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POSSIBLE ASSOCIATION BETWEEN GENETIC POLYMORPHISMS OF THE ADRENERGIC RECEPTOR GENES AND OBESITY AND HYPERTENSION IN SOUTH AFRICAN FEMALE VOLUNTEERS

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"The most beautiful thing we can experience is the mysterious. It is the source of all true art and all science. He to whom this emotion is a stranger, who can no longer pause to wonder and stand rapt in awe, is as good as dead"

Albert Einstein

ACKNOWLEDGEMENTS

I would like to express my heartfelt gratitude to the following persons and institutions who contributed to make this study possible:

KEY TERMS AND DEFINITIONS

Acanthosis nigricans — Skin condition characterized by dark (hyperpigmented), velvety thickening of the skin

Agonist — Binding of this molecule/substance to receptor increases the receptor-mediated effect

Agonist promoted down-regulation — Down-regulation is the process by which a cell decreases the number of receptors to a given hormone or neurotransmitter to reducits sensitivity to this particular molecule. In this case the down-regulation is caused by the binding of an agonist

Analgesia — Absence of the ability to feel pain, without loss of consciousness or touch sensation, in response to stimulation that would normally be painful

Antagonist — Binding of this molecule/substance to receptor decreases the receptor-mediated effect

Cardiomyopathy — The technical term for heart failure, means the heart muscle has lost its power to do the work it needs to do; it still functions, but not as effectively as it should

Central adiposity — Excessive fat tissue in and around the abdomen

Cistron — A DNA sequence that encodes a specific polypeptide chain

Desensitization — Decreasing sensitivity to a stimulus or substance

Diplotype — Two polymorphisms usually inherited as a unit

Dyslipidemia — Abnormality in/abnormal amount of, lipids and lipoproteins in the blood; can cause plaque buildups in artery walls

Essential hypertension — An elevated systemic arterial pressure for which no cause can be found. Often the only significant clinical finding; individuals with elevated blood pressure are at risk for cardiovascular disease

Genetic polymorphism
Variability in DNA sequence that occur with an allele frequency of >1% in the population

Haplotype
One of the alternative forms of the genotype of a gene complex. Different polymorphisms inherited as a unit. This term is applied to gene complexes rather than the term allele, which refers to one of the forms of a single gene

Linkage disequilibrium
Allelic association; when alleles at 2 distinct loci occur in gametes more frequently than expected, given the known allele frequencies and recombination fraction between the 2 loci, the alleles are said to to be in linkage disequilibrium

Negative feedback loop
A system responds to reverse the direction of change. Since this process tends to keep things constant, it is stabilizing and attempts to maintain homeostasis. When a change of variable occurs within the stable negative feedback system, it will attempt to establish equilibrium

NIDDM
Non-insulin dependent diabetes mellitus. Type 2 diabetes; the pancreas produces lots of insulin, but the body becomes resistant to its effects over time; develops gradually in adults; can be precipitated by obesity or severe stress or menopause or other factors; can usually be controlled by diet and hypoglycemic agents without injections of insulin

Non-alcoholic steatohepatitis
An extreme form of fatty liver

Nonsynonymous polymorphisms
Type of polymorphism that changes the encoded amino acid

Osteoarthritis
A form of arthritis in which one or many of the joints undergo degenerative changes like loss of articular cartilage

Polycystic ovarian syndrome	A common condition found in approximately 10 percent of women. This condition is characterized by infrequent menses (with cycles of greater than six weeks in length or 8 or fewer periods a year) and hyperandrogenism (increased serum levels of testosterone, androstenedione, DHEAS). Approximately 50 percent of women with polycystic ovarian syndrome will have obesity and insulin resistance. They may also have hirsutism, facial acne, or alopecia (thinning hair or male pattern baldness)
Polygenic predisposing genes	A combination of genes predisposing a person carrying a certain combination (for example polymorphisms) to a certain condition
Prothrombotic state	E.g., high fibrinogen or plasminogen activator inhibitor [-1] in the blood
Proinflammatory state	E.g., elevated high-sensitivity C-reactive protein (plays a role in the inflammatory respons) in the blood
Synonymous polymorphisms	Type of polymorphism that does not change the encoded amino acid encoded
Transgenic animals	Certain gene(s) have been altered; in order for the effects to be studied
Type 2 diabetes	Patients are not insulin-dependent or ketosis prone; two subclasses involve the presence or absence of obesity. In patients glucose tolerance is often improved by weight loss. Environmental factors superimposed on genetic susceptibility are probably involved in the onset. There are many health risks involved with diabetes, and it should be treated as well as possible

ABBREVIATIONS

AR	Adrenergic receptor
Arg	Arginine
BMI	Body mass index; calculated as body weight in kg divided by height squared
cAMP	Cyclic adenosine monophosphate
DBP	Diastolic blood pressure
FFA	Free fatty acids measured during the oral glucose tolerance test
g	Gram
Gln	Glutamine
Glu	Glutamate
Gly	Glycine
HDL	High density lipoprotein
Hm	Homozygote for the polymorphic allele of the gene
HOMA-IR	Homeostatic model of insulin resistance; index of insulin resistance
Ht	Heterozygote
HWE	Hardy-Weinberg equilibrium
INS	Insulin concentration measured during the oral glucose tolerance test
LDL	Low density lipoprotein
MS	Metabolic syndrome
N	Homozygote for the wild type allele of the gene
OGTT	Blood glucose concentration during the oral glucose tolerance test
PCR	Polymerase chain reaction
SBP	Systolic blood pressure
Ser	Serine
SNP	Single nucleotide polymorphism
Tc	Total blood cholesterol
Trig	Triglycerides
Trp	Tryptophan
WC	Waist circumference

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ABSTRACT

Introduction

Across the world the incidence of the metabolic syndrome increases annually at an alarming rate. Two conditions associated with this are obesity and hypertension (high blood pressure). Both have negative health and lifestyle consequences. Numerous studies on adrenergic receptor (AR) gene polymorphisms in various population groups have proved, although not exclusively, that these polymorphisms may be positively associated with susceptibility to and progression of obesity and hypertension. The AR encoding genes are attractive targets for such studies because the ARs, as part of the sympathetic nervous system, perform important functions like vasoconstriction, vasodilation, lipolysis and influence the heart's contraction. These functions accentuate the possible role of AR gene polymorphisms in the onset or progression of obesity and hypertension.

Obesity is a health concern especially among black South African women. The prevalence of obesity (BMI > 30 kg/m^2) in the North-West province of South Africa is high: 28.6%. The POWIRS (Profile of Obese Women with the Insulin Resistance syndrome) study was conducted in 2003 on 102 black South African female volunteers to search for possible associations of the β_2-AR Gln27Glu and β_3-AR Trp64Arg polymorphisms with parameters of the carbohydrate and lipid metabolism, the index of insulin resistance (HOMA-IR), body mass index (BMI) and body fat % (Schutte *et al.*, 2005). To our knowledge, this was the first study of its kind in South Africa and which led to this study and dissertation.

Objectives

The objectives of this study were to:

- Determine the incidence of the following polymorphisms in
    - 102 black South African female volunteers and calculate the minor allele frequency:
        - β_1-AR: Ser49Gly
        - β_2-AR: Arg16Gly

    - 115 white South African female volunteers and calculate the minor allele frequency:
        - β_1-AR: Ser49Gly
        - β_2-AR: Arg16Gly
          Gln27Glu
        - β_3-AR: Trp64Arg;
- identify possible diplotypes and haplotypes in the study groups;

- take relevant physiological parameters (measured in the POWIRS studies) into account in the search for possible associations of these polymorphisms, diplotypes and haplotypes with obesity and high blood pressure as characteristics of the metabolic syndrome;
- compare the black and the white study groups with regards to the above mentioned objectives.

Methods

DNA was isolated from blood spots on Guthrie cards (collected during the POWIRS studies) and the respective AR gene regions amplified by polymerase chain reaction (PCR). After restriction enzyme digestion, the DNA fragments were separated by agarose gel electrophoresis. Genotypic findings were examined along with measured physiological parameters (measured during the POWIRS studies) and statistically processed. Area under the curve (AUC) analysis was performed on parameters measured during the oral glucose tolerance test. Diplotype and haplotype analyses were also performed on both subject groups.

Results

The minor allele frequencies for both groups were calculated and compared to that reported in other published studies. For the black group, the minor allele frequencies were: 84% (β_1-AR Ser49Gly), 16% (β_2-AR Gln27Glu), 49% (β_2-AR Arg16Gly) and 28% (β_3-AR Trp64Arg) and for the white group: 94%, 46%, 50% and 7% respectively. The AUC differed in almost every instance of comparison, but was within normal ranges. Only a few significant differences were identified when the measured physiological parameters were compared between the genotypes, diplotypes and haplotypes in each group, most of which were found to be within normal ranges. When the two groups of test subjects were compared, only minimal differences were observed, most of which were still found to be well within normal ranges.

Conclusions

Although no associations were identified between the separate investigated AR gene polymorphisms, diplotypes or haplotypes and obesity and hypertension or high blood pressure, indications are present that they may act as contributors to risk factors for the onset and progression of these characteristics of the metabolic syndrome.

Keywords

Adrenergic receptor genes, polymorphism, the metabolic syndrome, obesity, hypertension, South Africa.

OPSOMMING

Inleiding

Wêreldwyd verhoog die voorkoms van die metaboliese sindroom teen 'n kommerwekkende tempo. Twee toestande wat hiermee geassosieer word, is obesiteit en hipertensie (hoë bloeddruk) wat albei negatiewe gesondheids- en lewenswyse gevolge het. Talle studies op adrenerge reseptor (AR) polimorfismes het in populasiegroepe in ander lande bewys, maar nie uitsluitlik nie, dat dit moontlik positief geassosieer word met vatbaarheid vir en progressie van obesiteit en hipertensie. Die AR gene is aanloklike teikens vir assosiasie studies omdat die ARs, wat deel vorm van die simpatiese senuweestelsel, belangrike funksies verrig soos vasokonstriksie, vasodilatasie, lipolise en die hart se kontraksies beinvloed. Hierdie funksies benadruk die moontlike rol van AR gene polimorfismes in die aanvang en progressie van obesiteit en hipertensie.

Obesiteit is veral 'n gesondheidskwessie onder veral swart Suid-Afrikaanse vroue. Die voorkoms van obesiteit (BMI > 30 kg/m^2) in die Noord-Wes provinsie van Suid-Afrika is hoog: 28.6%. Die POWIRS (Profile of Women with the Insulin Resistance Syndrome) studie is uitgevoer in 2003 op 102 swart Suid-Afrikaanse vroulike vrywilligers in die soeke na moontlike assosiasies tussen die β_2-AR Gln27Glu en β3-AR Trp64Arg en parameters van koolhidraat en lipied metabolisme, die indeks van insulien weerstandbiedendheid (HOMA-IR), liggaamsmassa indeks (BMI) en liggaamsvet % (Schutte *et al.*, 2005). Na ons kennis, was dit die eerste studie van hierdie soort in Suid-Afrika wat gelei het tot verdere investigation in hierdie studie en verhandeling.

Doelstellings

Die doelstellings van hierdie studie was om:

- Die voorkoms van die volgende polimorfismes in
  - 102 swart vroulike South Afrikaanse vrywilligers sowel as die polimorfiese alleel frekwensie te bepaal:
    - β_1-AR: Ser49Gly
    - β_2-AR: Arg16Gly

  - 115 blanke vroulike South Afrikaanse vrywilligers sowel as die polimorfiese alleel frekwensie te bepaal:
    - β_1-AR: Ser49Gly
    - β_2-AR: Arg16Gly
      Gln27Glu
    - β_3-AR: Trp64Arg;

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- 02 swart en 115 blanke vroulike Suid-Afrikaanse vrywillegers te bepaal en die polimorfiese allele frekwensie te bepaal:
  - β_1-AR: Ser49Gly
  - β_2-AR: Arg16Gly

      Gln27Glu
  - β_3-AR: Trp64Arg;
- moontlike diplotipes en haplotipes binne die twee groepe te identifiseer;
- relevante fisiologiese parameters (gemeet tydens die POWIRS studies) in berekening te bring in die soeke na moontlike assosiasies van hierdie polimorfismes, diplotipes en haplotipes met obesiteit en hoë bloeddruk as eienskappe van die metaboliese sindroom;
- die swart groep met die blanke groep te vergelyk ten opsigte van die voorafgaande doelstellings.

Metodologie

DNA is geïsoleer van bloed op Guthrie kaarte (versamel tydens die POWIRS studies) en die onderskeie AR gene geamplifiseer deur polimerase ketting reaksie (PCR). Na behandeling met geskikte restriksie ensieme, is die DNA fragmente geskei deur agarose gelelektroforese. Genotipiese bevindinge tesame met gemete fisiologiese parameters is ondersoek en statisties verwerk. Area onder die kurwe (AUC) analises is uitgevoer op parameters wat gemeet is tydens die glukose toleransie toets. Diplotipe en haplotipe analises is ook uitgevoer op albei studiegroepe.

Resultate

Die polimorfiese alleelfrekwensies vir beide groepe is bereken en vergelyk met ander gepubliseerde studies op ander populasie groepe s'n. Vir die swart groep, is die frekwensies gevind as: 84% (β_1-AR Ser49Gly), 16% (β_2-AR Gln27Glu), 49% (β_2-AR Arg16Gly) en 28% (β_3-AR Trp64Arg) en vir die blanke groep as 94%, 46%, 50% and 7%. Die AUC het deurgaans byne in elke vergelyking wat getref is verskil, maar was steeds binne normale grense. Slegs 'n paar statisties betekenisvolle verskille het aan die lig gekom met vergelyking van gemete fisiologiese parameters tussen genotipes, diplotipes en haplotipes in elk van die groepe, meeste steeds binne normale grense. In vergelyking van die twee groepe vroue, het slegs die minimum (vyf of minder) van die fisiologiese parameters betekenisvol verskil, maar dit is weereens gevind dat dit binne normale grense val.

Gevolgtrekkings

Alhoewel daar geen assosiasies gevind is tussen die aparte AR-geenpolimorfismes, diplotipes of haplotipes en obesiteit en hipertensie (hoë bloeddruk) nie, is daar indikasies dat hul mag optree as bydraende effektore tot risiko faktore vir die ontwikkeling en vordering van hierdie eienskappe van die metaboliese sindroom.

Sleutelwoorde

Adrenerge reseptor gene, polimorfisme, die metaboliese sindroom, obesiteit, hipertensie, Suid-Afrika.

Chapter 1: General introduction

Worldwide the incidence of the metabolic syndrome increases annually at an alarming rate (American Association for Clinical Chemistry, 2004; Van der Linde, 2004; Health24, 2005; News24, 2005). It is a multi-faceted disease of which the underlying contributing factors like sedentary lifestyle, obesity and type 2 diabetes have also been increasing (Sarti *et al.*, 2005). This disease has rapidly become one of the most important public-health concerns and challenges worldwide (Mehta & Reilly, 2004). Obesity and hypertension are two conditions associated with this syndrome, both having negative health and lifestyle consequences. Both are caused by (and contributed to) environmental and genetic factors possibly including inter-individual genetic diversity (Siani & Strazzullo, 2006).

The emerging literature on the prevalence of the metabolic syndrome in South Africa is rather scanty but there is progress. Results from various studies show that the situation in South Africa parallels that of the USA (Van der Linde, 2004; Health24, 2005; News24, 2005).
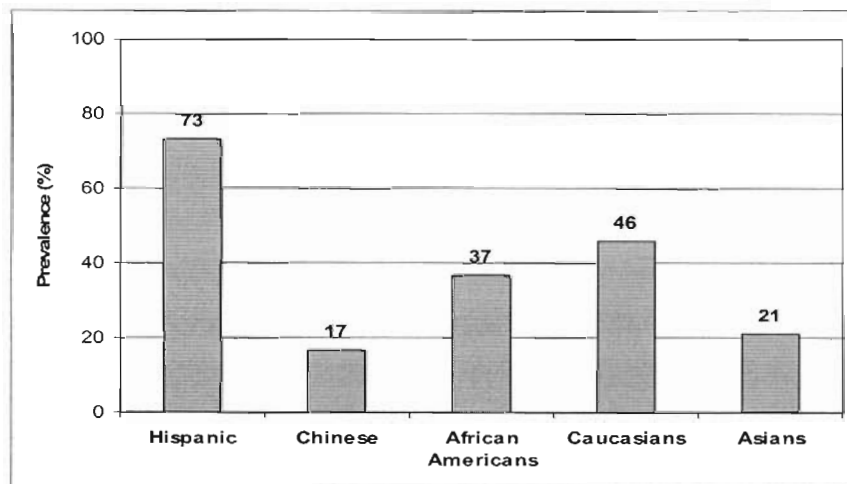
Figure 1.1: Prevalence of the metabolic syndrome in the USA with reference to ethnicity
(Adapted from American Association for Clinical Chemistry, 2004)

Figure 1.1 shows the prevalence of the metabolic syndrome in the American population of which about 25% of adults over 20 years of age are affected by the metabolic syndrome (Mehta & Reilly, 2004). The information given in figure 1.1 is of interest to this study, particularly that for African Americans and Caucasians, since the volunteers in this study are black and white South African women. The ethnic group in America with the highest prevalence of the metabolic syndrome is the Hispanic population with the African American and Caucasian prevalences differing by only 9%.

