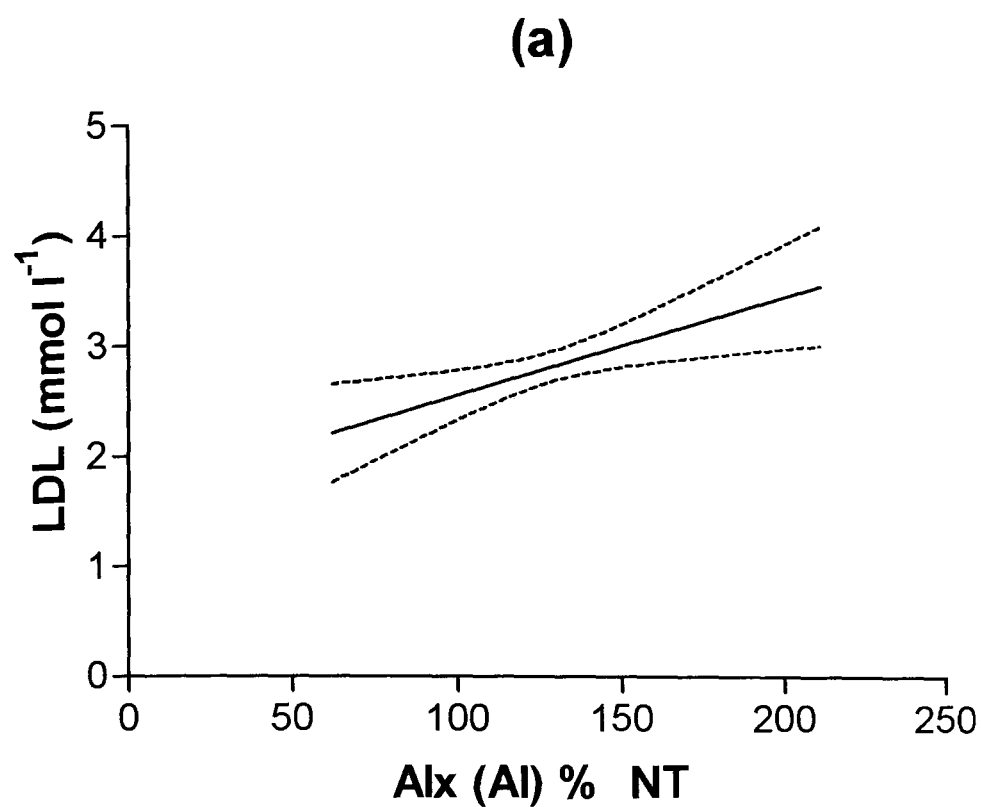
Figure 5.11 Linear regression of LDL against augmentation in (a)normotensive and (b) hypertensive African Trinidadians.

(a)

(b)
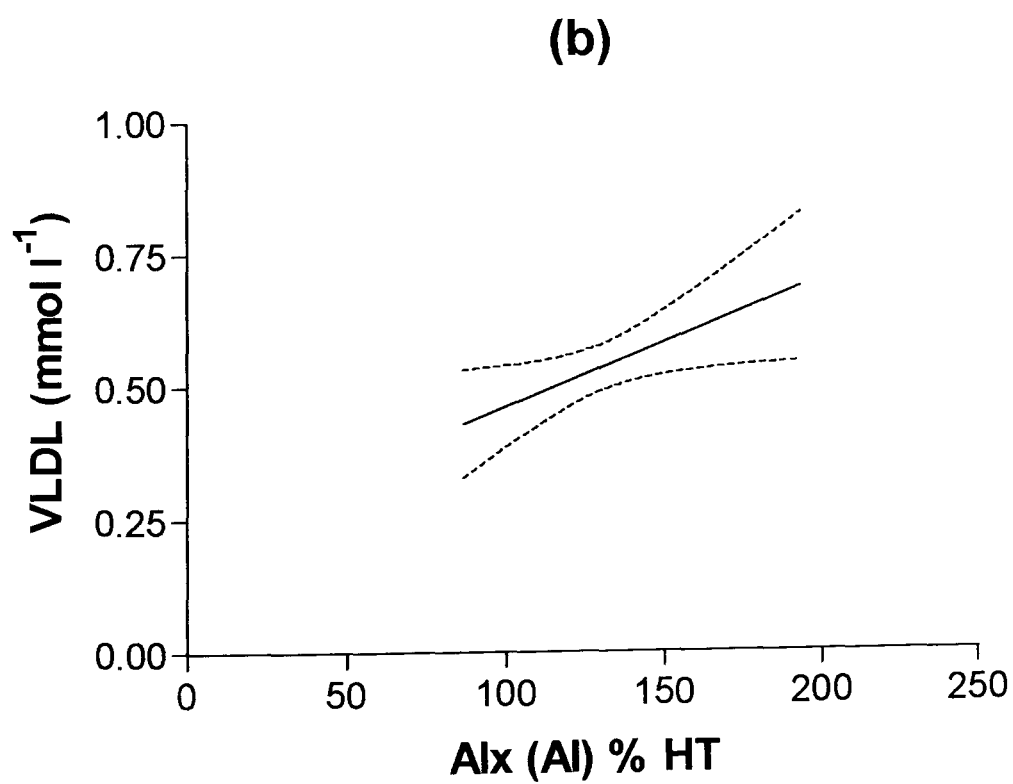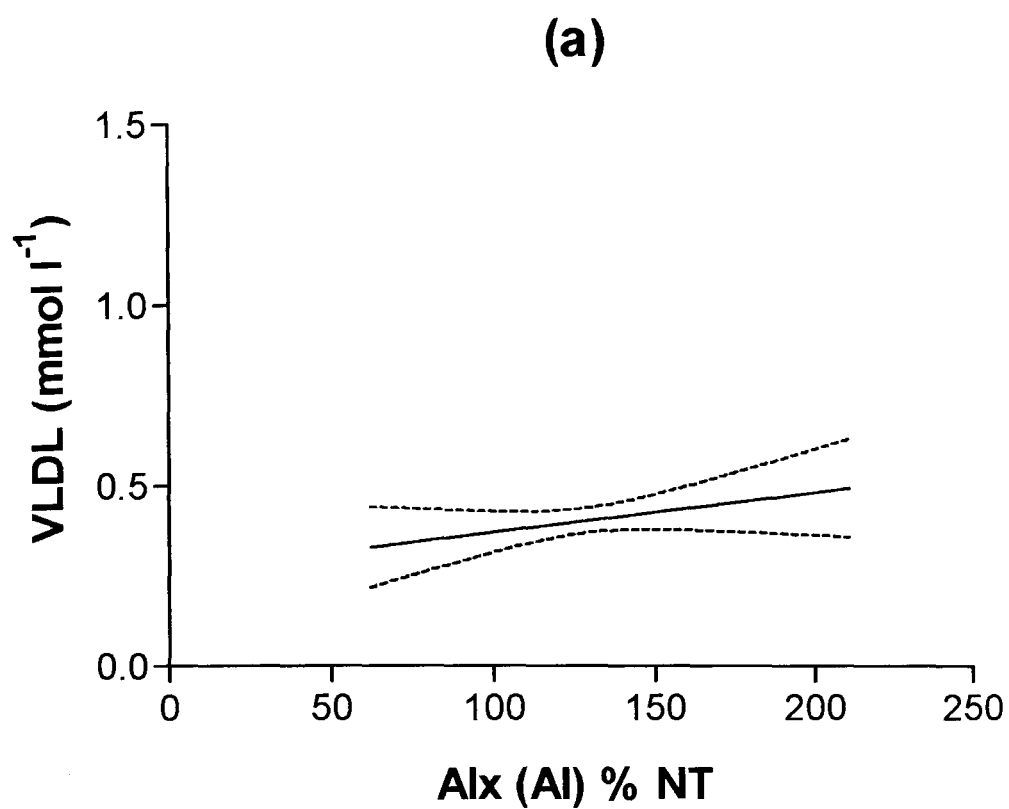
Figure 5.12 Linear regression of VLDL against augmentation in (a)normotensive and (b) hypertensive African Trinidadians.
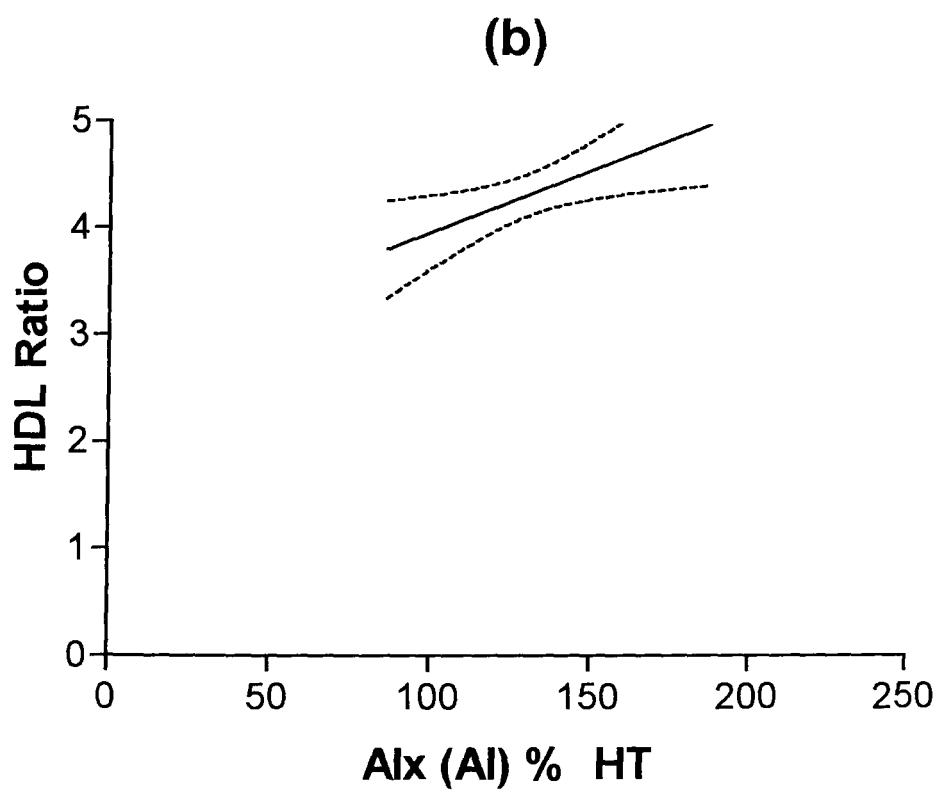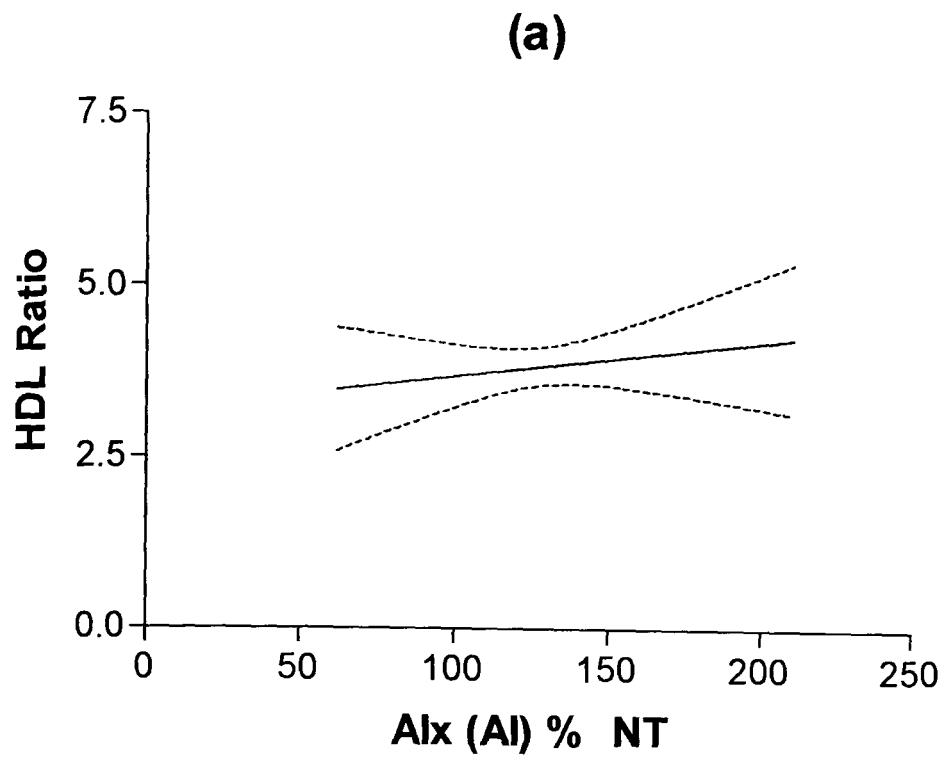
Figure 5.13 Linear regression of HDL Ratio against augmentation in (a)normotensive and (b) hypertensive African Trinidadians.
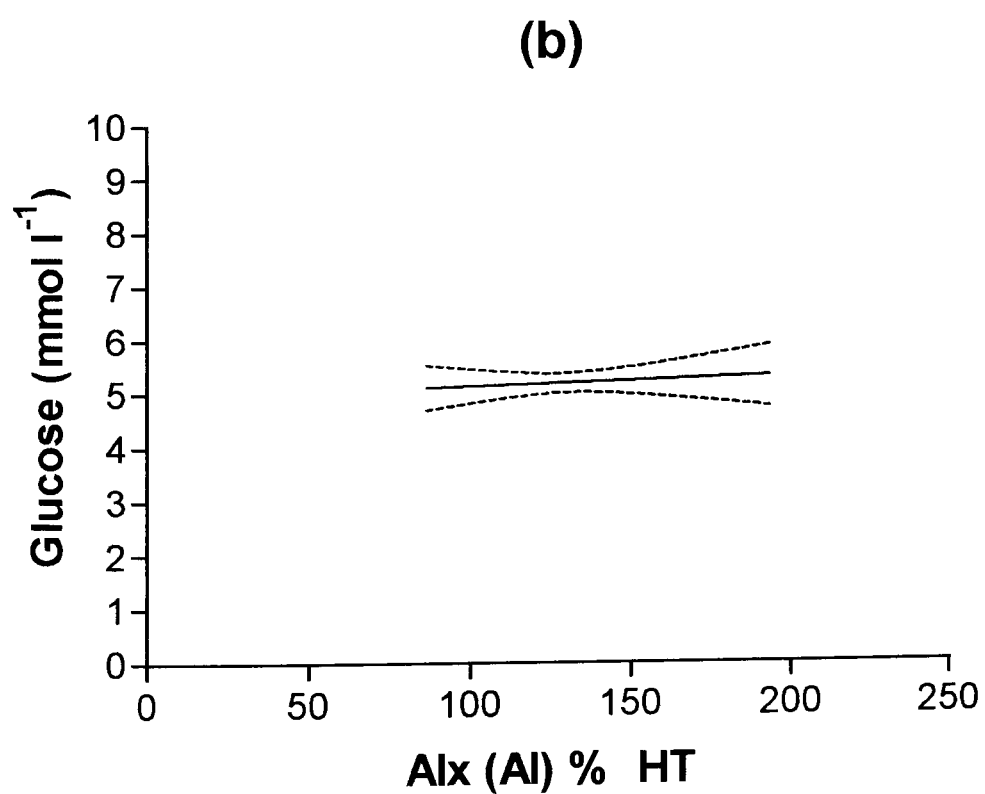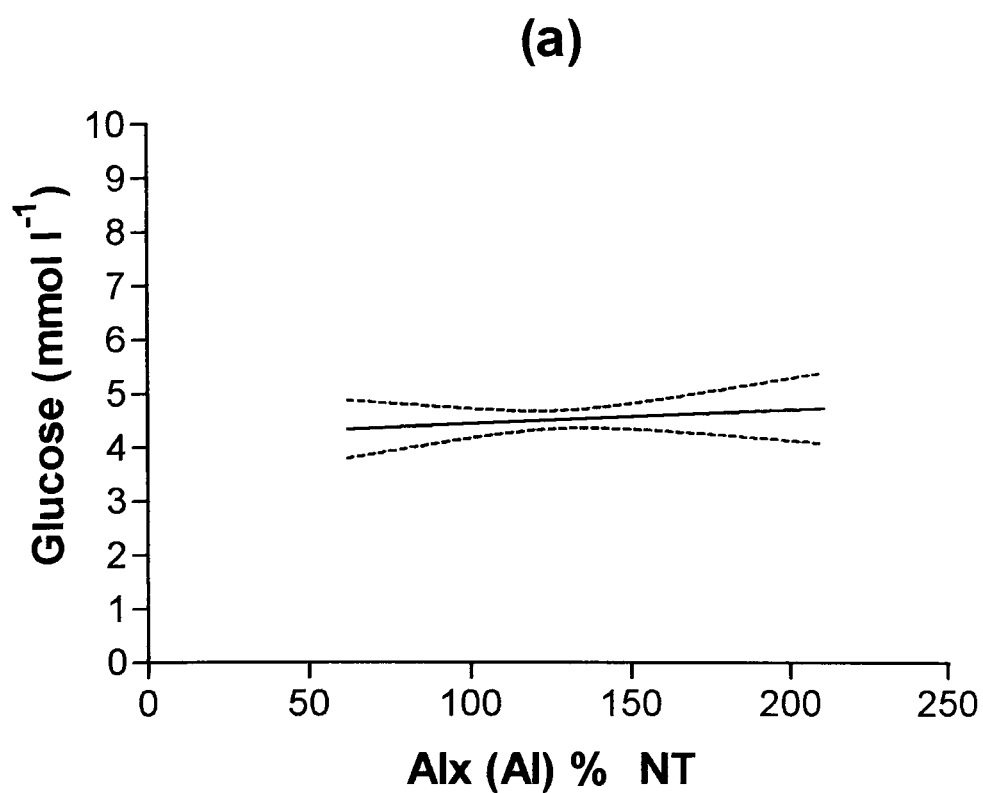
Figure 5.14 Linear regression of Glucose against augmentation in (a)normotensive and (b) hypertensive African Trinidadians.
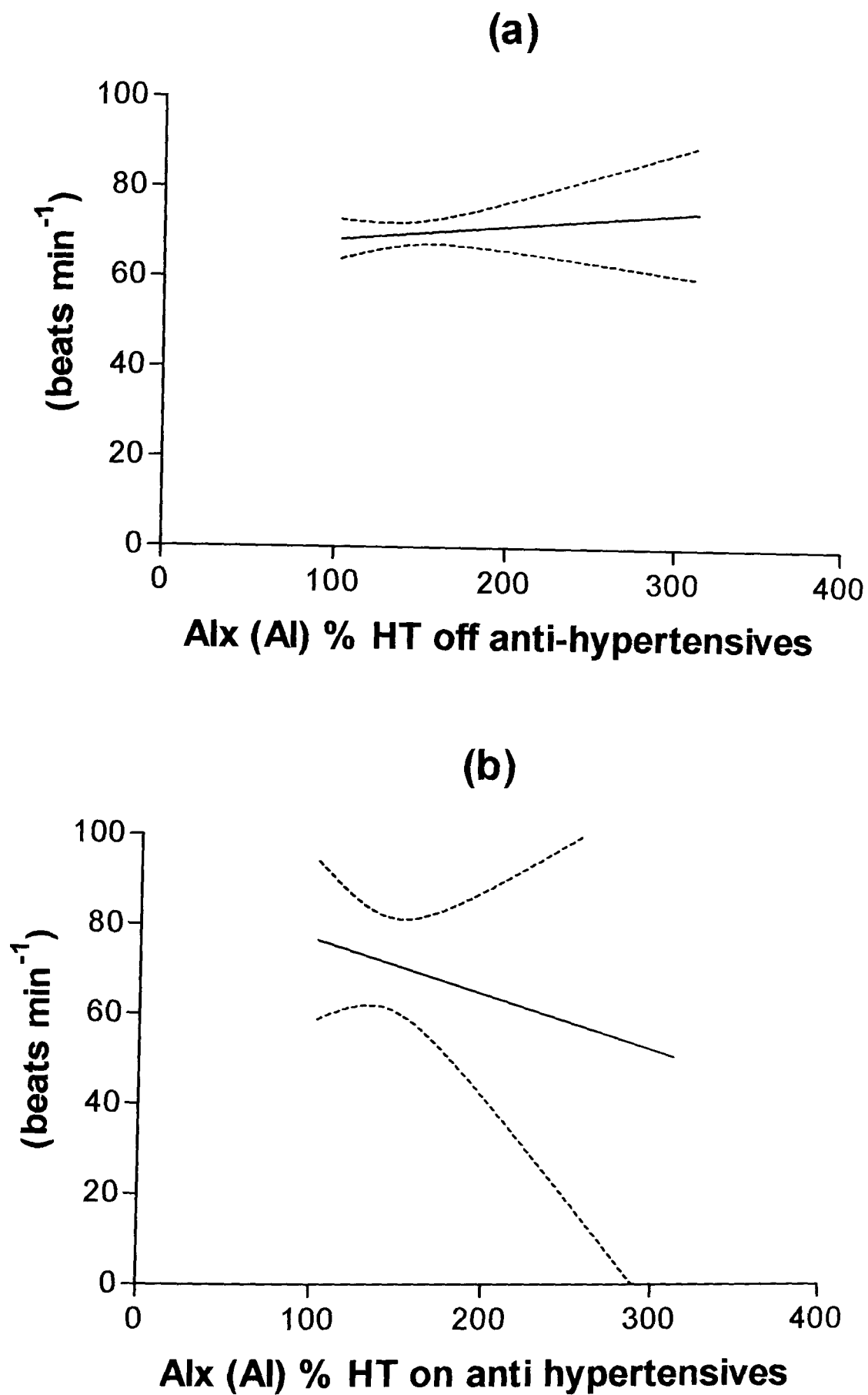
Figure 5.15 Linear regression of heart rate against augmentation in (a)hypertensives off anti hypertensive medication and (b) hypertensives on anti hypertensive medication in African Trinidadians.