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It has become apparent that the metabolic syndrome seems to result from the interplay between susceptible genes ("thrifty") and a society with increasing obesity and sedentary lifestyle (Isomaa, 2003). Obesity is rapidly becoming a worldwide epidemic. In the USA an estimated 1 in 3 overweight adults present with the metabolic syndrome. When the number of adults, adolescents and children suffering from the metabolic syndrome in the USA are combined, the total prevalence of the metabolic syndrome is 25% of all Americans (American Association for Clinical Chemistry, 2004; Mehta & Reilly, 2004; International Society of Hypertension in Blacks, 2006). Obesity is one of the risk factors for developing metabolic syndrome, and has many detrimental health effects such as hypertension and cardiovascular heart disease (Van der Linde, 2004; Health24, 2005; News24, 2005). If the rise in obesity is not halted, it will overtake smoking as the leading cause of preventable deaths in America (Van der Linde, 2004).

One might think that South Africa, a developing country, couldn't possibly be faced with the same problem of obesity as America. This notion is incorrect. Social class has no bearing on obesity, it occurs equally across all the classes in South Africa (Van der Linde, 2004; Health24, 2005; News24, 2005), even in the predominantly rural North-West Province of South Africa, where the prevalence of obesity is as high as 28.6% (Van Rooyen et al, article submitted for publication).

Variations in blood pressure have shown to be genetically determined, at least to some extent. It can therefore be said that a person's susceptibility to develop hypertension seems to be influenced by variations in different genes, each of which makes a small contribution (Ranade et al., 2001).

The 2003/2004 South African Health Review stated that chronic non-infectious diseases that are usually associated with lifestyle (cardiovascular disease, chronic obstructive pulmonary disease and diabetes) resulted in 37% of deaths in this country (Health24, 2005; News24, 2005). Some of the risk factors for these conditions include obesity, sedentary lifestyle, poor diet and hypertension. These are also risk factors for development of the metabolic syndrome, although it is in some cases unclear whether these factors gives rise to the onset of this syndrome, or develop as a result of it (Johnson & Terra, 2002; Van der Linde, 2004; Health24, 2005; News24, 2005).

Numerous studies on adrenergic receptor (AR) polymorphisms in population groups in other countries have shown, although not exclusively, that certain polymorphisms may be positively associated with susceptibility to and progression of obesity and hypertension. The genes encoding these receptors are therefore attractive targets for studies of the metabolic syndrome. They form

part of the sympathetic nervous system and carry out numerous important functions including energy homeostasis in the body.

Alarming is the fact that in obese children and adolescents the risk factors for development of the metabolic syndrome are already present. Early identification and optimal intervention is critical to minimize the mortality and healthcare burden associated with this condition (Mehta & Reilly, 2004; Haffner, 2006).

The aims of this study were to investigate the possibility of association between selected genetic polymorphisms of the adrenergic receptor genes and the metabolic syndrome. In this dissertation, the first chapter provides an overview of the current literature, followed by chapter 3 describing the materials and methods. The results are reported in chapter 4 and discussed in chapter 5. The final conclusions drawn from this study are given in chapter 6. In appendix F the results of the first round of statistical analyses are provided in full tables. Lastly the first draft of a research paper prepared for publication from this study is included.

CHAPTER 2: LITERATURE REVIEW AND APPROACH

THE METABOLIC SYNDROME AND THE INVOLVEMENT OF SPECIFIC ADRENERGIC RECEPTOR GENE POLYMORPHISMS

2.1. INTRODUCTION

In 1962, the *thrifty gene* theory was proposed by Neel as reported by Isomaa (2003). His hypothesis was that our forefathers lived in an environment with unstable food supplies and insufficiency at times, which increased their possibility for survival if they could maximize food storage. He further stated that genetic selection would therefore favour energy storing genes and when this energy storing genotype is then exposed to the abundance of food in the modern (westernized) lifestyle, it becomes disadvantageous causing obesity and diabetes. Thrifty genes could therefore possibly serve as predisposition for the development and progression of the metabolic syndrome.

Isomaa (2003) also emphisizes that in 1988 Reaven suggested that insulin resistance and the resulting compensatory hyperinsulinemia underlies the clustering of metabolic disturbances and that the so-called Syndrome X (the metabolic syndrome) was a contributing risk factor for cardiovascular disease.

The exact cause of type 2 diabetes and insulin resistance remains unknown, but studies have suggested that overweight and obesity are contributors (Chaplin, 2005). In insulin resistance, the body's cells become less sensitive to the action of insulin, eliciting increased insulin production to compensate for this in order to maintain normal blood glucose concentrations. Hyperinsulinemia results, which stimulates lipid storing (potentiating further weight gain), changes in lipoproteins (increase in cholesterol), increase in the risk of damage to the cardiovascular system and it stimulates triglyceride release from the liver which in turn could lead to dyslipidemia which then causes plaque formation in the arterial walls (Mehta & Reilly, 2004; Haffner, 2006; Safar *et al.,* 2006).

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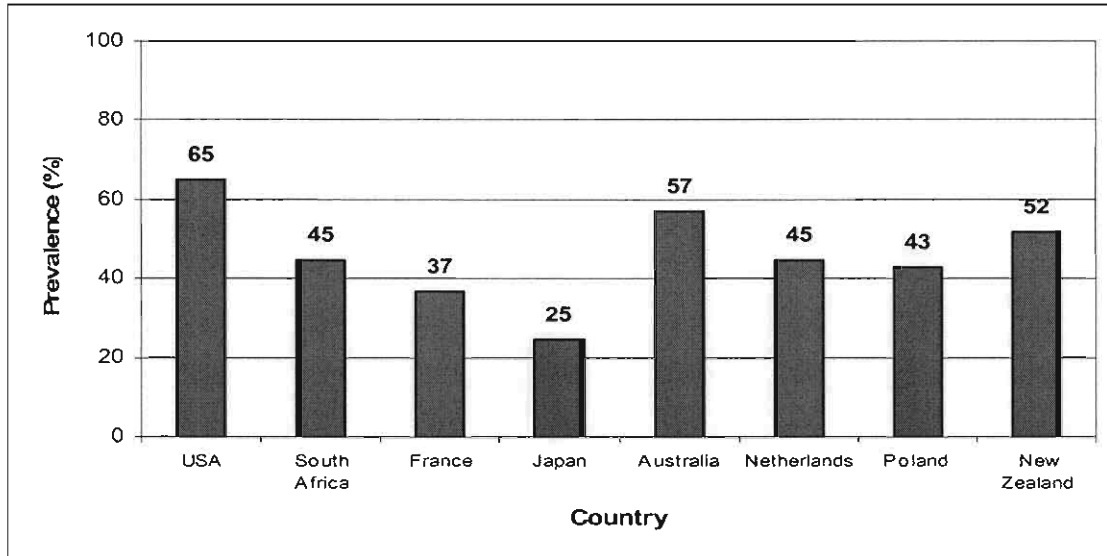
Figure 2.1: Prevalence of obesity in some countries

(Compiled using the most recent statistics obtained from Johnson & Terra, 2002; Small *et al.*, 2003; American Association for Clinical Chemistry, 2004, American Heart Association, 2004; Kraja *et al.*, 2005; Sarti & Gallagher, 2005; Haffner, 2006; Safar *et al.*, 2006 and Shen *et al.*, 2006)

The USA leads currently with 65% followed closely by several other countries, one of which is South Africa with a prevalence of 45% (figure 2.1).
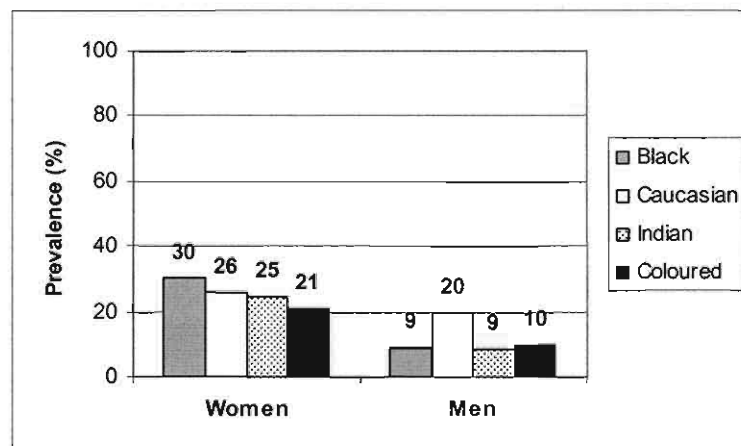
Figure 2.2: Prevalence of obesity in South Africa with reference to gender and race

(Compiled using the most recent statistics obtained from Johnson & Terra, 2002; Small *et al.*, 2003; American Heart Association, 2004; American Association for Clinical Chemistry, 2004; Kraja *et al.*, 2005; Haffner, 2006; Sarti & Gallagher, 2005; Safar *et al.*, 2006 and Shen *et al.*, 2006)

Within South Africa, the highest prevalence of obesity is observed in black women (figure 2.2). Amongst the males the Caucasians have the highest rate of obesity. It is also clear that the prevalence of obesity in South African women is higher than in men.

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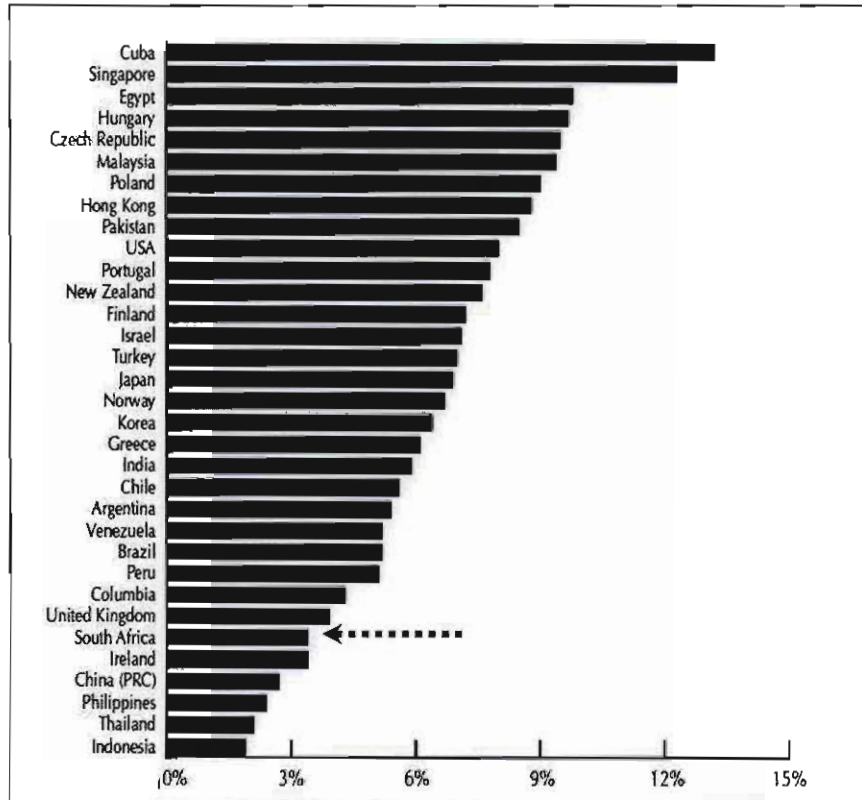
Figure 2.3: Prevalence of type 2 diabetes across the world

(Adapted from Chaplin, 2005)

In South Africa about 3.5% of the population present with type 2 diabetes, which is relatively low when compared to the other countries (figure 2.3). The actual percentage may be significantly higher, since the studies performed to obtain these numbers, often do not include people from rural communities.
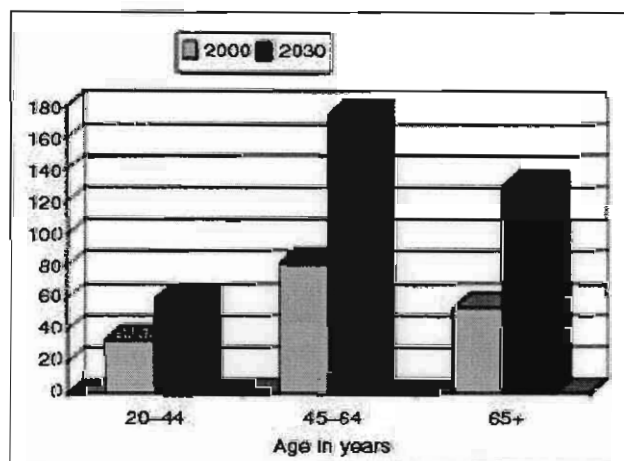
Figure 2.4: Number of people presenting with diabetes and the predicted increase

(Adapted from Chaplin, 2005)

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In figure 2.4, a table is provided of the current number of people (in millions) with diabetes worldwide as well as a prediction of the future situation. Obesity and metabolic syndrome statistics are following the same trend all around the world (Van der Linde, 2004; Health24, 2005; News24, 2005; International Society for Hypertension in Blacks, 2006). About 70-80% of people with type 2 diabetes are overweight and the prevalence of diabetes among people who are overweight is 3.8 times greater than in individuals with a healthy body weight (Chaplin, 2005). It has been said that obesity itself is not the problem, but rather the range of associated illnesses. Diseases (some potentially fatal) linked to obesity include cardiovascular diseases, stroke, gal bladder defects, osteoarthritis, sleep apnea, respiratory problems, insulin resistance, type 2 diabetes and hypertension among others (Busher *et al.*, 1999; Johnson & Terra, 2002; Girod & Brotman, 2003; Grundy, 2003; Small *et al.*, 2003; American Association for Clinical Chemistry, 2004; Van der Linde, 2004; Health24, 2005; News24).
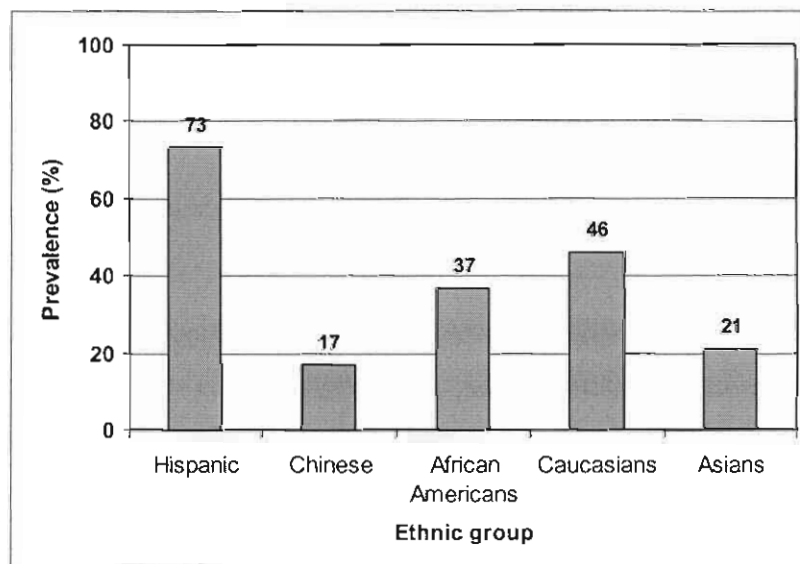
Figure 2.5: Prevalence of the metabolic syndrome in the USA with reference to ethnicity

(Compiled using the most recent statistics obtained from Johnson & Terra, 2002; Small *et al.*, 2003; American Heart Association, 2004; American Association for Clinical Chemistry, 2004; Kraja et al, 2005; Haffner, 2006; Sarti & Gallagher, 2005; Safar et al, 2006 and Shen et al, 2006)

The percentage of Americans (adults, children and adolescents) suffering from the metabolic syndrome, is reported as 25% of the population (American Association for Clinical Chemistry, 2004; Mehta & Reilly, 2004; International Society for Hypertension in Blacks, 2006). From figure 2.5, it is clear that the US Hispanic population has the highest prevalence, followed by the Caucasian and the African American groups. The Caucasian and the African Americans prevalence rates only differ by 9 % and are still relatively high when compared to the other ethnicities.
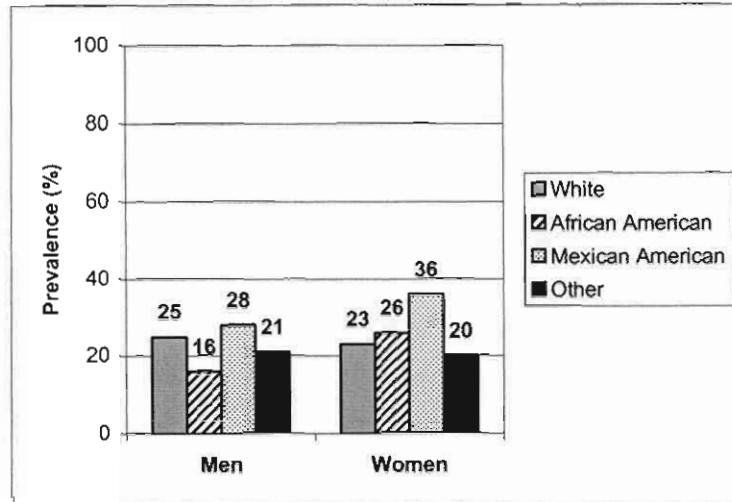
Figure 2.6: Prevalence of the metabolic syndrome in the USA with reference to gender and ethnicity

(Compiled using the most recent statistics obtained from Johnson & Terra, 2002; Small *et al.*, 2003; American Heart Association, 2004; American Association for Clinical Chemistry, 2004; Kraja *et al.*, 2005; Sarti & Gallagher, 2005; Haffner, 2006;Safar *et al.*, 2006 and Shen *et al.*, 2006)

Mexican American men and women have the highest prevalence of metabolic syndrome followed by white American men and African American women (figure 2.6). South African numbers have been said to be in accordance with these figures (American Association for Clinical Chemistry, 2004; Kiess *et al.*, 2006; International Society for Hypertension in Blacks, 2006).

It is estimated that the prevalence of the metabolic syndrome is likely to increase drastically over the next few decades, along with increasing rates of obesity and type 2 diabetes (American Heart Association, 2004; Chaplin, 2005). Obese children and adolescents already carry predisposition for developing this syndrome (Kiess *et al.*, 2006). Thus it is crucial that individuals at risk be identified and treated promptly and optimally to minimize or eliminate any future healthcare burdens (Grundy, 2003; Chaplin, 2005; Sarti & Gallagher, 2005; Kiess *et al.*, 2006; Safar *et al.*, 2006).

2.2. THE METABOLIC SYNDROME

2.2.1. CHARACTERISTICS AND CAUSES

The **metabolic syndrome** (also called Syndrome X) is the collective name given to a set of risk factors/characteristic components including central adiposity, dyslipidemia, hypertension and insulin resistance with or without glucose intolerance. Prothrombotic and proinflammatory states may or may not be present (Grundy, 2003; Girod & Brotman, 2003; American Association for Clinical Chemistry, 2004; Mehta & Reilly, 2004).

All the risk factors associated with the metabolic syndrome are interrelated. Obesity and lack of exercise tend to lead to insulin resistance (Girod & Brotman, 2003; American Association for Clinical Chemistry, 2004; Mehta & Reilly, 2004). The cells of the body become less sensitive to insulin, eliciting increased insuline production to maintain normal blood glucose levels, resulting in hyperinsulinemia. This in turn stimulates lipid storing and abnormal change in the nature of lipoproteins, for example, increased cholesterol. Insulin resistance has negative effects on lipid production, increasing VLDL (very low-density lipoprotein), LDL (low density lipoprotein) and triglyceride levels in the blood and decreasing HDL (high-density lipoproteins) (Mehta & Reilly, 2004). This can lead to the build up of fatty plaque deposits in the arteries which, over time, can lead to cardiovascular disease, blood clots and stroke. Insulin resistance also leads to increased insulin and glucose concentrations in the blood. Excess insulin increases sodium retention by the kidneys, which increases blood pressure and can lead to hypertension. Chronically elevated glucose levels in turn damage blood vessels and organs, for example the kidneys, and may lead to diabetes. There are also many diseases associated with the metabolic syndrome like polycystic ovarian syndrome, acanthosis nigricans (dark, velvety thickening of the skin) and non-alcoholic steatohepatitis (Grundy, 2003; Girod & Brotman, 2003; American Association for Clinical Chemistry, 2004).

Risk factors for the development of the metabolic syndrome include a poor diet, sedentary lifestyle, overweight/obesity, and genetic predisposition (Grundy, 2003; American Association for Clinical Chemistry, 2004; Safar *et al.*, 2006). One of the risk factors that has been receiving increasingly more attention is genetic predisposition, the susceptibility to certain diseases and/or physiological states as a result of our genetic makeup (Grundy, 2003; American Association for Clinical Chemistry, 2004; Hughes & Aitman, 2004; Safar *et al.*, 2006). Among others, the AR (adrenergic receptor) genes have been implicated as possible polygenic predisposing genes.

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2.2.2. DIAGNOSIS AND TREATMENT

Sinca there are currently no definitive markers for the diagnosis of the metabolic syndrome, a checklist of five criteria (shown in table 2.1, is generally applied and a diagnosis of metabolic syndrome is only made if a patient complies with three or more of these (American Association for Clinical Chemistry, 2004). Experts hope to add genetic markers to the list of criteria used to identify individuals with latent metabolic syndrome (Grundy, 2003; American Association for Clinical Chemistry, 2004).

Table 2.1: Diagnostic criteria for the metabolic syndrome

Feature	Criterion
Abdominal girth (circumference) Men Women	Waist circumference >102 cm >88 cm
Fasting plasma HDL-C Men Women	 <1.04 mmol/l < 1.29 mmol/l
Fasting plasma triglycerides	≥1.69 mmol/l
Fasting blood glucose	≥6.1 mmol/l
Blood pressure	≥130/≥85 mmHg

Table was compiled from American Association for Clinical Chemistry, 2004; American Heart Association, 2004 and Safar *et al.,* 2006

Features associated with the observed increase in the prevalence of the metabolic syndrome, especially in women, are increased waist circumference, high blood pressure, and hypertriglyceridemia. Although the best form of treatment is still under debate, the first-line of therapy remains lifestyle modification – a programme of weight loss and increased physical activity. In short, there are two main areas of treatment, namely lifestyle changes (weight loss and increased physical activity) and pharmacotherapy (Grundy, 2003; American Association for Clinical Chemistry, 2004; Safar *et al.,* 2006).

2.3. ADRENERGIC RECEPTORS

2.3.1. INTRODUCTION

The adrenergic receptors (ARs) are expressed by virtually every cell type in the human body and are receptors for the neurohormones (catecholamines), epinephrine and norepinephrine within the sympathetic nervous system (Hein & Kobilka, 1997; Johnson & Terra, 2002; Small *et al.*, 2003). Epinephrine is an adrenal medullary hormone and norepinephrine is a neurotransmitter (Mersmann, 2001; Flordellis *et al.*, 2004).

The sympathetic nervous system plays a key role in regulation of energy balance. The ARs are essential components of the sympathetic nervous system and form part of the autonomic nervous system which controls numerous physiological functions such as energy homeostasis and metabolism of carbohydrates and lipids (Small *et al.*, 2003; Yasuda *et al.*, 2006). The genes encoding these proteins are therefore considered "candidate genes" for the development of obesity and disorders in carbohydrate and lipid metabolism (Azuma *et al.*, 1998; Fujisawa *et al.*, 1998; Arner & Hoffstedt, 1999; Buscher *et al.*, 1999; Mershmann, 2001; Malczewska-Malek *et al.*, 2003; Small *et al.*, 2003; Yasuda *et al.*, 2006).

2.3.2. STRUCTURE

The ARs are members of the super family of cell surface receptors that carry out signalling functions via coupling to guanine nucleotide binding proteins (G-proteins). There are nine subtypes of these serpentine receptors: α_{1A}-, α_{1B}-, α_{1D}AR; α_{2A}-, α_{2B}-, α_{2C}-AR; β_1-, β_2-, β_3-AR (Hein & Kobilka, 1997; Small *et al.*, 2003). The subtypes are classified on the basis of selective agonist and antagonist binding (Gudermann *et al.*, 1997; Hein & Kobilka, 1997; Insel & Kirstein, 2003; Small *et al.*, 2003; Masuo *et al.*, 2005a).

The genes encoding the β_1- and β_2-ARs are intronless, while the β_3-AR gene contains introns (Leineweber *et al.*, 2004; Rozec & Gauthier, 2006). The ARs are proteins of > 400 amino acids in length, and their structure is characterized by the presence of seven hydrophobic regions, corresponding to seven relatively hydrophobic segments that traverse the cell membrane (transmembrane domains). These segments are connected by three extracellular and three intracellular loops (figure 2.7). These receptors possess an extracellular N-terminal tail and an intracellular C-terminal tail. The ligand binding site is composed of amino acids contributed by

several of the transmembrane segments. G-protein binding occurs primarily at intracellular loop 3. Phosphorylation is one mechanism by which receptor activity is reduced, with the phosphorylation sites on the C-terminal segment (Gudermann *et al.*, 1997; Mersmann, 2001; Malczewska-Malek *et al.*, 2003).
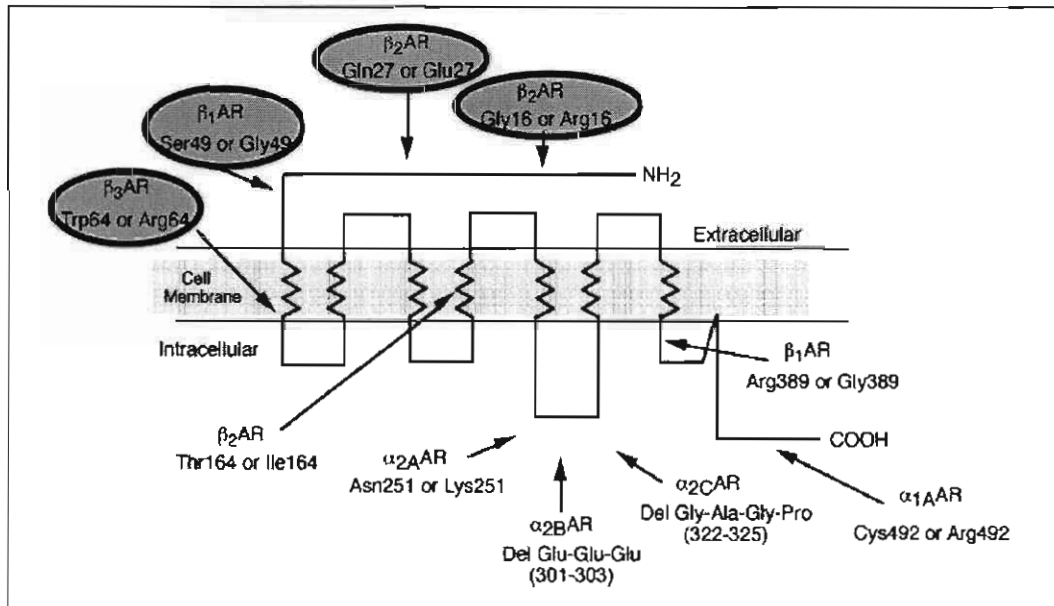
Figure 2.7: The adrenergic receptor (AR) structure and identified polymorphisms

(Adapted from Small *et al.*, 2003)

The seven transmembrane spanning domains of the AR as well as the position of SNPs are shown in figure 2.7. The general position of each selected AR gene polymorphisms being investigated in this project are highlighted (circled). Certain domains on the ARs are critical for binding of agonists and antagonists, G-protein coupling and "turning off" of the receptor signal known as desensitization. It is clear from studies that small changes in the amino acid sequence of the receptor gene (a polymorphism for example) can cause notable changes in its function, for example a polymorphism in the region critical to G-protein coupling could impair the subsequent cell signalling cascade (Buscher *et al.*, 1998).

There exists a marked interindividual variation in physiological responses, expression, and function of adrenergic receptors, as well as the response to adrenergic receptor agonists and antagonists. Although environmental factors and the heterogeneity of diseases, such as asthma and hypertension, for which drugs are used, are undoubtedly important, recent efforts have been under way to study the impact of genetic variations of the receptor genes in order to try to explain interindividual variation in phenotypes (Buscher *et al.*, 1999; Small *et al.*, 2003; Phillip & Hein, 2004).

The β_1-AR is the largest of the three subtypes, comprising of 460 amino acids. The β_2-AR has approximately 420 amino acids and the β_3-AR has approximately 410 amino acids. Homology amongst the three subtypes within a certain species is usually between 45 and 60%, whereas for a given subtype it is rather high across species (usually > 70%). This simply means that between different species, the amino acid number and sequence stay approximately the same. In spite of this strong homology in the ARs across species, variations in the amino acid sequence result in variation in ligand binding or functional properties of these homologous receptors (Mersmann, 2001).

The β_3-AR is different from the β_1- and β_2-AR subtypes in that it recognizes most of the β_1- and β_2-AR antagonists as agonists and presents with a lower affinity for catecholamines. This suggests that β_1- and β_2-AR's mediate the effect of circulating catecholamines, whereas the β_3-AR mediates only the effects of much higher concentrations of norepinephrine (Malczewska-Malec et al., 2003; Masuo et al., 2005a). Another distinguishing feature of the β_3-AR is that it appears to be relatively resistant to desensitization and down-regulation. Thus it can be hypothesized that the primary function may be to maintain signalling during periods of sustained sympathetic stimulation, such as during exercise (Malczewska-Malec et al., 2003; Yasuda et al., 2006).

2.3.3. FUNCTIONS

Individual tissues have different proportions of subtypes and subtype distribution within a tissue varies between species (Mersmann, 2001). The ARs serve critical roles in maintaining homeostasis under normal physiological as well as pathological conditions, as they are present on almost every cell type and control numerous physiological and metabolic functions, including synthesis and secretion of hormones, neuronal firing, cardiac function and blood pressure homeostasis (Buscher et al., 1998; Mersmann, 2001; Flordellis et al., 2004). Alterations of AR function may play a role in the pathophysiology of diseases and states such as obesity and related metabolic disorders.

Table 2.2: The tissue distribution and adrenergic response

Tissue	Adrenergic receptor	Effect
Blood vessels	α_1* and α_2	Constriction
	β_2*	Dilatation
Heart	β_1*	Tachycardia; increased contractility
	β_2	Positive inotropic and chronotropic effects
	α_1	Increased contractility
	α_2	Inhibits norepinephrine release
Bronchi	β_2	Relaxation
Thrombocytes	α_2	Agglutination
Kidneys	α_1 and α_2	Vasoconstriction
	β_1* and β_2	Rennin release; inhibition of tubular sodium reabsorption
Adipocytes	α_2	Inhibition of lipolysis
	β_1,β_2 and β_3	Lipolysis

* Dominant in the particular tissue.
Table compiled from references Brodde & Michel, 1999; Buscher *et al.*, 1999; Port & Bristow, 2001; Small & Liggett, 2001; Breitwieser, 2002; Adrenergic Receptor Database, 2003; Malczewska-Malec *et al.*, 2003; Small *et al.*, 2003; Bruck *et al.*, 2005 and Masuo *et al.*, 2005a.

The physiological role of the α_{2C}-AR has only come to light and studies have shown that it controls adrenaline release from the adrenal medulla and assists in presynaptically inhibiting norepinephrine release (Small & Liggett, 2001; Flordellis *et al.*, 2004).

Both lipolysis and fat tissue blood flow is stimulated by the β_2-AR (table 2.2). Evidence that β_3AR is expressed in visceral fat makes it a prime candidate for the regulation of lipolysis and insulin sensitivity in humans. This receptor, by stimulating the uncoupling protein UCP-1, alters respiration coupling and dissipates oxidation-derived energy as heat (Malczewska-Malek, 2003, Yasuda *et al.*, 2006).

The β-AR agonists have been shown to markedly increase lipid catabolism (adipocyte degradative lipid metabolism). Activation of the β-AR causes an increase in cAMP (cyclic Adenosine monophosphate) levels, which activates PKA (protein kinase A) which then phophorylates hormone-sensitive lipase. Phosphorylated lipase is the activated form that initiates the catabolic process, lipolysis. Fatty acids are produced and, to a large extent, exported from the adipocyte to be used as oxidative fuels by other tissues. Fatty acid synthesis and the esterification of fatty acids into triacylglycerol, the primary energy storage molecule in the adipocyte, are both inhibited by β-AR agonists. Thus, an increase in catabolic and a decrease in anabolic lipid metabolic processes in the adipocyte would both lead to decreased hypertrophy of the adipocyte with a consequent

decrease in fat deposition. *In vitro*, the synthetic β-AR agonist isoproterenol and the physiological agonists epinephrine and norepinephrine each increase adipocyte catabolic lipid metabolism and decrease synthetic lipid metabolism (Arner & Hoffstedt, 1999; Louis *et al.*, 2000; Mersmann, 2001).

The β-ARs are critical regulators of cardiac function in both normal and pathophysiological states. Under normal conditions, β-ARs and their signalling pathways modulate both the rate and force of myocardial contraction (table 2.2), allowing individuals to respond appropriately to physiological stress or exercise. Acute changes in myocardial function are controlled predominantly by β-AR pathways. Specifically, the signal transduction pathways triggered by agonist occupancy of β-ARs are key regulators of heart rate, systolic and diastolic function, and myocardial metabolism (Port & Bristow, 2001).