5.2.15 Summary of data values

The following tables are summary data tables of descriptive and correlation statistics of regression analysis of Augmentation index against all variables except genotype. Pearsons' correlation demonstrates the extent or incline of the positive or negative linear slope in the figures as previously described in this chapter.

Table 5.1 Normotensives descriptive and correlation statistics data values.

Parameter	Mean	Standard Deviation	Pearson's Correlation	P value
Age	43.41	11.55	0.489	< 0.001
BMI	26.59	4.74	0.078	< 0.001
SBP	118.2	12.9	0.143	< 0.001
DBP	73.15	7.88	0.067	< 0.001
PP	45.05	9.81	0.134	< 0.001
MAP	88.17	8.68	0.111	< 0.001
HR	67.02	9.90	-0.367	< 0.001
Total Cholesterol	4.56	1.24	0.172	< 0.001
Triglycerides	2.01	1.10	0.067	< 0.001
HDL	1.24	0.39	0.179	< 0.001
LDL	2.82	1.13	0.176	< 0.001
VLDL	0.41	0.28	0.087	< 0.001
HDL Ratio	3.83	2.54	0.051	< 0.001
Glucose	4.55	1.32	0.047	< 0.001

Table 5.2 Hypertensives descriptive and correlation statistics data values.

Parameter	Mean	Standard Deviation	Pearson's Correlation	P value
Age	53.14	10.47	0.138	< 0.001
BMI	29.2	5.5	0.024	< 0.001
SBP	146.0	21.96	0.065	< 0.001
DBP	89.95	9.69	0.049	< 0.001
PP	56.0	17.0	0.056	< 0.001
MAP	108.6	12.6	0.063	< 0.001
HR	71.0	34.2	0.082	< 0.001
Total Cholesterol	5.29	1.03	0.174	< 0.001
Triglycerides	1.38	0.73	0.144	< 0.001
HDL	1.31	0.37	-0.099	< 0.001
LDL	3.32	0.97	0.169	< 0.001
VLDL	0.52	0.29	0.181	< 0.001
HDL Ratio	4.32	1.28	0.195	< 0.001
Glucose	4.98	1.55	0.039	< 0.001

5.3 Discussion

5.3.1 Introduction

Pulse Wave Analysis (PWA) is a simple and reproducible technique in which to measure arterial stiffness, and is suitable for large-scale population and intervention studies investigating clinical relevance of vascular stiffness (Wilkinson et al, 1998b). It allows the accurate measurement of peripheral waveforms and generation of the corresponding waveform, from which augmentation index and central pressure is derived. In clinical studies (Wilkinson et al, 1998b) PWA has shown to be a highly reproducible technique and easy to apply. It provides important information about arterial stiffness, which is under the functional control of the endothelium, releasing a number of vasodilatory mediators. Increased stiffness impairs arterial function and is associated with most cardiovascular risk factors and established athersclerosis.

To date there are no studies of the use of PWA to examine augmentation of arterial compliance in African Trinidadians or African Caribbean's. However, it has been documented that PWA is a suitable methodology to be used for large-scale population and intervention studies, investigating the relevance of vascular stiffness (Wilkinson et al, 1998).

Aortic PWA is strongly related to the presence and extent of CHD, and has been shown to be a strong predictor of cardiovascular risk as determined by Framingham study (Kannel et al, 1967) and the National Service Framework (NSF) for CHD.

An important result of this study is that African Trinidadians may have an accelerated risk of CHD, over the age of forty-five, with increased arterial stiffness, associated with raised lipid levels (Cross et al, 2003). The results are of interest as most previous

studies relating PWV to cholesterol or dyslipidemia have found minimal or inconsistent correlations (Barenbrock et al, 1995).

As described in other studies (Cameron et al, 1998), raised augmentation in univariate analysis shows association with age, sex, height and heart rate. Results from this study with age and heart rate were as similar to Cameron et al, 1998. Even after adjustment with heart rate, pressure augmentation was not associated with any antihypertensive treatment investigated. This means that age and heart rate but not the classification of antihypertensive medication may affect the degree of pressure augmentation.

Similarly, the elevation of pulse pressure (PP) found mainly in hypertensive African Trinidadians, is regarded as a manifestation of increased arterial stiffness and augmentation. This combined with raised systolic blood pressure (SBP) are considered independent risk factors of CHD. These findings were also seen in studies conducted by Boutouyrie et al, 2001 and Benetos et al, 1997, who conducted large longitudinal studies in French populations.