2.3.4. MECHANISM OF FUNCTION

The following figures are simplified examples of AR function and figure 2.8b illustrates only one of the several functions of these receptors (opening of a Ca^{2+} channel).
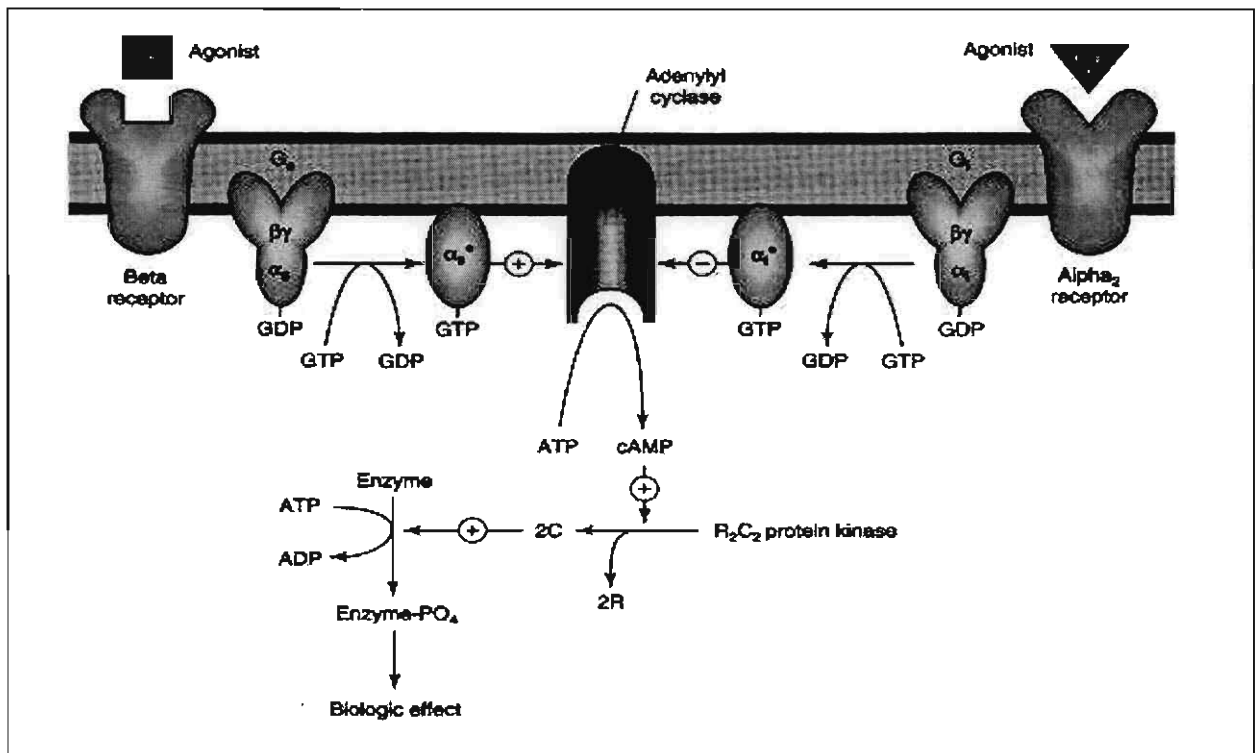
Figure 2.8a: Representation of the mechanism of adrenergic receptor functioning

(Adapted from Adrenergic Receptor Database, 2003)
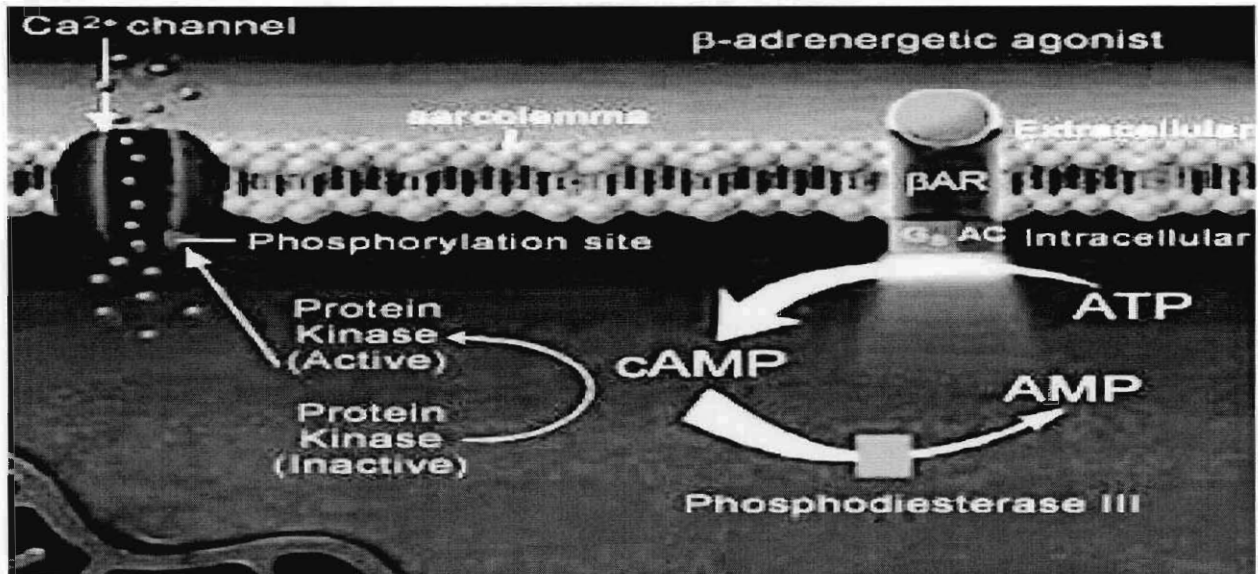
Figure 2.8b: Example of the mechanism and effect of β-adrenergic receptors stimulation

(Adapted from Adrenergic Receptor Database, 2003)

Figure 2.8a shows that the α_2- and β-AR subtypes are triggered by activation and inhibition of adenylyl cyclase with concomitant alterations in the intracellular levels of cAMP and the modulation of PKA activity. Hormones that bind to the AR activate adenylate cyclase (Mersmann, 2001; Malczewska-Malec *et al.*, 2003). Catecholamine binding induces the coupling of the receptor to a G-protein that then binds to GTP (Guanosine triphosphate). This either stimulates (Gs) or inhibits (Gi) adenylyl cyclase, thus stimulating or inhibiting the synthesis of cAMP (the second messenger). This of course happens as required: if enhanced effect is needed, then more cAMP is formed. In turn, a cascade of intracellular reactions is induced, which has the desired effect to maintain homeostasis. The Ca^{2+}-channels are also phosphorylated and open (figure 2.8b). More Ca^{2+}-ions enter the cell which could then promote contraction (Mersmann, 2001; Malczewska-Malec *et al.*, 2003; Small *et al.*, 2003; Flordellis *et al.*, 2004)

2.4. GENETIC POLYMORPHISMS

2.4.1. GENERAL

Polymorphisms are single base variations in a DNA sequence that occur at a frequency of >1% in a given population. Mutations on the other hand, are rare variants that may be the cause of an inherited disease, for example MCAD (medium-chain acyl-koA dehydrogenase) deficiency. In cases such as this, the mutation(s) are necessary and sufficient to cause the disease (Gregersen *et al.*, 1991).

Nucleotide deletions and insertions are observed in the human genome, but the most common variations are single nucleotide substitutions (single nucleotide polymorphisms or SNPs). If the polymorphisms are in the coding region of the gene, they may encode different amino acids (nonsynonymous polymorphisms) or, because of the redundancy of the genetic code, may have no effect on the encoded amino acid sequence (synonymous polymorphism). Polymorphisms also occur in the 5' UTR (untranslated region), promoter, 3' UTR as well as in introns and in general, these are more common than coding polymorphisms (Cadman & O'Connor, 2003; Insel & Kirstein, 2003; Small *et al.*, 2003).

2.4.2. ADRENERGIC RECEPTOR (AR) GENE POLYMORPHISMS

The functional consequences of polymorphisms in the AR genes could be either impaired function or enhanced activity that could impact on its physiological function which then may cause an increase in the person's risk for developing, for example, the metabolic syndrome (Hein & Kobilka, 1997; Buscher *et al.*, 1999; Small *et al.*, 2003). In other words, polymorphisms may have no effect, have effects that are clinically silent but can be revealed with physiologic testing, have an increased prevalence in certain diseases and therefore act as low level risk factors, they can act in modifying diseases, or they can alter the response to therapy (Hein & Kobilka, 1997; Buscher *et al.*, 1999; Cadman & O'Connor, 2003; Small *et al.*, 2003; Phillip & Hein, 2004). More specifically, the functional consequence of a polymorphism in the AR gene could impact upon that AR's physiological function. This could be impaired function (as in the case of the Del322-325 of the α_{2C}-AR) or over-activity (in the case of the Arg389Gly of the β_1-AR). These changes in turn may be basis for an increase a person's risk for developing, for example metabolic syndrome (Small *et al.*, 2002). In the specific case of the Del322-325 of the α_{2C}-AR it has been shown that this variant of the receptor has a marked decreased coupling to its G-protein (specifically Gi), with decreased

inhibition of adenylyl cylase and decreased stimulation of MAPK (mitogen-activated protein kinase). Thus the signal transduction pathway becomes derailed resulting in the impairment of the physiological functioning of the receptor, and ultimately leads to a pathoglogical state (Ryden *et al.*, 2001; Small *et al.*, 2003; Small *et al.*, 2004).

Thus, polymorphisms could, but not necessarily, manifest in a specific phenotype. If a polymorphism in the β_3-AR gene causes essential hypertension in certain individuals for instance, this genotype manifests in a distinct phenotype. It is important to determine whether a particular polymorphism causes a particular phenotype or if it is just simply a marker for that phenotype. The clinical importance of the AR polymorphisms is not only whether the genetic variants influence physiologic and pharmacologic responses, but also whether they contribute to disease phenotypes. This entails onset, severity, progression and complications of such a disease (Arner & Hoffstedt, 1999; Insel & Kirstein, 2003).

Studies on polymorphisms are being performed with the goal of developing allele-specific responsive drugs and identifying patients who will benefit while sustaining only minimal side effects and to optimize the treatment of an individual. An interesting example which is currently being explored, is the potential of individuals with the β_3-AR polymorphism Trp64Arg being treated with an agonist to promote weight loss (Flordellis *et al.*, 2004).

A study on the phenotypic linkage between SNPs of the β_3-AR gene and NADH (nicotine adenine dinucleotide) dehydrogenase subunit-2 (ND2) gene, with special reference to eating behaviour, showed that a combination of SNPs in the nuclear β_3-AR gene and the mitochondrial ND2 genes may affect eating behaviour, besides just the biochemical and metabolic process of signal transduction and the electron transfer system. This combination of SNPs could determine some phenotypes including eating behaviour, which could have future applications in prevention of life-style related diseases (Aoyama *et al.*, 2003).

An important feature to emphasize is the phenomenon of haplotypes. Polymorphisms should not only be considered as individual players, but as a team. It has been reported in recent studies that, for instance, a SNP could maximize the effect of another SNP at a completely different location, for instance, in another gene. It can therefore be said that knowledge of a single SNP may not provide enough predictive power in recognizing genetic predisposition (Small *et al.*, 2003; Mehrian-Shai & Reichardt, 2004). It is possible that haplotypes could result in functional consequences that would then have a direct or indirect effect on the phenotype. It can, therefore, be safe to say that

haplotypes will not always be neutral genetic markers but could in fact act as functional alleles in their own right (Reichardt, 2006).

2.4.3. RELATIONSHIP BETWEEN AR GENE POLYMORPHISMS AND THE METABOLIC SYNDROME

The two characteristics of the metabolic syndrome being investigated in this study are obesity and hypertension. The AR genes are being considered as candidate genes for the onset and / or progression of these two conditions or as contributing factors. Literature linking these genes and their altered forms to obesity and hypertension are the focus of the following paragraphs.

The β_1-AR has been implicated in hypertension and cardiovascular phenotypes in several studies. However, these are inconclusive, since results obtained have not always been replicated by other studies on the same population groups (Johnson & Terra, 2002; Small *et al.*, 2003; Flordellis *et al.*, 2004; Leineweber *et al.*, 2004; Small *et al.*, 2004).

Genetic polymorphisms of the β_2- and β_3-AR genes have been shown, although not exclusively, to have possible effects on an individual's predisposition to some of the features of the metabolic syndrome, i.e. obesity and hypertension (Fujisawa *et al.*, 1998; Arner & Hoffstedt, 1999; Buscher *et al.*, 1999; Candy *et al.*, 2000; Kato *et al.*, 2000; Bengtsson *et al.*, 2001; Strazzullo *et al.*, 2001; Johnson & Terra, 2002; Malczewska-Malec *et al.*, 2003; Small *et al.*, 2003). The involvement of β_2- and β_3-AR genes in metabolic disorders suggests that a polymorphism in the encoding genes might be an inter-individual susceptibility factor for these disorders and a spectrum of related disorders (Malczewska-Malec *et al.*, 2003; Leineweber *et al.*, 2004). This makes these genes candidates for determining genetic predisposition and therefore a risk for the development and/or progression of obesity and hypertension. In recent years, researchers have become interested in the β_2- and β_3-ARs encoding genes as candidate loci for obesity and insulin resistance in humans because of their importance in lipolysis and thermogenesis (Stahl, 1999; Santos *et al.*, 2002; Masuo *et al.*, 2005a). Currently, it seems that the β-AR polymorphisms are more likely to be risk factors rather than disease-causing genes (Leineweber *et al.*, 2004; Siani & Strazzullo, 2006).

Overweight, in particular visceral adiposity, and high blood pressure, tend to manifest together in the same individuals. Both disorders have a high degree of heritability and share a polygenic model of inheritance. High sympathetic nervous system activity has been associated with both overweight and hypertension and is believed to have pathogenic significance. One or more functional genetic abnormalities at this level could have a myriad of metabolic and cardiovascular effects, making the

genes involved in adrenergic regulation good candidates to enhance our understanding of the etiology of this association. Among the genes involved in catecholamine activity, the one encoding the β_3-AR is of particular interest, given the demonstration of functional β_3-ARs (list of functions shown in table 2.2) in human adipocytes (Strazzullo *et al.*, 2001; Masuo *et al.*, 2005a). The Trp64Arg polymorphism of the β_3-AR has been linked not only to adiposity, but also to hypertension as a secondary effect. It can therefore be said that if one could be prevented, so could the other (Azuma *et al.*, 1998; Fujisawa *et al.*, 1998; Masuo *et al.*, 2005a; Strazzullo *et al.*, 2005).

It is evident that the β_1-, β_2- and β_3-ARs are relevant in studies as candidate genes involved in creating a predisposed phenotype related to the metabolic syndrome. These polymorphisms have the potential to serve as markers for this disease as well as for its risk factors such as obesity and hypertension.

2.4.4. AR GENE POLYMORPHISMS AND OBESITY

Central and peripheral factors work together in maintaining body weight. These central and peripheral components of weight control are activated by receptors (including the ARs) for several key neurotransmitters and hormones. Since obesity results from an imbalance between caloric intake and energy expenditure, these features of the human body suggest that treatment of obesity can be based on central mechanisms that decrease the urge to eat and on peripheral mechanisms that increase the mobilization of energy. These findings then also suggest that a strategy can be developed for treating obesity by stimulating metabolism and peripheral burning of fat, rather than by inducing depression of appetite (Stahl, 1999; Yasuda *et al.*, 2006).

Adipose tissue is regulated mainly by catecholamines like epinephrine, which stimulates lipolysis and hydrolysis of triglycerides. Catecholamines also acutely regulate as well as increase the expression of mitochondrial uncoupling protein (UCP1), which generates a nucleotide-inhibitable conductance pathway in the mitochondrial inner membrane, which is stimulated by the fatty acids generated upon β-adrenergic receptor activation, thereby adjusting the amount of energy production or wasting by mitochondria (Breitwieser, 2002; Yasuda *et al.*, 2006).

Obesity is a complex metabolic disorder which is the result of the combined effects of genes and behaviour. Although there has been a search for candidate genes ("obesity genes"), the exact genes have not been identified. One possible reason for this is that obesity is determined not only by a number of different genes, but also by a large number of environmental factors. β_1-, β_2- and β_3-ARs stimulate lipolysis in human fat cells (table 2.2). Genes encoding these receptors may be

interesting candidates to explain part of the genetic predisposition to human obesity (Louis *et al.*, 2000; Kim *et al.*, 2002).

The AR genes have recently been intensely studied in connection with the metabolic syndrome and associated conditions, because of their important roles in regulation of energy mobilization and utilization (Yasuda *et al.*, 2006). An altered function of these genes could lead to energy saving in situations where stored energy has to be used over a long period of time. Where obesity is involved, it is possible that not only altered presynaptic sympathetic nerve activity but also lowered responsiveness to sympathetic activation could be the cause of a reduced basal metabolic rate and increase in weight as a result of a decline in sympathetic-mediated energy expenditure (Arner & Hoffstedt, 1999; Yasuda *et al.*, 2006).

Although the β_1-AR is indicated as being the most abundant AR in human adipose tissue, it has been proven by some studies that the AR gene polymorphism, Arg389Gly, has no significant effect on obesity in human subjects (Ryden *et al.*, 2001). On the other hand, there have been studies indicating that this polymorphism is present in subjects with a greater body weight, BMI (body mass index) and fat mass (Yasuda *et al.*, 2006).

The β_2-AR is abundant in human adipose tissue (table 2.2) and stimulates fat tissue blood flow as well as lipolysis. It is therefore the subject of studies on genes associated with obesity (Ryden *et al.*, 2001; Malczewska-Malec *et al.*, 2003). The β_2-AR is the dominant lipolytic receptor in white human adipose tissue and treatment of obese animals with selective β_2-agonists promotes a marked redistribution of body-composition with a decrease in the fat mass and increase in muscle mass (Silver *et al.*, 1997; Arner & Hoffstedt, 1999; Macho-Azcarate *et al.*, 2003). Some studies have reported that genetic variation in the human β_2AR gene could be of major importance for obesity, energy expenditure, and lipolytic β_2AR function in adipose tissue, but there is still considerable debate on this association (Louis *et al.*, 2000; Kim *et al.*, 2002).

Brown fat adipocytes express primarily α_1- and β_3-ARs, which mediate their effects through distinct signalling pathways. β_3-ARs elevate cellular cAMP, increase the rate of lipolysis and up-regulate UCP1 expression (Breitwieser, 2002). The main effect of β_3-AR stimulation is said to be the acceleration of lipolysis *in vitro* and *in vivo* (Arner & Hoffstedt, 1999; Strazzullo *et al.*, 2001). The β_3-AR stimulates the mobilization of lipids from the white fat cells and increases thermogenesis in brown fat cells; treatment of obese animals with selective β_3-adrenergic agonists reduces fat stores most effectively. However, experimental results obtained with animal models should not be taken

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as necessarily applicable to humans (Brodde & Michel, 1999; Mersmann, 2001; Malczewska-Malec *et al.*, 2003; Phillip & Hein, 2004). The role of brown fat (or adipocytes in general) in the regulation of metabolism in humans is less clear than in rodents (where it is crucial to the maintenance of nutritional homeostasis). Nonetheless the human β_3-AR gene polymorphism has been linked to obesity and early onset of type 2 diabetes also known as non-insulin dependent diabetes mellitus (NIDDM). Agonists for the β_3-AR are being considered for treatment of obesity and insulin resistance (Breitwieser, 2002).

The β_3-AR is expressed particularly in visceral adipose tissue. This receptor is perceived to play an important role in lipolysis, energy expenditure and thermogenesis in reaction to sympathetic activation (Yasuda *et al.*, 2006). An impairment of β_3-AR function could potentially lead to obesity through its effect on energy expenditure of adipose tissue. For example: as a result of altered β_3-AR function (by polymorphisms), energy storage could result instead of expenditure. This AR gene polymorphism has also been abundantly studied and found to be associated with obesity (Fujisawa *et al.*, 1998; Yasuda *et al.*, 2006)

2.4.5. AR GENE POLYMORPHISMS AND HYPERTENSION

Essential hypertension is a complex trait in which genetic, environmental, and demographic factors contribute (Kato *et al.*, 2000; Cadman & O'Connor, 2003; Siani & Strazzullo, 2006). Although extensive efforts have been made to explore genetic susceptibility to essential hypertension, consistent results have rarely been found for particular candidate genes. One possible explanation is that because individual genes play a modest role in the pathogenesis of hypertension, confounding influences of non-genetic factors may decrease (or increase) the possibility of identifying a relation between the genes and hypertension, depending on the population studied (Kato *et al.*, 2000).

The β_2-ARs mediate relaxation of blood vessels. Therefore it can be speculated that dysfunction of these receptors (as a result of a polymorphism) could possibly lead to inadequate relaxation or even constriction of vessels. At present, the physiological role of the β_3-AR in this regard remains not clearly identified (Rozec & Gauthier, 2006).

The β_2-AR gene has drawn significant attention in studies for candidate genes for hypertension. The sympathetic nervous system is a major regulator of blood pressure (BP) through mediated changes of sodium handling, vascular resistance, and cardiac output. When considering the physiological importance of the β_2-AR gene, "functional" molecular variations might cause

insufficient vasodilatation, leading to increased total peripheral resistance and ultimately resulting in hypertension (Kato *et al.*, 2000; Gumaraes & Moura, 2001; Bruck *et al.*, 2005).

Hypertension arises from an imbalance between vasoconstrictive and vasodilatory mechanisms. This may, at least partly, be due to pre- and post-sympathetic dysfunctions. In various human and animal models a generalized decrease in β-adrenergic response has been observed. Main causes of this include β-AR down-regulation, changes in G-protein levels and impaired coupling of β-AR-G protein effectors. The data on the potential role of vascular β_3-AR in hypertension remains limited. A polymorphism such as the Trp64Arg in this AR has been linked to hypertension. Some studies show that this variant of the β_3-AR may serve as a predictor for the development of abdominal obesity and hypertension with advancing age (Rozec & Gauthier, 2006). One particular study concludes that through their findings and given the location of the β_3-AR in visceral adipose tissue, its role in lipolysis and thermogenesis, the Trp64Arg polymorphism may contribute to the development of hypertension via the development of central obesity and insulin resistance, rather than a direct effect on the vascular system (Watson *et al.*, 1996).

The fact that β-blockers are so widely used in the management of chronic heart failure and hypertension suggests that activation of the β-ARs, or the sympathetic nervous system, plays an important role in the etiology of most cardiovascular diseases (Johnson & Terra, 2002, Small *et al.*, 2003).

2.5. COMPILATION OF RESULTS FROM VARIOUS PUBLISHED STUDIES

2.5.1. MINOR ALLELE FREQUENCIES

Various studies have been performed on numerous population groups in order to obtain the minor allele frequency for AR gene polymorphisms. The controversy of the results from these studies is illustrated in figure 2.9.
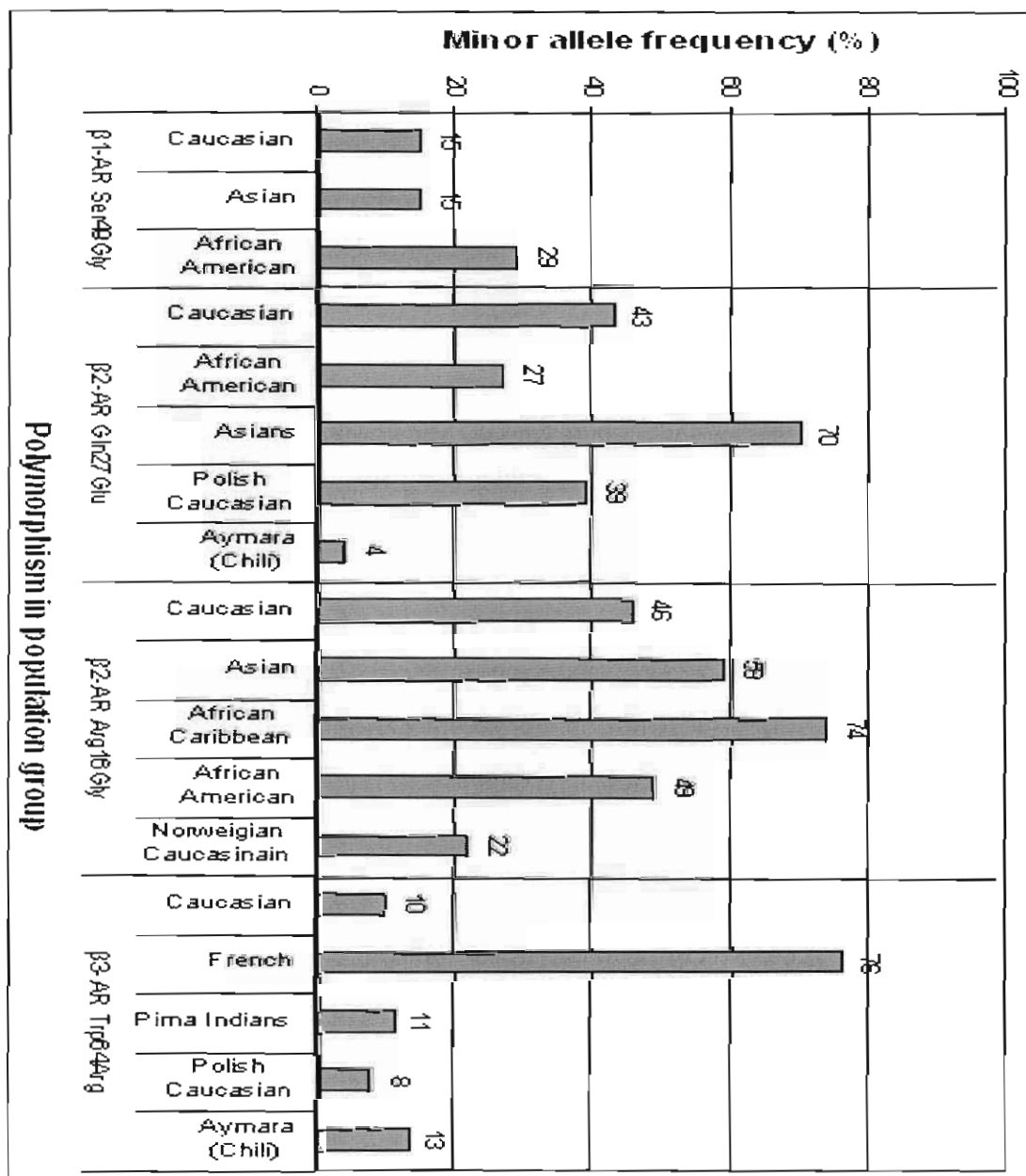
Figure 2.9: Minor allele frequencies of selected AR polymorphisms

(Compiled from: Buscher et al., 1999; Johnson & Terra, 2002; Santos et al., 2002; Malczewska-Malec et al., 2003 and Small et al., 2003)

Of relevance to this study, is the minor allele frequencies for the β_1-AR Ser49Gly, β_2-AR Gln27Glu and Arg16Gly and the β_3-AR Trp64Arg polymorphisms published for the black and white populations since the two study groups in this investigation represent black and white individuals. Three of the frequencies are relatively high, i.e. the β_2-AR Gln27Glu polymorphism in Asians, the Arg16Gly polymorphism in African-Carribeans and the β_3-AR Arg64Gly polymorphism in the French.

The differences in reported minor allele frequencies can possibly be explained by the diversity in study groups used with respect to the number of subjects. Gender, age and ancestral descent have also been mentioned as possible causes for discrepant results (Small *et al.*, 2003; Leinweber *et al.*, 2004).

2.5.2. POSSIBLE ASSOCIATIONS OF THE AR GENE POLYMORPHSISMS WITH OBESITY AND HYPERTENSION

It is important to note that although many studies have been performed on using animal models, results obtained on adrenergic gene polymorphisms from animal models (knockout mice and transgenic animals) cannot be directly extrapolated to humans, because of a number of species-related differences in the receptors (Mersmann, 2001; Malczewska-Malec *et al.*, 2003; Phillip & Hein, 2004). The results from different studies have been combined to prove the point that different associations as well as conflicting results have been obtained from different studies in different population groups. It is by far not a complete picture of all studies conducted on this subject, but is representative examples.

Table 2.3: Association of AR polymorphisms in various populations and ethnic groups with obesity

Polymorphism	Ethnic Group	
	Association	No association
β_1-AR Ser49Gly	American Caucasian (Beitelshees *et al.*, 2004)	
	African American (Beitelshees *et al.*, 2004)	
β_2-AR Gln27Glu	Japanese (Masuo *et al.*, 2005b)	Polish Caucasian (Malczewska-Malec *et al.*, 2003)
	Spanish (Macho-Azcarate *et al.*, 2003)	Korean (Kim *et al.*, 2002)
β_2-AR Arg16Gly	Japanese (Masuo *et al.*, 2005b)	Aymara from Chile (Santos *et al.*, 2002)
β_3-AR Trp64Arg	Japanese* (Breitwieser, 2002; Masuo *et al.*, 2005b)	Japanese* (Yuan *et al.*, 1997; Arner & Hoffstedt, 1999)
	German (Masuo *et al.*, 2005a)	Polish Caucasian (Malczewska-Malec *et al.*, 2003)
	Italian (Strazzullo *et al.*, 2001)	Caucasian (Arner & Hoffstedt, 1999)
	Finns* (Arner & Hoffstedt, 1999)	Finns* (Arner & Hoffstedt, 1999; Buscher *et al.*, 1999))
	French (Buscher *et al.*, 1999)	American Caucasian (Buscher *et al.*, 1999)
	Swedes (Arner & Hoffstedt, 1999)	Spanish (Del Moral *et al.*, 2002)
		Aymara from Chile (Santos *et al.*, 2002)

*Discrepancy in results from different studies on the same population group.

It has been reported that gender plays a role in an individual's susceptibility for and progression of obesity (Small *et al.*, 2003). From the compilation of published studies shown in table 2.3 it is clear that much controversy remains with respect to obesity and the presence of the β_3-AR Trp64Arg polymorphism in some population groups (Japanese and Finns).

Table 2.4: Association of AR polymorphisms in various populations and ethnic groups with hypertension

Polymorphism	Ethnic Group	
	Association	No association
β_1-AR Ser49Gly	European-Americans (Flordellis *et al.*, 2004)	Caucasians (Johnson & Terra, 2002; Leineweber *et al.*, 2004)
	Asians (Flordellis *et al.*, 2004)	
β_2-AR Gln27Glu	Japanese* (Masuo *et al.*, 2005b)	Japanese* (Kato *et al.*, 2000)
		Black African (Cockcroft *et al.*, 1998; Candy *et al.*, 2000)
		Australian Caucasian (Yasuda *et al.*, 2006)
β_2-AR Arg16Gly	Chinese (Ranade *et al.*, 2001)	American Caucasian (Herrmann *et al.*, 2000)
	Japanese (Kato *et al.*, 2000; Masuo *et al.*, 2005a; Masuo *et al.*, 2005b)	African American (Herrmann *et al.*, 2000)
	Swedish Caucasian (Bengtsson *et al.*, 2001)	Black African (Candy *et al.*, 2000)
	Norwegian Caucasian (Buscher *et al.*, 1999)	
β_3-AR Trp64Arg	Japanese* (Masuo *et al.*, 2005b)	Japanese* (Azuma *et al.*, 1998)
	Italian (Strazzullo *et al.*, 2001)	German (Buettner *et al.*, 1998)

*Discrepancy in results from different studies on the same population group

The following observations can be made from tables 2.3 and 2.4:

- The β_1-AR Ser49Gly polymorphism has been linked to obesity, but more strongly to hypertension and cardiovascular phenotypes (Small *et al.*, 2003; Flordellis *et al.*, 2004; Leineweber *et al.*, 2004)

- The β_2-AR Arg16Gly polymorphism has been studied more extensively with regards to hypertension; however, so far only indications of an association with obesity exist.

The information in tables 2.3 and 2.4 also highlight the fact that different studies have proved or disproved the mentioned AR polymorphism-phenotype associations, even in the same population group. One possible explanation for this could be the differences between the selected study groups, for example the number of participants may have been insufficient numbers for statistically significant results (statistical power). It has been mentioned previously (paragraph 2.5.1), that some degree of inconsistency occurs in the results from several studies in this regard on various population groups and ethnicities. The following tables summarize the results of some of these studies.

Table 2.5: Summary of a selection of studies proving or refuting association with obesity

AR gene Polymorphism	Obesity	
	Association	**No association**
β_1-AR Ser49Gly	Beitelshees *et al.*, 2004	
β_2-AR Gln27Glu	Santos *et al.*, 2002	Malczewska-Malec *et al.*, 2003
	Bengtsson *et al.*, 2001	Kim *et al.*, 2002
	Arner & Hoffstedt, 1999	
	Masuo *et al.*, 2005a	
	Leineweber *et al.*, 2004	
	Macho-Azcarate *et al.*, 2003	
	Lin *et al.*, 2001	
	Masuo *et al.*, 2005b	
β_2-AR Arg16Gly	Masuo *et al.*, 2005a	Santos *et al.*, 2002
	Leineweber *et al.*, 2004	
	Masuo *et al.*, 2005b	
β_3-AR Trp64Arg	Small *et al.*, 2003	Buscher *et al.*, 1999
	Buscher *et al.*, 1999	Del Moral *et al.*, 2002
	Del Moral *et al.*, 2002	Santos *et al.*, 2002
	Santos *et al.*, 2002	Malczewska-Malec *et al.*, 2003
	Arner & Hoffstedt, 1999	Arner & Hoffstedt, 1999
	Masuo *et al.*, 2005a	Buettner *et al.*, 1998
	Aoyama *et al.*, 2003	Yuan *et al.*, 1997)
	Azuma *et al.*, 1998	
	Silver *et al.*, 1997	
	Masuo *et al.*, 2005b	
	Rozec & Gauthier, 2006	

Epidemiological studies have revealed inconsistencies regarding the contributions of the β-ARs to predisposition to overweight/obesity in different populations, with a possible sex-specific effect of the Gln27Glu polymorphism with obesity only presenting in males (Bengtsson *et al.*, 2001; Santos *et al.*, 2002; Leineweber *et al.*, 2004).