As approximately one quarter of the participants had already been treated for hypertension at baseline, the predictive value of PWA observed in the entire population of African Trinidadians may not be a time estimate. However, the predictive value of PWA as represented in tables 5.1, 5.2 and 5.3 suggests it may operate as a significant and independent association for an increased risk of developing hypertension.

5.4 Summary

In conclusion, the cohort of normotensive and hypertensive subjects whether untreated or treated demonstrates the following points.

- ❖ The collected phenotypic variables do have an impact on augmentation index.

- ❖ Heart rate has a significant effect on augmentation index

- ❖ HDL is shown to have a protective measure against an increase in augmentation index, in particular those who have hypertension.

Chapter 6

Multiple Regression

6.1 Introduction

The data shown in Chapter 5 demonstrated linear regression analysing the relationship between two variables. The figures and results determined the relationship between augmentation (AIx %) and the phenotypic variables, singularly.

In this chapter multiple regression is employed as a statistical procedure to analyse the simultaneous effects of two or more independent variables on the dependent variable of augmentation (AIx %). This is accomplished by developing a model to define a response or function between the dependent variable and fitting data to a linear equation with two or more independent variables.

Therefore the data described in this chapter make use of multiple regression analysis approaches to examine this issue.

Statistical Package for the Social Sciences (SPSS) was used to combine multiple linear regression and analyse bio statistics.

6.2 Analysis

The process of constructing a linear equation that predicts the values of the dependent variable, (augmentation index) from knowledge of the specified values of an independent variable is extended in this chapter to situations where the data has two or more independent variables. Multiple regression is the construction of a linear regression equation with two or more independent variables.

In this case simultaneous multiple regression was used whereby all the independent variables, which are known predictors of hypertension, as described in chapters 3, 4 and 5 were entered directly.

6.3 Results

6.3.1 Total Population Regression Model

Firstly, simple scatterplots were developed to eliminate outliers in data sets and deselecting independent variables that showed no significant contribution to the model. The variables that showed to be significant contributing factors to AIx (AI), appeared to be correlated with a scatterplot. These were systolic blood pressure, heart rate, height, age and gender.

Table 6.2 shows the regression ANOVA, which tested goodness of fit between the variables. F is the test statistic, being the ratio of the residual mean square and the residual mean squared. This means that the predictors or variables have a confirmed relationship with the dependent variable of central AIx.

However, the examination of the scatterplots of the variables against AIx can confirm a genuine linear relationship. Figures 6.1, 6.2, 6.3 and 6.4 are scatterplots showing the correlations of the independent variables used in the model with AIx.