Table 2.6: Summary of a selection of the studies proving or refuting association with hypertension

AR gene Polymorphism	Hypertension	
	Association	**No association**
β_1-AR Ser49Gly	Small *et al.*, 2003	Small *et al.*, 2003
	Flordellis *et al.*, 2004	Johnson & Terra, 2002
		Flordellis *et al.*, 2004
		Leineweber *et al.*, 2004
β_2-AR Gln27Glu	Kato *et al.*, 2000	Candy *et al.*, 2000
	Bengtsson *et al.*, 2001	Masuo *et al.*, 2005a
	Ranade *et al.*, 2001	Lin *et al.*, 2001
	Masuo *et al.*, 2005a	Cockcroft *et al.*, 1998
β_2-AR Arg16Gly	Kato *et al.*, 2000	Cockcroft *et al.*, 1998
	Bengtsson *et al.*, 2001	Herrmann *et al.*, 2000
	Masuo *et al.*, 2005a	
	Phillipson, 2002	
	Ranade *et al.*, 2001; Santos *et al.*, 2002	
	Hermann *et al.*, 1998	
β_3-AR Trp64Arg	Strazzullo *et al.*, 2001	Buettner *et al.*, 1998
	Ayoama *et al.*, 2003	Yuan *et al.*, 1997
	Azuma *et al.*, 1998	
	Silver *et al.*, 1997	
	Masuo *et al.*, 2005b	
	Rozec & Gauthier, 2006	

Tables 2.3 and 2.4 summarize results from studies conducted on AR gene polymorphisms and are sorted by population groups of different ethnicity. Tables 2.5 and 2.6 summarize some of the studies conducted to ascertain whether or not an association exists between AR gene polymorphisms and two main features of the metabolic syndrome. Some observations and results from various studies on the subject are listed and analysed in the following paragraphs. Note that in many studies, obesity and hypertension are both considered and investigated in association with the AR gene polymorphisms.

A possible association between the Arg389Gly β_1-AR gene polymorphism and obesity has been disproved and there has been great controversy in studies about its association with hypertension (Ryden *et al.*, 2001; Beitelshees *et al.*, 2004; Leineweber *et al.*, 2004). This is why it has not been

taken into account in this particular study. It has also been reported that the Gly389/Gly49 haplotype occurs very rarely, if at all (Johnson & Terra, 2002; Flordellis *et al.,* 2004; Leineweber *et al.,* 2004). Contradictory results have been reported by studies on the β_1-AR Ser49Gly polymorphism. Strong association with hypertension and resting heart rate have been reported by studies on European-Americans and Asians. This polymorphism has also been reported to have myocardial protective effects in patients presenting with heart failure (Small *et al.,* 2003; Flordellis *et al.,* 2004).

Studies have proven that variation in blood pressure is genetically determined to some extent. An individual's susceptibility to develop hypertension seems to be influenced by variations in many different genes, each one making a small contribution (Ranade *et al.,* 2001). Stimulation of the β_2-AR by epinephrine causes vasodilation and lipolysis (Lin *et al.,* 2001). Masuo *et al.* (2005b) stated that genes responsible for or contributing to obesity are also candidate genes for diseases related to obesity, such as hypertension.

Several polymorphisms in the β_2-AR have been identified. Three of these occur in the USA with a high frequency, i.e. Cys19Arg, Arg16Gly and Gln27Glu and are in close linkage disequilibrium. What this means is that the Arg16 rarely occurs with Arg19 or Glu27, but Arg19 almost always occurs with Glu27. This supports the notion of examining haplotypes for disease association (Lima *et al.,* 2003). The Arg16Gly and Gln27Glu polymorphisms do not have an effect on ligand binding nor adenylyl cyclase activity; however, they do have an effect on the receptor desensitization. Studies have demonstrated that these forms of the receptor resisted agonist-promoted down-regulation. However, conflicting results have been obtained concerning the codon 16 polymorphism (Johnson & Terra, 2002; Lima *et al.,* 2003; Small *et al.,* 2003). The haplotype Arg16Glu27 occurs in less than 1% of the population. Position 16 determines the phenotype of 3 haplotypes of the β_2-AR polymorphisms namely Arg16Gln27, Gly16Gln27 and Gly16Glu27 with regard to agonist-promoted receptor down-regulation (Bruck *et al.,* 2005).

It has been reported that insulin resistance and hyperinsulinemia are associated with heightened sympathetic nerve activity and that this, as seen in elevated plasma norepinephrine levels, predicts insulin resistance, subsequent weight gain and blood pressure elevation. The β_2-ARs are also expressed on pancreatic β-cells to modulate insulin secretion (Phillipson, 2002; Lima *et al.,* 2004; Masuo *et al.,* 2005a). It has been suggested that the Gly16 polymorphism of the β_2-AR gene could influence insulin secretion. Studies have reported that subjects with the Gly16 allele also had higher plasma insulin and norepinephrine levels, which suggests that this allele is closely linked to the insulin-resistant status which is associated with increased sympathetic nerve activity shown as

higher plasma norepinephrine levels and elevated blood pressure. Thus the Gly16 allele of the β_2-AR gene could possibly lead to increased sympathetic over-activity, insulin resistance, elevated blood pressure and adiposity. The argument can therefore be made that the presence of this polymorphism could predict these developments in non-obese, non-hypertensive individuals (Phillipson, 2002; Masuo et al., 2005a).

Results from studies have implied that sympathetic over-activity is associated with genetic determinants on the β-AR that may be a contributing factor to insulin resistance. The findings of the study by Masuo et al. (2005a) suggest that insulin resistance could be partially determined by the Gly16 variant of the β_2-AR gene and that this polymorphism along with elevated plasma norepinephrine levels could increase insulin resistance, obesity and elevated blood pressure. This study also stated that the Gln27Glu polymorphism was not observed to be associated with insulin resistance, which may be attributed to the fact that the frequency of the Glu27 allele is so low.

Some studies have shown the β_2-AR polymorphisms to be associated with hypertension in black and white subjects (Kato et al., 2000; Ranade et al., 2001); while others claim that the association between β_2-AR polymorphisms and hypertension is still unclear (Bengtsson et al., 2001). It remains vague whether these polymorphisms are causative, or contributing to hypertension (Johnson & Terra, 2002).

In a five year study, an association was observed between the polymorphisms Arg16Gly of the β_2-AR and Trp64Arg of the β_3-AR and higher frequencies of weight gain and blood pressure elevation. Subjects with the Gln27Glu polymorphism also showed a higher frequency of blood pressure elevation (Masuo et al., 2005b).

There have been a number of studies exploring the possible association between the Trp64Arg variant of the β_3-AR and various features of the metabolic syndrome, including overweight and the tendency to gain weight over time, insulin resistance and high blood pressure. Results have been controversial and firm conclusions on the possible role of this polymorphism have not yet been reached or validated (Silver et al., 1997; Yuan et al., 1997; Azuma et al., 1998; Strazzullo et al., 2001; Leineweber et al., 2004). When expressed in artificial cell systems (cell lines), this polymorphism is associated with alterations in the β_3-AR. The genetic allele variance influences the native receptor function when measured in isolated human adipocytes. This polymorphism has been shown by studies to be of functional significance by lowering receptor responsiveness to agonists (Arner & Hoffstedt, 1999).

The β_3AR 64Arg allele seems to be a thrifty gene candidate as it is more frequent among women and is associated with menarche at earlier age and with a decrease in energy expenditure (Malczewska-Malec et al., 2003). It can be postulated that if energy expenditure decreases, energy saving will increase and subsequent weight gain may follow.

It has been reported that the β_3-AR not only functions in lipolysis and thermogenesis in adipocytes, but also modulates the peripheral vascular tone in dogs. It induces vasodilation predominantly in skin and fat. One of the studies done reported the difference in adiposity had a significant effect on the difference in blood pressure, raising the possibility that the higher blood pressure could be at least partly secondary to the effect of the variant allele on body fat mass and distribution (Strazzullo et al., 2001). Studies of a large sample of unselected male subjects, concluded that there is an indication that the Trp64Arg variant of the β_3-AR predicts a greater tendency to develop overweight, abdominal adiposity and high blood pressure with advancing age (Silver et al., 1997; Azuma et al., 1998; Strazzullo et al., 2001 Ayoama et al., 2003; Rozec & Gauthier, 2006), while other studies contradicted these findings (Buscher et al., 1997; Buettner et al., 1998).

2.5.3. POSSIBLE REASONS FOR THE ALLEGED DISCREPANCIES

Small differences in minor allele frequencies reported by various studies conducted on the same population group (ethnic group) might be attributed to the difference in the number of subjects used in the particular studies (Small et al., 2003; Leineweber et al., 2004). Other possible reasons for differences in results obtained from the various studies include differences in the age, gender and ethnicity (region-specific polymorphisms) of the persons in the study groups (Arner & Hoffstedt, 1999; Strazzullo et al., 2001; Santos et al., 2002; Malczewska-Malec et al., 2003; Flordellis et al., 2004). Another reason could be that there was insufficient statistical power (study group too small) and a lack of standardized definition for the phenotype for hypertension (Johnson & Terra, 2002). It is known that β-AR sensitivity decreases with age (Malczewska-Malec et al., 2003) while blood pressure increases (Strazzullo et al., 2001). Another possible reason for the observed discrepancy which deserves mention, is the fact that a genetic variant may interact with others (polymorphisms) to influence body fat. It is said that genes do not speak in monologues, but sing in choirs, from there the idea of haplotypes progressed (Buscher et al., 1999; Malczewska-Malec et al., 2003; Mehrian-Shai et al., 2004; Reichardt, 2006). Phenotypes (such as obesity and hypertension) are not solely determined by our genetic make-up, but also by environmental factors. These factors (like urbanization, sedentary lifestyle and diet), however, remain elusive and unclear at present (Flordellis et al., 2004; Saini & Strazzullo, 2006).

2.5.4. GAPS STILL EXIST IN ASSOCIATION STUDIES ON THE AR GENE POLYMORPHISMS

Still unclear: Whether the β_2-AR polymorphisms play a role in disease susceptibility (disease-causing gene), progression (disease-modifying gene) or variations in drug response (treatment-response gene) (Leineweber *et al.,* 2004). The question also remains the same for the other AR gene polymorphisms as well as for potential diplotypes and haplotypes.

Future approaches: A better way of studying the functional role and interaction of the polymorphic ARs could possibly not focus on single SNPs for individual AR genes as has been mostly the case till now. The focus of studies needs to shift more towards haplotypes (combinations of SNPs within one gene) and "functional haplotypes" (combinations of SNPs within and between genes). This notion is supported in the literature (Mehrian-Shai & Reichardt, 2004; Reichardt, 2006). Furthermore, population studies with the maximum number of subjects should be undertaken to obtain statistically significant results with less room for doubt (increased statistical power).

2.6. AIMS AND APPROACH

The general aim of this study was to search for possible association between AR gene polymorphisms and obesity and hypertension (high blood pressure).

The objectives of this study were to:

- Determine the incidence of the following polymorphisms in 102 black and 115 white South African female volunteers and calculate the minor allele frequency:
    - β_1-AR: Ser49Gly
    - β_2-AR: Arg16Gly

        Gln27Glu
    - β_3-AR: Trp64Arg;
- identify possible diplotypes and haplotypes in the study groups;
- take relevant physiological parameters (measured in the POWIRS studies) into account in the search for possible associations of these polymorphisms, diplotypes and haplotypes with obesity and high blood pressure as characteristics of the metabolic syndrome;
- compare the black and the white study groups with regards to the above mentioned objectives.