Table 6.3 shows coefficients and is the representation of the regression analysis, regression equation and associated statistics. Column B indicates the relative weight of the independent variable in the regression equation. The remaining columns are t-tests (analysing the difference between two means) with p values.
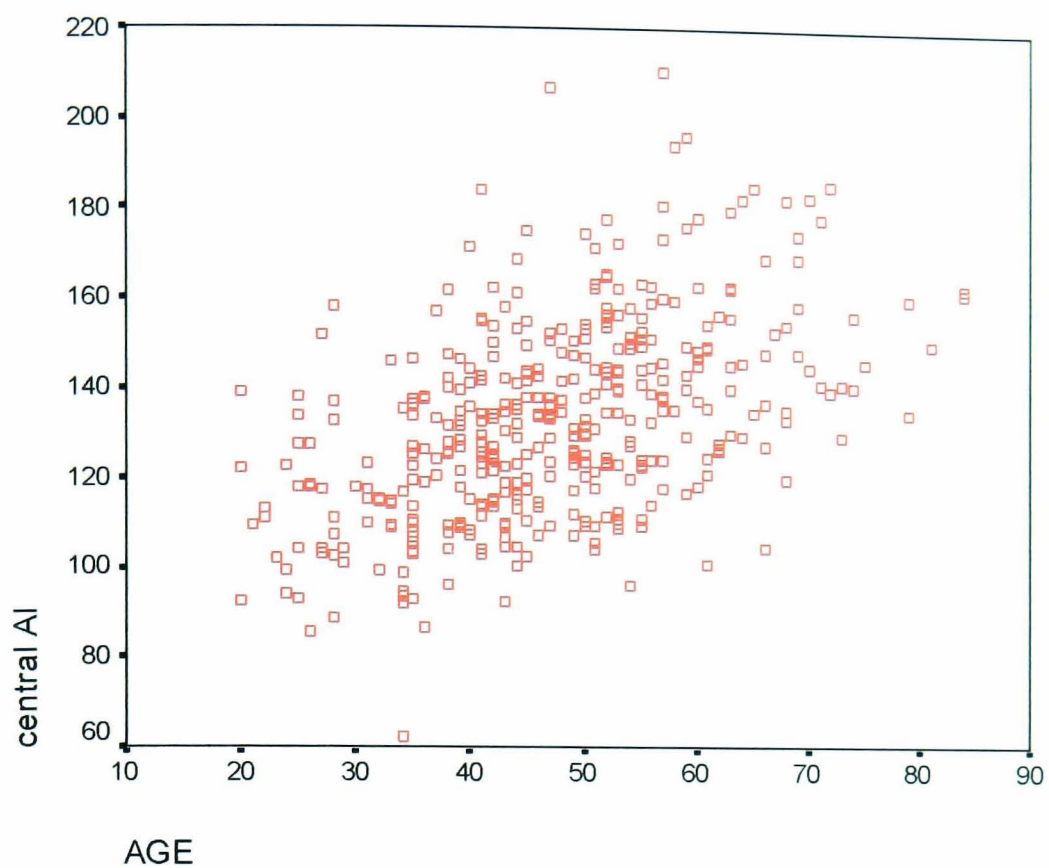
Figure 6.1 Scatterplot of correlation between age and AIx (AI).
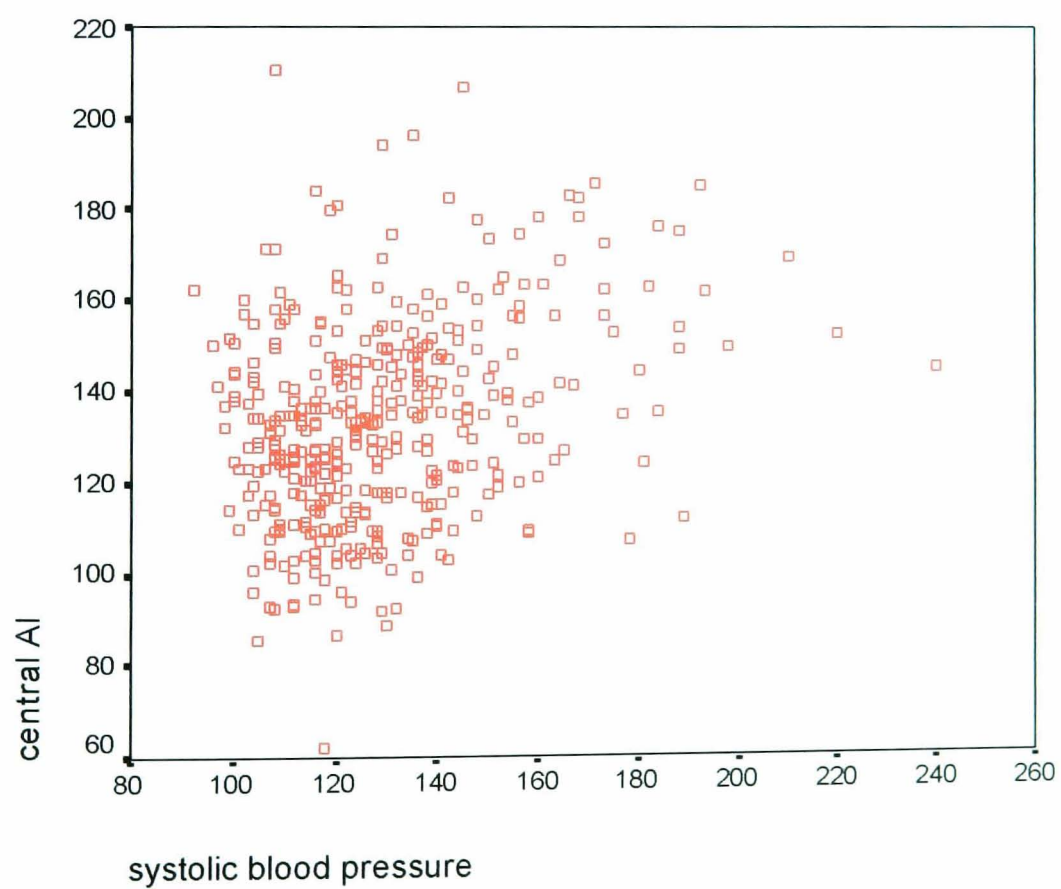
Figure 6.2 Scatterplot of correlation between systolic blood pressure and AIx (AI).
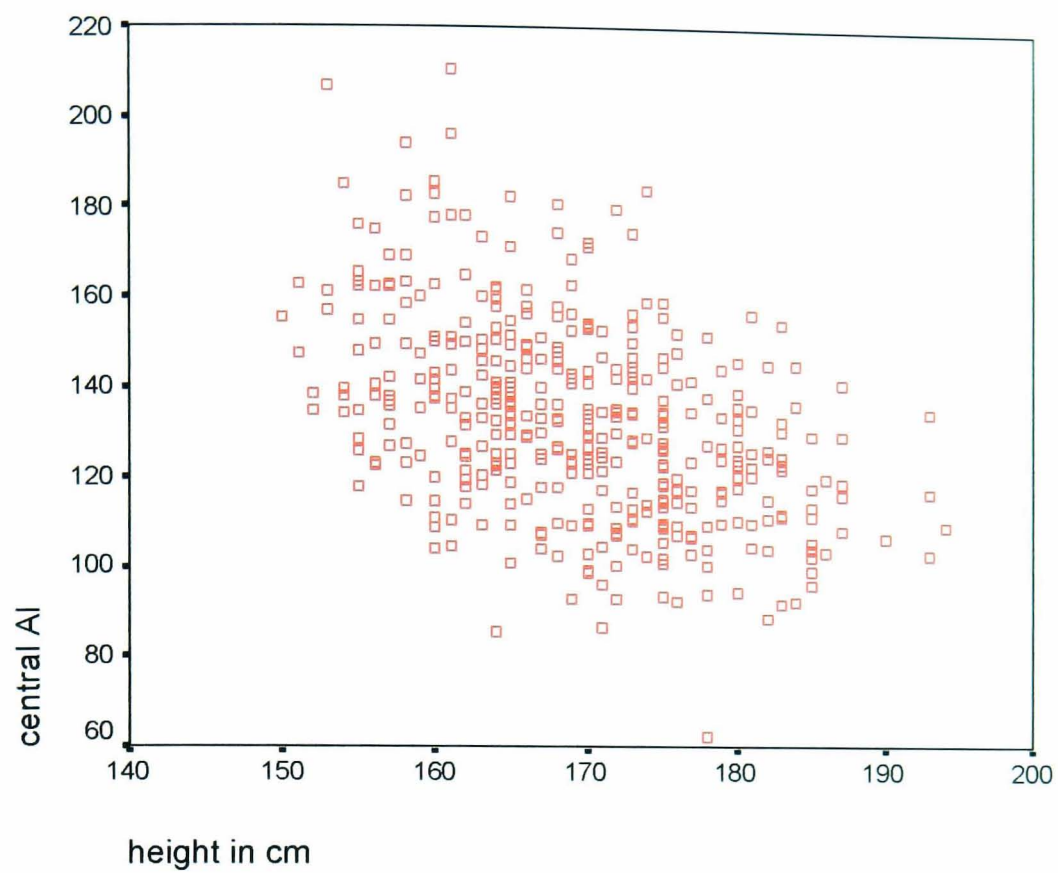
Figure 6.3 Scatterplot of correlation between height in cms and AIx (AI).
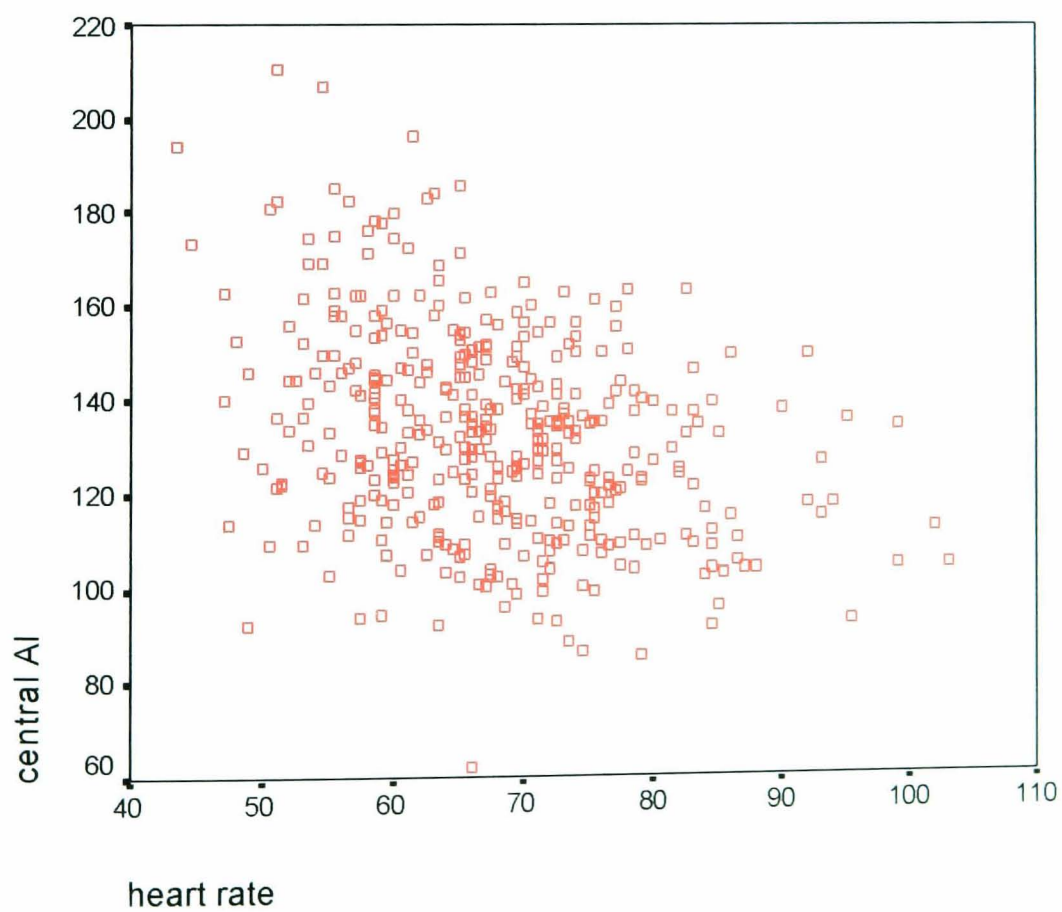
Figure 6.4 Scatterplot of correlation between heart rate and AIx (AI).

Table 6.1 R coefficient approach, indicating a good measure of fit.

Model	R	R Square	Adjusted R Std. Square	Error of the Estimate
1	.752[a]	.565	.560	14.65368

a Predictors: (Constant) systolic blood pressure, heart rate, height in cms, age, gender.

Table 6.2 ANOVA[b] for confirming significance association of regression line with AIx (AI).

Model 1	Sum of Squares	df	Mean Square	F	Sig
Regression	109504.535	5	21900.907	101.993	.000
Residual	84174.300	392	214.730		
Total	193678.835	397			

a Predictors: (Constant) systolic blood pressure, heart rate, height in cms, age, gender.

b Dependent Variable: central AIx (AI).

Table 6.3 Contribution of each predictor to the model.

Model 1		Unstandardized Coefficient B	Std. Error	Standardized Beta	t	Sig
	(Constant)	234.435	22.554		10.394	.000
	Gender	11.964	1.969	.266	6.076	.000
	Age	.635	.072	.350	8.822	.000
	Height in cms	-.641	.110	-.258	-5.837	.000
	Heart rate	-.887	.074	-.412	-12.054	.000
	Systolic blood pressure	.139	.039	.137	3.518	.000

a Dependent Variable: central AIx (AI).

6.3.2 Linear Regression Analysis for Arg→Gly16 and Gln→Glu 27 genotypes groups using AIx (AI) Model

Using the model for AIx (AI) demonstrated previously (Tables 6.1 and 6.2) I sought to establish whether genotype significantly improved the model. That is to say, whether possession of the polymorphism Arg→Gly16 or Gln→Glu 27 was significantly associated with AIx (AI) having taken the other model variables into account. This was performed using the general linear model function in SPSS.

Tables 6.4 and 6.5 are the number of individuals in each gender and genotype combined.

Table 6.4 Number of individuals for Arg→Gly16.

		Value Label	N
Gender	1.00	male	161
	2.00	Female	237
Arg→Gly16	1	Homozygote Arg	88
	2	Heterozygote	216
	3	Homozygote Gly	94

Table 6.5 Number of individuals for Gln→Glu 27.

		Value Label	N
Gender	1.00	male	161
	2.00	Female	237
Gln→Glu 27	1	Homozygote Gln	257
	2	Heterozygote	124
	3	Homozygote Glu	17

Table 6.6 and 6.7 are the test results for the independent variables or subject effects on the dependent variable, this time with the added effect of Arg→Gly16 and Gln→Glu 27 genotype. This analysis was conducted as a preliminary to examine if Arg→Gly16 and Gln→Glu 27 genotype had an association with the phenotypic variables as well as the dependent variable of central AIx (AI). The F ratio and p value of significance demonstrates that there is no significant influence on AIx (AI) in this model.

Table 6.6 Test between variables and the dependent variable central AIx (AI).

Source	Type III Sum of Squares	df	Mean Square	F	Sig
Corrected Model	109681.968	7	15668.853	72.751	.000
Intercept	31544.458	1	31544.458	146.462	.000
Gender	7802.426	1	7802.426	36.227	.000
Age	16772.458	1	16772.458	77.875	.000
Heart rate	31048.273	1	31048.273	144.158	.000
Height	7245.329	1	7245.329	33.640	.000
Systolic BP	2605.230	1	2605.230	12.096	.001
Arg→Gly16	177.433	2	88.716	.412	.663
Error	83996.867	390	215.377		
Total	7206396.750	398			
Corrected Total	193678.835	397			

a R Squared = .566 (Adjusted R Squared = .559)

Table 6.7 Test between variables and the dependent variable central AIx (AI).

Source	Type III Sum of Squares	df	Mean Square	F	Sig
Corrected Model	109573.770	7	15653.396	72.586	.000
Intercept	31820.471	1	31820.417	147.553	.000
Gender	7984.915	1	7984.915	37.027	.000
Age	16736.640	1	16736.640	77.609	.000
Heart rate	31068.163	1	31068.163	144.065	.000
Height	7337.331	1	7337.331	34.024	.000
Systolic BP	2630.162	1	2630.162	12.196	.001
Gln→Glu 27	69.236	2	34.618	.161	.852
Error	84105.064	390	215.654		
Total	7206396.750	398			
Corrected Total	193678.835	397			

a R Squared = .566 (Adjusted R Squared = .558)

6.3.3. Regression Model for Hypertensive African Trinidadians

The model for the hypertensive population used was that described in section 6.3.1 of this chapter. However, systolic blood pressure was not included, as this variable is already known in hypertensives. In this population AIx (AI) is negatively correlated with weight (see figure 6.5).

The results of analysis and significance are demonstrated in tables 6.8, 6.9 and 6.10. Figure 6.5 is the scatterplot to confirm that the variables have genuine linear relationship.
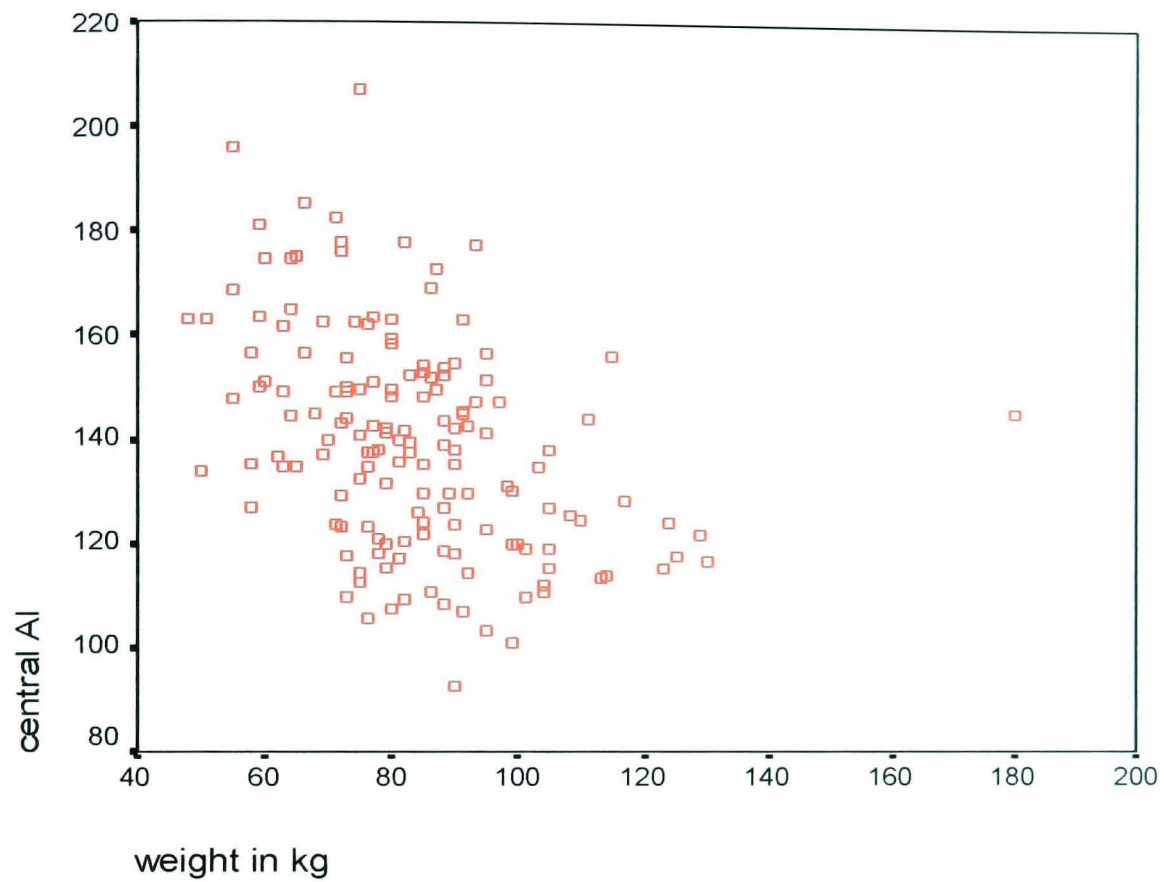
Figure 6.5 Scatterplot of correlation between weight in kilograms (kg) and AIx (AI) of the HT group.

Table **6.8** R coefficient approach, indicating a good measure of fit for African Trinidadian hypertensives.

Model	R	R Square	Adjusted R Std. Square	Error of the Estimate
1	.734[a]	.538	.523	14.47052

a Predictors: (Constant) weight in kg, heart rate, height in cms, age, gender.

Table **6.9** ANOVA_b for confirming significance association of regression line with Aix(AI) in African Trinidadian hypertensives.

Model 1	Sum of Squares	df	Mean Square	F	Sig
Regression	36140.337	5	7228.067	34.519	.000[a]
Residual	30990.604	148	209.396		
Total	67130.942	153			

a Predictors: (Constant) weight in kg, heart rate, height in cms, age, gender.

b Dependent Variable: central AIx (AI).

Table **6.10** Contribution of each predictor to the model in African Trinidadian hypertensives.

		Unstandardized Coefficient		Standardized	t	Sig
Model 1		B	Std. Error	Beta		
	(Constant)	236.762	33.434		7.081	.000
	Gender	14.009	3.056	.330	4.585	.000
	Age	.419	.115	.211	3.657	.000
	Height in cms	-.426	.181	-.184	-2.356	.020
	Heart rate	-.775	.112	-.396	-6.924	.000
	Weight in kg	-.201	.074	-.173	-2.720	.007

a Dependent Variable: central AIx (AI).

6.3.4 Multiple Linear Regression Analysis for Hypertensive African Trinidadians

The following analysis was performed to demonstrate if the Arg→Gly16 or Gln→Glu 27 polymorphisms are associated with AIx (AI) in hypertensive African Trinidadians.

Table 6.11 Multiple Linear Regression Analysis For African Trinidadians hypertensives for Arg→Gly16.

Source	Type III Sum of Squares	df	Mean Square	F	Sig
Corrected Model	36888.140[a]	7	5269.734	25.440	.000
Intercept	13045.397	1	13045.397	62.978	.000
Gender	4788.944	1	4788.944	23.119	.000
Age	3013.631	1	3013.631	14.549	.000
Heart rate	8887.590	1	8887.590	42.906	.000
Height	922.228	1	922.228	4.452	.037
Weight	1559.642	1	1559.642	7.529	.007
Genotype16	747.803	2	373.902	1.805	.168
Error	30242.801	146	207.142		
Total	3063172.500	154			
Corrected Total	67130.942	153			

[a]Dependent variable: Central AIx (AI).

R Squared = .549 (Adjusted R Squared = .528)

Table 6.12 Multiple Linear Regression Analysis For African Trinidadians hypertensives for Gln→Glu 27.

Source	Type III Sum of Squares	df	Mean Square	F	Sig
Corrected Model	36291.287	7	5184.470	24.544	.000
Intercept	14668.691	1	14668.691	69.444	.000
Gender	4440.850	1	4440.850	21.024	.000
Age	2683.095	1	2683.095	12.702	.000
Heart rate	10080.301	1	10080.301	47.722	.000
Height	1193.383	1	1193.383	5.650	.019
Weight	1521.353	1	1521.353	7.202	.008
Genotype27	150.950	2	75.475	.357	.700
Error	30839.654	146	211.231		
Total	3063172.500	154			
Corrected Total	67130.942	153			

[a]Dependent variable: Central AIx (AI).