The approach formulated to reach these goals is provided in the following flow diagram.

```
┌──────────────────────────────────────────────────────────┐
│        Isolate DNA from blood spot on Guthrie card       │
└──────────────────────────────────────────────────────────┘
                            │
                            ▼
┌──────────────────────────────────────────────────────────┐
│               Amplify desired genes by PCR               │
└──────────────────────────────────────────────────────────┘
                            │
                            ▼
┌──────────────────────────────────────────────────────────┐
│      Digest PCR product with specific restriction enzyme │
└──────────────────────────────────────────────────────────┘
                            │
                            ▼
┌──────────────────────────────────────────────────────────┐
│      Separate DNA fragments by agarose gel electrophoresis│
└──────────────────────────────────────────────────────────┘
                            │
                            ▼
┌──────────────────────────────────────────────────────────┐
│  Visualize ethidium bromide stained fragments and assign genotypes │
└──────────────────────────────────────────────────────────┘
                            │
                            ▼
┌──────────────────────────────────────────────────────────┐
│   Statistical analysis of measured physiological parameters │
└──────────────────────────────────────────────────────────┘
                            │
                            ▼
┌──────────────────────────────────────────────────────────┐
│  Comparison of physiological parameters between genotypes,│
│  diplotypes, haplotypes and between both groups of subjects│
└──────────────────────────────────────────────────────────┘
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Figure 2.10: Experimental approach for this study

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2.7. THE POWIRS STUDIES

2.7.1. INTRODUCTION

During 2003 – 2004 the POWIRS (Profile of Obese Women with the Insulin Resistance Syndrome) case-control studies were conducted by the School of Physiology, Nutrition and Consumer Sciences of the Faculty of Health Sciences, North-West University, on 102 black (POWIRS I) and 115 white (POWIRS II) female volunteers from the North-West Province of South Africa. The aim of this part of the overall study was to look for a possible association between the β_2-AR Gln27Glu and β_3-AR Trp64Arg gene polymorphisms with parameters of carbohydrate and lipid metabolism, index of insulin resistance, BMI and percentage body fat in African women and white women in South Africa.

The POWIRS studies were approved by the Ethics Committee of the North-West University (Potchefstroom Campus) and the study protocols conformed to ethical guidelines prescribed by the Helsinki Declaration of 1975 (ethics approval number 03M03). The subjects were all fully informed about the study objectives and procedures prior to their inclusion and with assistance the information could be provided to them in their own home language when required. An informed consent form was signed by all the volunteers (further details pertaining to the POWIRS studies provided in paragraphs 3.2 and 3.7).

2.7.2. CONCLUSION

In the POWIRS I study (102 black women) no direct association was found between the investigated polymorphisms (β_2-Gln27Glu and β_3-Trp64Arg AR gene polymorphisms) and parameters of lipid and carbohydrate metabolism or other characteristics/indices of the metabolic syndrome in these South African female volunteers.

2.7.3. RELEVANCE TO THIS STUDY

In the POWIRS I study on the black South African female volunteers, certain physiological parameters were measured as well as the genotypes determined for the β_2-AR Gln27Glu and the β_3-AR gene polymorphisms. In the POWIRS II study, the physiological parameters were also measured for white South African female volunteers. The genotypes were not yet determined.

For this study, and in conjunction with the POWIRS studies, we determined the genotypes for the same polymorphisms in the 115 white women that were determined in the black woman, namely the β_2-AR Gln27Glu and the β_3-AR gene Trp64Arg polymorphisms. We also determined the genotypes in both groups for the β_1-AR Ser49Gly and the β_2-AR Arg16Gly. The possible association of these four AR gene polymorphisms, as well as diplotypes for the $\beta2$-AR gene and haplotypes for all four, are presented here.

CHAPTER 3: MATERIALS AND METHODS

3.1   INTRODUCTION

DNA was isolated from blood collected on Guthrie cards obtained from volunteers participating in the POWIRS projects. The desired gene fragments were amplified using the standardized PCR protocol outlined in this chapter. After restriction enzyme digestion the presence or absence of the AR gene polymorphism (particular alleles) were visualized through agarose gel electrophoresis. Genotypic findings were statistically processed along with measured physiological parameters (POWIRS I and II studies).

3.2   STUDY SUBJECTS

The subjects for the POWIRS studies were "selected volunteers" because they are all female employees of a governmental institution in Potchefstroom, North-West Province of South Africa. The POWIRS I study comprised of 102 urban African female volunteers (these black women were not of homogenous descent, for instance only Zulu, but mixed). Inclusion criteria was apparently healthy African women 19 to 55 years of age and the exclusion criteria was pregnancy, lactation and ear temperatures above 37ºC. For the POWIRS II study, 115 white South African female volunteers were used with inclusion criteria being apparently healthy white women (homogenous group of caucasian South African women) between 19 and 55 years of age and the same exclusion criteria applying (Schutte *et al*, 2005).

3.3   STANDARDIZATION OF PCR

The method employed for amplification of the desired gene fragments needed to be standardized for each of the PCR oligonucleotide sets. This was done in a similar way for each of the four investigated polymorphisms using genomic DNA, but only a single example will be presented. The oligonucleotides used were synthesized by Inqaba Biotechnologies Inc. from published sequences (provided in Appendices A and F). The following parameters for the PCR method were standardized: temperature gradient, annealing temperature and agarose gel concentration (method and results for the latter not shown).

3.3.1 TEMPERATURE GRADIENT

PCR was initially performed at 2 mM $MgCl_2$ (the concentration of the buffer supplied with the commercially obtained Taq DNA polymerase, table 3.2) to determine the optimal annealing temperatures of the various oligonucleotide primer sets and the reactions were carried out using genomic DNA isolated with the Wizard kit (Promega, A1120). The principle followed was to set up a temperature gradient 4 – 5 °C to both sides of the calculated temperature provided on the synthesis reports of the oligonucleotide primers.

The β_2-AR Gln27Glu polymorphism is described as an example (PCR product 310 bp). The annealing temperatures given by the synthesis report of the primers from Inqaba were: forward 69.53 °C and reverse 59.9 °C. In an initial experiment (results not shown) it was determined that the annealing temperature is lower than 66 °C (no DNA bands were visible at this and above temperatures) and thus the temperature range was adjusted accordingly.

Table 3.1: Different annealing temperatures tested

Tube number	Temperature (°C)
1	55.4
2	57.4
3	58.6
4	60.2
5	62.0
6	63.8

Before the PCR mastermixes could be made up, the following dilutions had to be made: the dNTPs and oligonucleotide primers. This was done in order to prevent contamination and to improve on accuracy by increasing the volume added to the reactions.

For 20 reactions:

dNTP mix
The initial concentration of 20 mM of the dNTP mix was diluted to 2 mM (5 times dilution) by adding 10 μl dNTP mix to 80 μl ddH$_2$O.

Primers
To 36 μl of ddH$_2$O ,4 μl of primer was added to make up a solution with 20 pmoles in 0.2 μl.

Table 3.2: PCR mastermix with given dilutions

Component	Supplier and catalog number	Volume for 1 reaction (μl)
$MgCl_2$ free buffer (10x)	Promega, M1865	5
$MgCl_2$ (25mM)	Promega, M1865	4
dNTP (dilution)	New England Biolabs, N0447S	5
Forward primer (dilution)	Inqaba Biotechnologies	2
Reverse primer (dilution)	Inqaba Biotechnologies	2
8 % DMSO*	Sigma-Aldrich, D8418	4
ddH_2O		2.6
Taq DNA polymerase	Promega, M1865	0.4
	Total volume for 1 reaction:	25

*DMSO was added to the reactions for the β_1-AR Ser49Gly and the β_2-AR Arg16Gly polymorphisms to allow the polymerase enzyme to gain access to the DNA after it has been boiled off the card (these genes are apparently prone to folding). Not too high a concentration should be added, because it could lessen the activity of the polymerase enzyme by up to 50 %. This was not necessary for the other 2 tested polymorphisms.

To have enough mastermix for all the reactions, the volumes shown in the second column were multiplied by the number of reactions plus two to compensate for experimental errors.

Approximately 300 ng of genomic DNA (3 μl) was added to 22 μl ddH_2O to make up a final volume of 25 μl. To this was added 25 μl PCR mastermix (table 3.2) and PCR was performed on a Hybaid thermocycler with temperature being the only variable. The PCR program was configured as follows: initial denaturation at 94 °C for 5 minutes, 30 cycles of the following: denaturation at 94 °C for 1 minute, annealing for 1 minute – at selected temperatures (table 3.1) and extension at 72 °C for 1 minute, followed by a final extension step at 72 °C for 10 minutes.

The temperature providing the best defined band of the expected length (expected length for the specific AR gene fragment) with no/the least amount of background in the agarose gel was selected.

3.3.2 $MgCl_2$ CONCENTRATION

The concentration of $MgCl_2$ in a PCR reaction mixture is very crucial and needs to be standardized. For this purpose genomic DNA was used and a $MgCl_2$ concentration series ranging from 0 – 4mM was tested at the experimentally determined temperature. The reaction components are provided in tables 3.3 and 3.4.

Table 3.3: Mastermix for standardization of the MgCl₂ concentration

Component	Volume for 1 reaction (μl)
dNTP mix	5
Forward primer	4
Reverse primer	4
MgCl₂ free buffer (10x)	5
Taq DNA polymerase	0.4
Total volume	18.4

To have enough mastermix for all the reactions, the volumes shown in the second column were multiplied by the number of reactions plus two to compensate for experimental errors.

Table 3.4: Components of each PCR reaction mixture for standardization of MgCl₂ concentration

Tube number	1	2	3	4	5	6	7	8
[MgCl₂] mM	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
Volume of reagent added (μl)								
MgCl₂	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0
ddH₂O	27.6	26.6	25.6	24.6	23.6	22.6	21.6	20.6
Genomic DNA	3	3	3	3	3	3	3	3
Total volume	31.6	31.6	31.6	31.6	31.6	31.6	31.6	31.6

The mastermix (table 3.3) was added to each reaction tube (table 3.4) and PCR performed using the standardized annealing temperatures and the same program configuration, only the MgCl₂ concentration differed between the reaction tubes. Of the PCR product 15μl was loaded onto the agarose gel. The MgCl₂ concentration at which the strongest band (thus highest yield of PCR product) with no/the least amount of background in the agarose gel was chosen.

3.4 AMPLIFICATION OF DESIRED GENE FRAGMENTS USING THE STANDARDIZED METHOD

The method below was adapted from the one described by Gregersen *et al.* (1991) with the standardized annealing temperatures (table 4.1) and optimal MgCl₂ concentration determined to be 2 mM.

3.4.1 EXTRACTION OF DNA FROM GUTHRIE CARDS

Two 2 mm^2 punches from each Guthrie card (samples collected in the POWIRS I and II studies) were transferred to a PCR reaction tube. To this was added 50 μl 100% methanol (M3641, Sigma-Aldrich) and the tubes left to stand at room temperature for approximately 10 minutes. The card punches were then dried in a Speedvac for one hour at medium heat. To the dried paper was added 25 μl ddH$_2$O and boiled at 95 °C for 15 minutes.

3.4.2 PCR AMPLIFICATION OF THE DESIRED GENE FRAGMENTS

After the boiled solution had cooled down to room temperature, 25 μl PCR mastermix (table 3.2) was added. PCR was then performed on a computerized Hybaid Thermocycler. The PCR program was configured with the same steps used in standardization, except that the temperature for annealing was set at that determined as optimal for the specific primer set (table 4.1).

3.5   RESTRICTION ENZYME DIGESTION OF PCR PRODUCTS

Genetic polymorphisms either abolish a restriction enzyme recognition site (for example the β_3-AR Trp64Arg polymorphism) or create a restriction site (for example the β_1-AR Ser49Gly polymorphism). This makes it possible to distinguish between the different genotypes with the known lengths of the DNA fragments resulting from restriction enzyme digestion of the PCR product. In Appendix E the expected DNA fragment sizes for the polymorphisms are provided by which the various genotypes can be assigned.

Table 3.5: Restriction enzyme mastermix

Component	Volume for 1 reaction (μl)
ddH$_2$O	2.25
Restriction enzyme buffer(10x)	2.5
Restriction enzyme	0.25
Total volume for 1 reaction:	5

From the PCR product 20 μl was transferred to a fresh tube and 5 μl of the restriction enzyme mastermix (table 3.5) added to it. The mixture was then incubated at 37 °C for 2 hours.

3.6   SEPARATION OF DNA FRAGMENTS BY AGAROSE GEL ELECTROPHORESIS

The following solutions needed to be prepared before agarose gel electrophoresis of restriction enzyme digested PCR products.

TAE buffer

A 50 times concentrated stock solution was prepared by adding the following to 900 ml ddH$_2$O, adjusting the pH to 7 with HCl$_c$ and then filling up to 1 L:

242.0 g Tris base

57.1 g Glacial acetic acid

18.61 g Na$_2$EDTA·H$_2$O

Loading buffer

For 40% w/v loading buffer, 0.4 g sucrose was added to 1 ml ddH$_2$O and the tube vortexed until the sucrose dissolved completely. No dye was added.

A 3 % gel was prepared by adding 4.5 g of agarose (Roche, 1 816 586) to 150 ml TAE buffer. 5 μl ethidium bromide solution is added for every 100 ml of gel. Before loading the gel, 4 μl (40% w/v sucrose solution) loading buffer was added to the restriction enzyme digested PCR product. The total volume of the tube was loaded onto the gel. The gel was run at 60 V for one and a half hour before visualization on a SynGene Geldoc system.

3.7   PHYSIOLOGICAL PARAMETERS

The cut-off point of BMI was set at 25 in dividing the subjects into a non-obese and obese group for comparison. Parameters of carbohydrate and lipid metabolism, HOMA-IR (fasting glucose x fasting insulin/22.5), BMI and percentage body fat were measured. Weight, height and waist circumference were measured with calibrated instruments and standard methods (Schutte et al, 2005). The percentage body fat and HOMA-IR (index of insulin resistence) were calculated. A stethoscope and mercury sphygmomanometer were used to monitor blood pressure. Fasting blood samples were taken, after which a two-hour oral glucose tolerance test was done. Blood samples were collected every 30 minutes after glucose ingestion. Plasma glucose, insulin and various lipid levels were determined using standard methods (hexokinase method for plasma glucose concentration, enzyme immunoassay for insulin levels). Table 3.6 lists the physiological parameters measured in the POWIRS studies.

Table 3.6: Physiological parameters measured in the POWIRS studies

Parameter	Description	Motivation for measurement
BMI (kg/m^2)	Body mass index	Indicator of obesity
WC (cm)	Waist circumference	Indicator of abdominal obesity
Body fat %	Body fat percentage	Indicator of obesity
SBP (mmHg)	Systolic blood pressure	Blood pressure parameters
DBP (mmHg)	Diastolic blood pressure	
Tc (mmol/l)	Total blood cholesterol	Parameters of lipid metabolism
HDL (mmol/l)	High density lipoprotein concentration	
LDL (mmol/l)	Low density lipoprotein concentration	
Trig (mmol/l)	Triglyceride concentration	
OGTT0 (mmol/l)	Blood Glucose concentration	Parameters of carbohydrate metabolism Measured over time at 0, 30, 60, 90 and 120 minutes during the oral glucose tolerance test
OGTT30		
OGTT60		
OGTT90		
OGTT120		
FFA0 (mmol/l)	Blood free fatty acid concentration	
FFA30		
FFA60		
FFA90		
FFA120		
INS0 (pmol/l)	Blood insulin concentration	
INS30		
INS60		
INS90		
INS120		
HOMA-IR	Homeostatic model of insulin resistance; Insulin resistance index	Indicator of insulin resistance

3.8 STATISTICAL ANALYSIS

After the genotype for each test subject for the selected AR gene polymorphisms were determined, it was combined with the physiological parameters measured in the POWIRS studies (paragraph 3.7). Data was analyzed using the Statistica 7 software package (StatSoft, Inc., 2000, Microsoft Excel 2002, Microsoft, Inc., 2002 and Graphpad Prizm 4 (Graphpad software, Inc. 2003). Hardy-Weinberg equilibrium was tested. The t-test, independent by group analysis was performed in order to compare the different physiological parameters obtained from subjects between the genotypes of the four AR gene polymorphisms. Observed diplo- and haplotypes were documented. The physiological parameters were compared for the different diplo-and haplotypes using the unpaired t-test. The level of statistical significance was set at P ≤ 0.05. Area under the curve (AUC) analysis was performed to compare the parameters measured during the oral glucose tolerance test (OGTT), significant difference set at $\geq 5\%$. More detail will be provided with reporting of the results.

CHAPTER 4: RESULTS