R Squared = .541 (Adjusted R Squared = .519)

6.4 Discussion

The main questions asked from multiple regression were if (i) the Arg→Gly16 or, (ii) the Gln→Glu 27 polymorphism are associated with AIx (AI) in African Trinidadians. Multiple regression analysis shows that neither the Arg→Gly16 polymorphism nor the Gln→Glu 27 polymorphism is independent variables of AIx (AI). The data suggest the polymorphisms do not have a significant influence on contributing to arterial compliance in African Trinidadians. These findings are complimentary to those as described by Tomaszewski et al, 2002; Kato et al, 2001; Candy et al, 2000 and Xie et al, 2000. These studies suggest that although there are marked differences as regards ethnic distribution, the Arg→Gly16 and Gln→Glu 27 genetic polymorphisms of the β_2-adrenergic receptor gene are not associated with hypertension in either European, American, Japanese and African populations.

This is however, in contrast with the findings as described by Jindra et al, 2002; Dishy et al, 2001; Meirhaeghe et al, 2001; Timmermann et al, 1998; Kotanko et al, 1997, suggesting that genetic variability of the β_2-adrenergic receptor gene is implicated in the predisposition to essential hypertension.

The apparent lack of consistency of these studies may be, at least partially, attributed to ethnic differences. Other possible explanations for discrepancies between these studies are a pleiotropic effect exerted by the β_2-adrenergic receptor gene (Tomaszewski et al, 2002). Its possible contribution to other cardiovascular and metabolic phenotypes (insulin resistance, cardiac failure, obesity) of different prevalence among hypertensive and normotensive individuals may act to confound studies (Bray et al, 2000).

Interestingly, in this study, obesity was a feature, as body mass index was significantly higher ($P < 0.001$) in the HT group. Therefore, analysis of weight was

investigated to assess whether arterial stiffness is associated with increasing body weight in this population. Body weight is correlated to AIx (AI) in both groups, but not associated to the β_2-adrenergic receptor gene (P> 0.05). These results are in contrast to findings of Meirhaeghe et al, 2001, who suggested, that genetic variants in the β_2-adrenergic receptor gene may be associated with obesity, altered physical activity profiles, nonesterified fatty acid levels and decreased insulin sensitivity. However, the findings reported here are consistent with Kato et al, 2001, in that there was no confounding influence of obesity on the postulated association.

Phenotypical variables such as age, gender and heart rate were proven to be predictors of AIx (AI), in both groups (P < 0.001). These findings are consistent with other studies. (Benetos et al, 1997; Sa Cunha et al, 1997; Mangoni et al, 1996; O'Rourke 1995; Kelly et al, 1989).

6.5 Summary

❖ There is no association between Arg→Gly16 and Gln→Glu 27 and AIx (AI) in normotensive African Trinidadians even when other known predictors of AIx (AI) are taken into account.

❖ There is no association between Arg→Gly16 and Gln→Glu 27 and AIx (AI) in hypertensive African Trinidadians.

❖ Gender, age, heart rate and height have a significant influence on AIx (AI) in African Trinidadians.

Chapter 7

General Discussion and Conclusions

7.1 General Discussion

The National Health Service Framework for Coronary Heart Disease (DoH, 2000) had laid down the United Kingdom government's strategy for tackling heart disease and stroke. The national target is to reduce mortality by at least 50% by the year 2010, identifying groups or individuals at most risk and in greater need. It has been known for a long time that African Caribbean's are at great risk of essential hypertension even at an early age with an accelerated risk as age advances. The impact of further disease complications such as stroke, left ventricular hypertrophy and end stage renal failure that arise not only have a financial burden on society, but an emotional and social misery to their families.

The evidence suggests that genetic and environmental factors predispose African Caribbean people to accelerated arterial stiffness, and this is reflected in African Trinidadians. In the current study, the influence of racial dilution was excluded as selection was based on screening the participants by asking for information on three forefathers who had to have a strong African Trinidadian background maternally and paternally and permanent residency in Trinidad or Tobago. Structural inequalities such as financial, social classification and the impact of environmental factors have previously been suggested to contribute towards increased cardiovascular risk.

A comparison of the data obtained in several different studies, in the U.K., U.S.A., South Africa and Caribbean demonstrates that essential hypertension is common in African Caribbean's despite the differences in the political, socio-economic and environmental climate in their different countries (Jindra et al, 2002; Tomaszewski et al, 2002; Meirhaeghe et al, 2001; Kato et al, 2001; Xie et al, 2000; Cockcroft et al, 2000; Candy et al, 1999; Kotanko et al, 1997). In view of this, I set out to investigate

the potential contribution of genetic factors, which previously has been shown in at least some studies to alter vascular reactivity.

Few studies have used pulse wave analysis (PWA) to assess vascular function and hypercholesterolaemia in particular ethnic groups identified as having a morbid predisposition to essential hypertension. Epidemiological interpretation of the data published to date (Jindra et al, 2002; Tomaszewski et al, 2002; Meirhaeghe et al, 2001; Kato et al, 2001; Xie et al, 2000; Cockcroft et al, 2000; Candy et al, 1999; Kotanko et al, 1997), demonstrate that hypertension in this ethnic group has a multifactorial aetiology.

With regards to this, the major aim of this thesis was to investigate whether there was a genetic predisposition to vascular function in African Trinidadians, and also to validate the use of PWA as a diagnostic and effective research tool, in order that large groups of people could be studied.

7.2 The β_2–Adrenoceptor

This thesis was designed to investigate the frequency of β_2 -adrenoceptor coding region polymorphism in a random African Trinidadian population and the contribution of these polymorphisms (if any) to pathogenesis of hypertension and or altered AIx (AI). The β_2 –adrenoceptor is important in the control of vascular function and relevant mutations may be responsible for altering vascular behaviour.

7.2.1 β-2 adrenoceptor polymorphism and vascular function

Hypertension is not generally due to a single gene abnormality. Since clear Mendelian models of inheritance cannot readily be assigned in essential hypertension it is probable that there is variable penetrance of susceptibility genes.

More recent investigations have focused on the two common polymorphisms of the β_2 adrenoceptor: Arg→Gly16, and Gln→Gln27. *In vitro* studies of Arg16 receptors demonstrate altered down regulation (Green et al, 1995). Consistent with the *in vitro* data, clinical studies have demonstrated increased responsiveness to β-agonist stimulation, and an increased risk of hypertension among Arg homozygotes (McNamara et al, 2002). Functional changes which are associated with β_2 adrenoceptor polymorphisms become even more complex when the strong linkage disequilibrium between the known polymorphisms is considered (Barbier, 2001). Investigation into the vasodilatory responses to isoprenaline and desensitisation in patients with three different genotypes for two common alleles at positions 16 and 27 revealed that the Glu27 polymorphism is associated with enhanced responsiveness to agonists (Cockcroft et al, 2000). However, the Arg16 polymorphism is associated with increased desensitisation (Dishy et al, 2001). The Glu27 polymorphism is found more often in Caucasians than African populations, and could contribute to the well-documented differences in vascular reactivity between the two racial groups. Further investigation of the clinical implications of these common variants of β-adrenoceptors is needed and this was a major aim of the work described in this thesis. My findings suggest that the two common β_2-receptor polymorphisms do not contribute significantly to the determination of augmentation index (AIx), (i.e. arterial compliance). Importantly, the pharmacogenetic impact of these variants of effectiveness of β-adrenergic blockage remains unknown.

7.3 PWA and the Implementation in a Clinical Setting

The application of Pulse Wave analysis (PWA) has offered a simplistic, reliable, efficient and non-invasive tool to detect sub-clinical, vascular data on cardiovascular

disease process. As it is more sensitive and specific than conventional blood pressure monitoring it can facilitate precise treatment and management of not only vascular disease but also drug therapy and risk stratification. The use of a non-invasive approach enabled study on a large cohort of subjects encompassing a wide range of ages and blood pressures and was appealing to subjects, as it was not regarded as physically an uncomfortable experience. This had an attraction not only to the public but also to health professionals. Alongside lipid and blood pressure analysis and possibly genetic screening a tailor made service on cardiovascular risk assessment could potentially be employed to identify and implement advice and intervention strategies to meet client's needs. This does not just apply to minority ethnic groups but to all ethnic backgrounds where cardiovascular disease is widespread. The validity of the application of such a technology is the amount of data that is possible to collect in a short time, efficiently, reliably, and cost effectively with minimal discomfort to subjects. However, there is significant opposition in the use of PWA/V as a clinical tool for the diagnosis and management of hypertension or other cardiovascular diseases. In the case of hypertension, ambulatory blood pressure has been viewed as the 'gold standard' and a conclusive diagnostic tool for hypertension, and is widely used (Verdecchia et al, 1998). PWV is considered a more accurate method of assessing arterial stiffness. However, ambulatory blood pressure monitors may not be as accurate or conclusive as some health practitioners may believe. The equipment is temperamental and may be uncomfortable for the patient, whilst intermittent blood pressure measurements are taken over a twenty-four hour period. It is not subjected to rigorous controlled environments and cannot predict precisely the impact of therapeutic drug intervention over a short duration of time (Pickering et al, 1996). Some patients need drug therapy intervention, in advance of monitoring to cover

potential cardiovascular risks, due to the long waiting list for an appointment for fitting and monitoring. Such intervention of therapy may mask the patients '*true*' blood pressure readings (Pickering et al, 1996).

The advantages of using pulse wave analysis (PWA) or pulse wave velocity (PWV) would enable the health practitioner a more efficient tool for not only seeing many patients in a shorter time-frame, but effective and conclusive evidence for the specific diagnosis and management of hypertension. This does not mean that PWA/V should replace the use of ambulatory blood pressure monitoring, but be complementary. The health practitioner should therefore consider the patient condition with regards to hypertension carefully and use either monitoring tool or both, to ascertain the true cause and successful treatment regime.

7.4 Study Strengths and Limitations

As described in section 7.3, the use of PWA, allows a comprehensive and detailed collection of cardiovascular data, quickly, effectively and reliably with exceptional validity. However, the additional data or use of pulse wave velocity (PWV) would have been strengthened by giving an indication of the compliance of the artery. Serial measurement of PWV in a section of artery indicates the magnitude of change in arterial compliance. Similarities and differences between augmentation index and pulse wave velocity in the assessment of arterial stiffness have been identified (Yasmin et al, 1999). However, augmentation measurement is technically the easiest and quickest of available methods to assess arterial stiffness and is sufficient to be incorporated into a clinic visit. The justification in this instance is that a large cohort of people were able to participate in this study, with augmentation measurement being sufficient alone to assess arterial stiffness. Augmentation and PWV are influenced by

gender and height (Yasmin et al, 1999), yet the focus of study of arterial stiffness was ethnicity and genetic determination. PWV has technical difficulties of application are as the location and measurement of the carotid artery may be laborious and there is a slight risk of carotid rubbing. As emergency cover was not always readily available, PWA was used in this study due to the ease of augmentation measurement.

Practically, PWA is very operator dependent and requires experience to obtain reliable measurements. It is also argued that AIx is a composite of both vascular tone and arterial stiffness, but lacks addition information to accurately interpret such pathology. Others will add that AIx has not been validated and cannot be considered a robust parameter (Kelly et al, 2001). However, augmentation alone can predict long-term morbidity to justify measurement of arterial stiffness in everyday practice (Yasmin et al, 1999).

The limitation of nurse prescribing meant delays in getting newly diagnosed hypertensive participants emergency treatment, before referral. This led to a dichotomy of responsibility and accountability of the investigator. Specialist nurses should have authority to prescribe anti-hypertensive medication in such emergency situations with an appropriate protocol and procedures in place, with follow-up clinics for the participant who has become a patient. Some newly diagnosed hypertensive participants were seen by a cardiologist, but this was not always consistent and African Trinidadians did fear any additional cost to treatment in perusing this offer, despite it being free. The investigator did provide follow up after treatment whether commenced by the cardiologist or general practitioner.

Pseudohypertension and 'whitecoat hypertension' are features of blood pressure studies and clinics. In accordance to strict adherence of procedure of blood pressure measurement (described in Chapter 2) detection was limited. If participants were

suspected of having pseudohypertension, especially if the participant was relatively young or was over anxious, they were asked if they wished to return to the clinic at another time. This allowed familiarisation with the procedure to allay apprehension and to check if a young participants' plateau of systolic blood pressure and relatively low central aortic pressure augmentation corresponded on separate occasions.

It is in these incidences that the use of ambulatory blood pressure monitoring would have been beneficial and could have determined over a longer period of time with changeable environmental factors if a diagnosis of hypertension was appropriate. However, the technology and computer software for analysis was not available. Despite this limitation, referral was available. All participants were asked prior to participating to adhere to the inclusion criteria as regards dietary and fluid intake. There was no confirmed and clear indication that the patient had adhered to the dietary requirement and the investigator had to be sure that the participant had conformed to the requirement of entry by close questioning prior to PWA. Salt restriction was not a prerequisite for entry, as this would have taken considerable time and effort to enforce and would have decreased the cohort size if implemented.

The renin-angiotensin system (RAS) plays an integral part in the control of blood pressure, but polymorphisms of the angiotensin converting enzyme gene, although associated with left ventricular hypertrophy, do not have a clear association with hypertension, and thus was not investigated. Again, PWV may have been beneficial to examine this question in this case as PWV and MAP are greater in populations with high salt intake, increasing age and high prevalence of hypertension compared to populations with low salt intake, increasing age and low prevalence of hypertension (Avolio et al, 1985; Ergul et al, 2000; Safar et al, 2000).

Subjects treated with antihypertensive medications contribute important information regarding genetic components on blood pressure variance. This is demonstrated when treated subjects are excluded by the reduction in both genetic and shared environmental components of variance compared with other approaches (Cui et al, 2003). Valuable information is not only lost, but statistical power is weakened because of reduced numbers of subjects. The use of measured pressures from treated subjects is not ideal and represent a biased distortion in a quantitative analysis because treatment lowers blood pressure and is usually applied to those with the highest values. Treatable subjects might be expected to provide genetic and environmental clues to elevated blood pressure, that relate to blood pressure phenotypes and genotypes. A potential solution is to adjust measured pressures in treated subjects so that they reflect the inherent untreated levels (Cui et al, 2003). However this was not undertaken in this study, but subjects were analysed for arterial stiffness and genetic predisposition separately. There are less robust data on the effect of treatment on AIx and this was corrected for in the current study.

Data did reflect an ethnic difference in the frequency of the Glu 27 homozygote genotype in African Trinidadians, which is compatible when compared to other findings of studies (Candy et al, 2000; Xie et al, 2000). A population based case controlled association study, or comparison with a different ethnic group would have demonstrated marked ethnic differences in their distribution of the genetic polymorphisms. However, this was not feasible due to the geographical location of the study and the time available.

7.5 Future Work

Some published data have suggested that the effect of the β_2-adrenoceptor polymorphism is more dependent upon haplotypes than single nucleotide polymorphisms (SNPs) (Drysdale et al, 2000). Four common haplotypes exist which appear in part to predict clinical responsiveness in the airways (Drysdale et al , 2000). These haplotypes can be defined by genotyping for the four SNPs at bp –367, -47, codon 16 and codon 27, due to strong linkage disequilibrium between these SNPs and other SNPs forming extended haplotypes. No studies to date have used haplotype-based approaches to delineate contribution of this locus to vascular reactivity. Therefore, determination of haplotypes for all individuals in these two African Trinidadian cohorts could have examined if individuals were carrying a specific haplotype that increases vascular stiffness. Similarities in phenotypic expression between hypertension in blacks and patients with Liddle's syndrome (Skimkets et al, 1994), a monogenic form of hypertension, have suggested that abnormalities of the distal tubular epithelial sodium channel, a major regulator of overall control of sodium balance, may underlie the development of high blood pressure in African Trinidadians and Caribbean's (Schild et al, 1995). A number of variants of the sodium channel β-subunit coding sequence have been identified in subjects with hypertension (Chang et al, 1996; Dong et al, 1997; Persu et al, 1998) and it would have been interesting to study this locus, perhaps in combination with the β_2- adrenoceptor locus in this population.

A further polymorphism, G46OW, of the α-adducin gene has been associated with essential hypertension (O'Byrne et al, 1998; Cusi et al, 1997). Also, the TM59M polymorphism in the gene for the beta2-subunit of the epithelial sodium channel has been found to have an association hypertensive black people in London U.K. (Baker

et al, 1998). This may influence blood pressure response to sodium loading or depletion and response to long-term treatment with a thiazide diuretic. Further studies are needed to clarify this, which may determine the reasons why monotherapy using thiazides is predominant and successful in treating hypertension in African Trinidadians.

Overall, findings from the BRIGHT (BRItish Genetics of HyperTension) study, (Caulfield et al, 2003), suggest that essential hypertension has an oligogenic element with interaction or relation to more minor genetic predispositions.

7.6 Summary

❖ In summary, the data presented in this thesis suggest that common SNPs at codon 16 and 27 of the β_2-adrenoceptor receptor locus do not contribute to AIx either in normotensive or hypertensive African Trinidadians.

❖ The use of pulse wave analysis is an effective and efficient and non-invasive diagnostic tool for diagnosing and managing hypertension in fast track cardiovascular clinics. African Trinidadians would benefit from CHD and CVD risk factor screening, after the age of forty-five. This achieved by primary prevention strategies incorporated in cardiovascular screening clinics.

❖ Essential hypertension in African Trinidadians may have an oligogenic element.

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