4.1 STANDARDIZATION OF THE PCR METHOD AND CONDITIONS

The methods employed for the amplification of the different gene fragments needed to be standardized to facilitate the accurate genotyping of the investigated AR gene polymorphisms.

4.1.1 ANNEALING TEMPERATURE
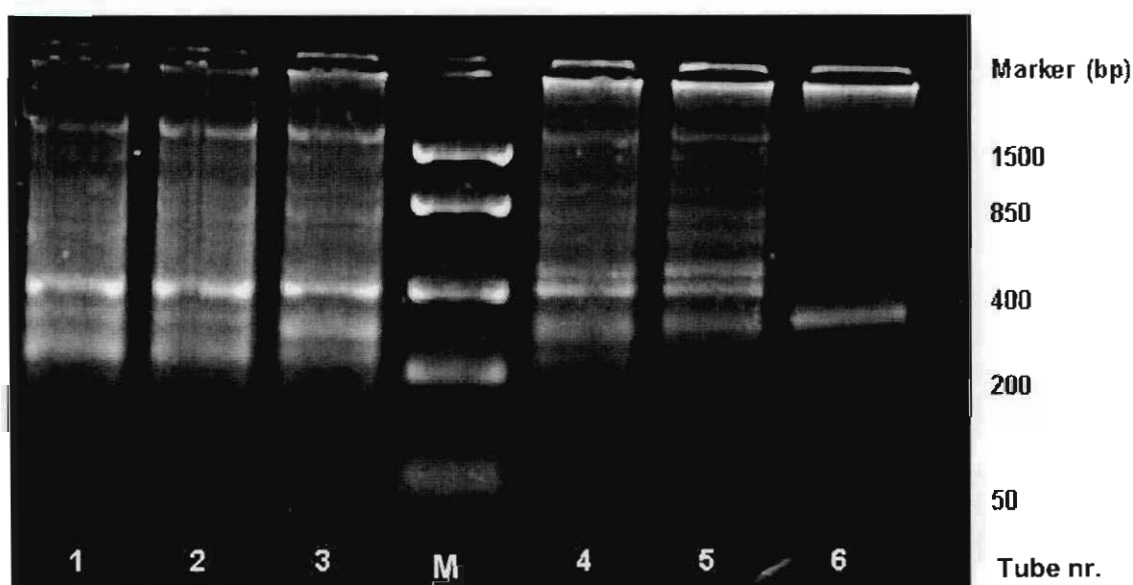
Figure 4.1: PCR product yield for the β_2-AR gene fragment at different annealing temperatures for the specific set of oligonucleotides

The DNA marker used (M) was the low range FastRuler from Fermentas (SM1103)

To demonstrate the methods followed for all four of the investigated polymorphisms the β_2-AR Gln27lu polymorphism will be taken as an example. The specific temperatures at which the DNA fragment was amplified in each tube are provided in table 4.1. It can clearly be seen that the optimal annealing temperature for PCR of the gene fragment of β_2-AR is the temperature represented by tube 6. In the case of the β_2-AR Gln27lu polymorphism, the primers have multiple annealing sites, therefore it is clear from the results given in figure 4.1 why standardization of the annealing temperature was necessary – to eliminate non-specific binding and unwanted PCR products. The fragment of interest (PCR product) is 310 bp in size and can be seen distinctly in lane 6 with minimal non-specific PCR products.

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Table 4.1: Optimal annealing temperatures for the oligonucleotide sets

Polymorphism	Annealing temperature (°C)
β_1-AR Ser49Gly	59.0
β_2-AR Arg16Gly	61.7
β_2-AR Gln27Glu	64.0
β_3-AR Trp64Arg	66.0

Table 4.1 provides the optimized annealing temperatures for the specific sets of oligonucleotides for the specific AR gene polymorphisms investigated in this study. These were standardized to ensure accurate and maximum PCR product yield of the desired gene fragments.

4.1.2 MgCl₂ CONCENTRATION

The next step was to determine the optimal $MgCl_2$ concentration for each set of oligonucleotides. This was performed at the above determined optimal annealing temperatures.
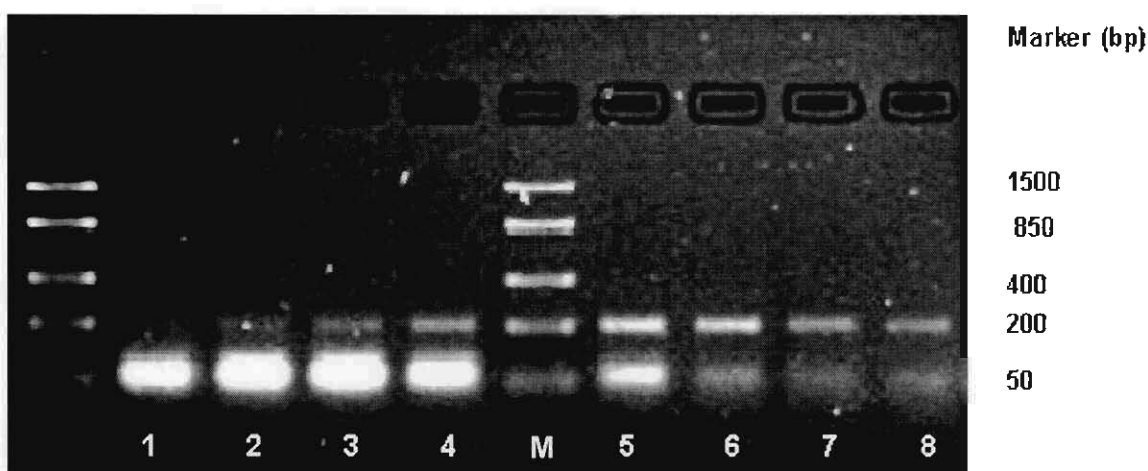
Figure 4.2: PCR product yield for the β_2-AR gene fragment at different MgCl₂ concentrations

The DNA marker used (M) was the low range FastRuler from Fermentas (SM1103)

The PCR product yield in lane 5 in figure 4.2 is clearly the brightest and was therefore selected as the optimal $MgCl_2$ concentration, which was 2 mM. In the same way, the optimal $MgCl_2$ concentration was determined for the remaining three AR gene polymorphisms and found to be 2 mM for each of them. The bright bands visible at the bottom of all 8 lanes are the so-called "primer dimers" which form as a result of a certain degree of complementarity between the two oligonucleotide primers used in the PCR reactions (virtually unavoidable, no matter how well the oligonucleotides are designed). Different primer concentrations were tested, and the concentration described in section 3.3.1 were found to be optimal for maximum PCR product yield. As the optimal

annealing temperature is approached, the primer dimer bands fade as more and more of the oligonucleotides are incorporated into the PCR product.

4.2   STANDARDIZED METHOD FOR DETERMINING THE GENOTYPES

The expected DNA fragments for each polymorphism after restriction enzyme digestion of the particular PCR product are shown in Appendix E. The fragments present in the agarose gel were analyzed with the Genetools program (version 3.06, SynGene 2005) to confirm their size and to ensure that the genotype of each individual was correctly assigned. It was found that in almost all of the cases accurate genotype identification could be performed with the naked eye from the picture taken of the agarose gels with the GelDoc system.
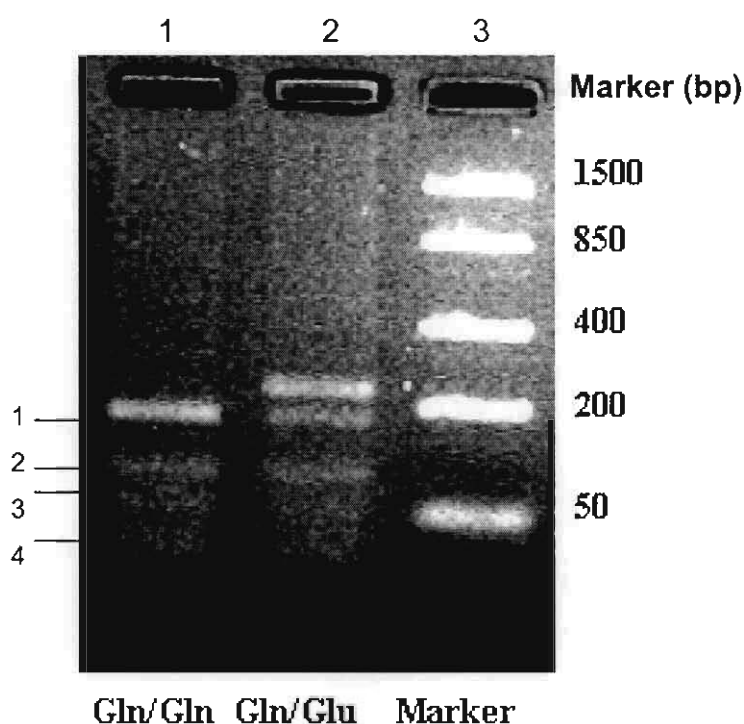
Figure 4.3: Resolution of DNA fragments in an agarose gel for genotypes of the β_2-AR Gln27Glu polymorphism

(The 3% agarose gel was run at 60V for one and a half hour and visualized on a Geldoc system)

To illustrate the point that the differentiation can easily be made, two genotypes for the β_2-AR Gln27Glu polymorphism are given in figure 4.3. In lane 1, 4 bands can be distinguished (numbers 1 to 4 on the far left of this figure), representing a homozygote for the wild type (Gln/Gln) at this locus. Lane 1 clearly shows the following bands (length identified with the help of the marker in lane 3): 171 (1), 84 (2) and 55 (3) bp. For the heterozygote genotype in lane 2 (Gln/Glu) the bands visible

are the same bands as the Gln/Gln genotype (1-4), with an additional 226 bp band. The band at the very bottom (numbered 4) represents the primer-dimers.

To minimize space taken up by the number of tables and in order to present only the relevant results in this chapter, the following abbreviations were used for the genotypes:

- Hm: homozygous for the polymorphism, the individual possesses two (identical) polymorphic alleles, e.g. Gly/Gly for the β_1-AR Ser49Gly polymorphism.
- Ht: heterozygotes possessing one wild type and one polymorphic allele, e.g. Ser/Gly in the case of the β_1-AR Ser49Gly polymorphism.
- N: homozygous for the wild type of the AR, the individual has two copies of the wild type alleles of the AR gene, e.g. Ser/Ser for the β_1-AR Ser49Gly polymorphism.

Table 4.2: Observed genotypes in the two study cohorts

Female group	Polymorphism	Genotype		
		Hm	Ht	N
Black	β_1-Ser49Gly	71	30	1*
	β_2-Gln27Glu	5	23	74
	β_2-Arg16Gly	20	61	21
	β_3-Trp64Arg	5	48	49
White	β_1-Ser49Gly	102	13	0**
	β_2-Gln27Glu	28	49	38
	β_2-Arg16Gly	28	61	26
	β_3-Trp64Arg	0**	16	99

*too few subjects present in the group with this genotype for further statistical analysis
**no subject identified with this genotype

Not all the expected genotypes for the AR gene polymorphisms involved in this investigation were represented in the study subjects (table 4.2). In the case of the β_1-AR Ser49Gly polymorphism, only one homozygote for the wild type of the receptor gene was present in the group of black women and amongst the white women, none was identified. For the β_3-AR Trp64Arg polymorphism there were no homozygotes among the group of white women. These genotypes were therefore not considered in the statistical analysis and comparisons.

In the POWIRS studies (see paragraph 2.7) various parameters were measured. In table 3.3 the relevant parameters to this study, pertaining to obesity and blood pressure are listed. The abbreviations are listed briefly below each table.

Table 4.3 reports the statistical information (descriptive statistics, means and standard deviations of all the relevant measured parameters) of the black and the white study subjects as well as the comparison of the values between the two groups.

Table 4.3: Statistical description of the study groups

Parameter	Black women (n=102)		White women (n=115)		P-values
	Mean	SD	Mean	SD	
Age (years)	31.25	8.64	31.34	9.18	0.945
BMI (kg/m^2)	27.98	6.33	28.48	7.15	0.588
WC (cm)	87.18	14.77	86.00	14.94	0.558
SBP (mmHg)	129.82	19.60	125.41	11.69	**0.043**
DBP (mmHg)	77.68	10.69	72.47	9.01	**0.000**
Tc (mmol/l)	4.25	0.93	4.94	1.03	**0.000**
HDL (mmol/l)	1.25	0.33	1.21	0.32	0.287
LDL (mmol/l)	2.85	0.87	3.15	0.93	**0.017**
Trig (mmol/l)	0.71	0.42	1.28	0.70	**0.000**
OGTT0 (mmol/l)	5.19	1.19	5.04	0.41	0.190
OGTT30	7.19	1.42	7.74	1.33	**0.004**
OGTT60	6.99	2.23	7.65	1.89	**0.021**
OGTT90	6.76	2.18	7.01	1.90	0.381
OGTT120	6.59	1.99	6.60	1.66	0.990
FFA0 (mmol/l)	0.54	0.27	0.56	0.19	0.621
FFA30	0.30	0.21	0.34	0.21	0.167
FFA60	0.09	0.09	0.08	0.08	0.858
FFA90	0.04	0.04	0.05	0.06	0.328
FFA120	0.03	0.02	0.04	0.07	**0.026**
INS0 (pmol/l)	92.94	41.47	92.66	33.47	0.956
INS30	659.05	444.11	603.47	375.75	0.326
INS60	597.84	400.91	576.99	361.49	0.692
INS90	477.61	373.92	479.23	330.75	0.973
INS120	428.58	325.75	385.33	277.42	0.300
HOMA-IR	3.09	1.55	3.04	1.29	0.791

Bold print indicates significant statistical difference (P ≤0.05)
BMI: body mass index; WC: waist circumference, SBP: Systolic blood pressure, DBP: diastolic blood pressure, Tc: total blood cholesterol, HDL: high density lipoproteins, LDL: low density lipoproteins, Trig: triglycerides, OGTT: oral glucose tolerance test, FFA: free fatty acids, INS: insulin, HOMA-IR: index of insulin resistance

Normal distributions were observed in both the study groups. Both the SBP and DBP were significantly higher in the black group compared to the white group. The Tc, LDH and Trig, however, were higher in the white women than the black. Only three of the parameters measured during the oral glucose tolerance test were significantly higher in the white women. The measured physiological parameters, although found to be significantly different, were still within the normal ranges.

4.3　HARDY-WEINBERG EQUILIBRIUM

The Hardy-Weinberg principle (also Hardy–Weinberg equilibrium (HWE), or Hardy–Weinberg law, named after G. H. Hardy and Wilhelm Weinberg) states that the frequency of alleles and genotypes at a single gene locus remains relatively unchanged from generation to generation in a large, interbreeding population characterized by random mating, Mendelian inheritance and the absence of migration, mutation and selection. In most cases not all of these criteria are met, but this does not necessarily mean a deviation from the Hardy-Weinberg equilibrium. This principle also states that under these conditions, the ratio of individuals homozygous for a dominant allele to those heterozygous and to those homozygous for a recessive allele is 1:2:1 (Hickman *et al.*, 2001:124; McClean, 1997).

In diploid organisms, in the case of a single gene locus with two alleles A and a, with allele frequencies of p and q, Hardy-Weinberg Equilibrium predicts that the genotype frequencies for AA will be p^2 and for aa will be q^2. The number of individuals would then be for example p^2 x n (n=number of subjects). A homozygote for the mutation contributes 2 polymorphic alleles, a heterozygote only one because the other allele is of the wild type or the AR gene and a homozygote for the wild type contributes no polymorphic allele (thus only the wild type alleles), because they don't possess it. The minor allele percentage is calculated by adding up the number of polymorphic alleles present in the group, dividing that by the total number of alleles (twice the number of individuals in the group since each individual inherits 2 copies of everyu locus – one from maternal and one from paternal origen) and then multiplying that number by 100 to get a minor allele percentage. If the true number observed is equal to what is expected, then the locus is said to be in Hardy-Weinberg equilibrium.

The Hardy-Weinberg Principle disproves the popular misconception that a characteristic associated with a dominant allele increases in frequency because it is genetically dominant and a rare allele does not disappear from a large population simply because it is rare.

This principle also states that the hereditary process alone does not produce evolutionary change. In large biparental populations, equilibrium is established in one generation between allelic frequencies and genotype ratios which remains constant in following generations, unless disturbed by recurring mutations, natural selection, migration, non-random mating or genetic drift (random sorting).

This is explained in terms of dominant and recessive alleles, but can also be applied to wild type (normal) alleles and polymorphic alleles. Therefore the expected frequencies can be calculated and compared to the frequencies observed in the group of test subjects. The Pearson's chi-squared test is used to test deviation from the Hardy-Weinberg principle, comparing the observed genotype frequencies from data of the test subject group to the expected values obtained using the Hardy-Weinberg principle (Hickman *et al.*, 2001:124, McClean, 1997).

Table 4.4: Compliance with Hardy-Weinberg equilibrium

Group	Polymorphism	Genotype	Observed number with genotype	Expected number with genotype	Results of Chi-squared test
Black women	β_1-Ser49Gly	Hm	71	73	
		Ht	30	27	
		N	1	3	0.5
	β_2-Gln27Glu	Hm	5	3	
		Ht	23	28	
		N	74	72	0.2
	β_2-Arg16Gly	Hm	20	25	
		Ht	61	51	
		N	21	26	0.1
	β_3-Trp64Arg	Hm	5	8	
		Ht	48	42	
		N	49	52	0.3
White women	β_1-Ser49Gly	Hm	102	102	
		Ht	13	12	
		N	0	0	0.8
	β_2-Gln27Glu	Hm	28	24	
		Ht	49	57	
		N	38	34	0.3
	β_2-Arg16Gly	Hm	28	30	
		Ht	61	57	
		N	26	28	0.8
	β_3-Trp64Arg	Hm	0	1	
		Ht	16	15	
		N	99	100	0.7

A value < 0.05 obtained from the Chi-squared test indicates inconsistency with HWE
Hm: homozygote for the polymorphism, Ht: heterozyogte, N: homozygote for the wild type allele

Hardy-Weinberg equilibrium was observed in all the AR gene polymorphisms as the chi-squared test returned values of > 0.05 in all the instances for both groups. The numbers used in testing the HWE were then employed to calculate the minor allele frequency for each of the polymorphism in both groups.

4.4   MINOR ALLELE FREQUENCIES

A minor allele frequency is the frequency (prevalence) of the polymorphic allele in the test subject group. The minor allele frequency was calculated for all four of the selected AR gene polymorphisms for both groups. This was done by adding up the number of polymorphic alleles present in the group, dividing this by the total number of alleles (twice the number of individuals in the group because two alleles are present for each gene – one maternally inherited and one paternally) and then multiplying that by 100 to get a minor allele percentage. (Hm = 2 polymorphic alleles, Ht = 1 polymorphic allele and N = 0 polymorphic alleles). Thus the minor allele frequency is the prevalence of the minor allele (the polymorphic allele) for a given polymorphism in a test group and not the genotype frequency (the number of individuals homozygous for the polymorphism).
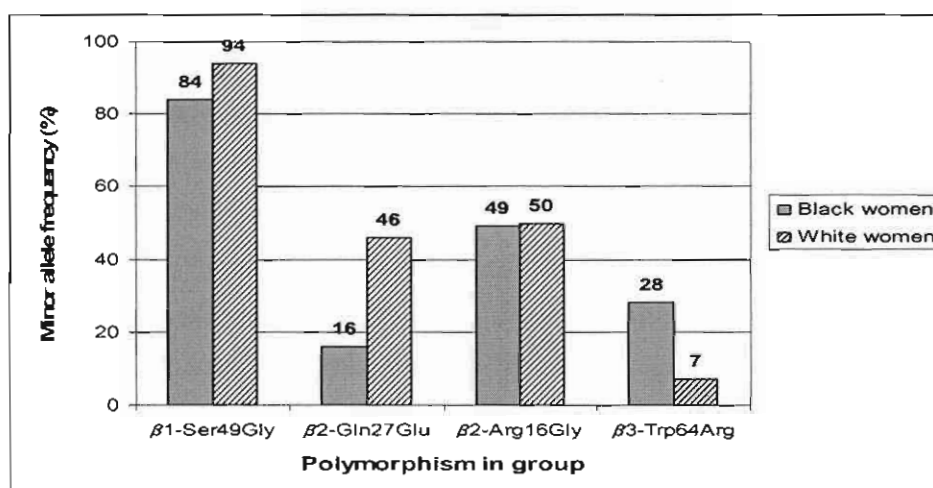
Figure 4.4: Minor allele frequencies of the subjects involved in the POWIRS studies

The minor allele frequencies for the β_1-AR Ser49Gly polymorphism are the highest in both the black and the white women (figure 4.4). In the case of the β_1-AR Ser49Gly the minor allele frequencies observed for the two groups differed by 10 %. For the β_2-AR Arg16Gly polymorphism the allele frequencies are similar (only 1% difference). However, for both polymorphisms the frequency for the white women is the highest. It is also clear from figure 4.4 that the β_2-AR Gln27Glu and β_3-AR Trp64Arg polymorphism minor allele frequencies differ to a great extent between the groups. In the case of the β_2-AR Gln27Glu polymorphism minor allele frequency of the white women exceeded that of the black women by 30 %. In the case of the β_3-AR Trp64Arg polymorphism the black women have a minor allele frequency of 21% higher than the white women. This is the only polymorphism of the four investigated where the minor allele frequency for the black women was higher than that of the white women.

4.5   COMPARISON OF MEASURED PHYSIOLOGICAL PARAMETERS

Additional notes on some of the parameters measured:

- The oral glucose tolerance test (OGTT). The body's ability to process or absorb the glucose is monitored by drawing a fasting blood sample, and drawing blood every thirty minutes after a ratio of glucose per body weight is given for two hours and determining the glucose concentration at each time interval.

- The insulin levels (INS) are measured in conjunction with the glucose levels. This was performed to determine whether the body is processing the glucose normally – a process for which insulin is necessary (for the cells of the body to absorb glucose).

- The free fatty acid levels (FFA) in the blood are also measured at the same time intervals as the glucose and insulin levels. If cells cannot absorb / use glucose (primary energy source), they resort to free fatty acids. These have to be mobilized from the liver into the blood. High levels of FFA at the time / in conjunction with the glucose loading would indicate an energy shortage in the body and hence pointing towards ineffective glucose uptake and utilization by the cells.

- The index of insulin resistance (HOMA-IR) is calculated as follows: (fasting glucose x fasting insulin) / 22.5. The current accepted cut-off point for diagnosis of insulin resistance is reported as 3.16 (Keskin *et al.*, 2006).

The divisions of the test group on the basis of BMI (with a cut-off point of 25) as well as the entire group, irrespective of BMI, were statistically processed. The latter was performed to gain statistical power. The tables containing these results are in Appendix F and only the parameters that showed significant differences are reported and discussed.

Certain parameters showed a statistical difference only when the group was divided according to BMI, others came to light only when the division was done away with and then there were some that persisted throughout. This phenomenon of appearance, disappearance and persistence of significance, is described in the following paragraphs. Where there was no significant difference found between measured parameters for genotypes, it is indicated by a "-"next to the genotypes that were compared. Where there were no individuals present with a particular genotype, that comparison was left out from those listed.

4.5.1 GROUP OF BLACK WOMEN

In the case of the β_1-AR Ser49Gly polymorphism there were only 2 individuals with the Hm genotype that fell into the BMI ≤25 group, and only a single N genotype present in the entire group of black females. Only one parameter, i.e. OGTT30 differed significantly when the entire group was compared but wasn't observed in the BMI divided groups.

For the β_2-AR Gln27Glu polymorphism several significant differences were observed between the genotypes and measured parameters when the entire group was compared. Only 5 individuals represented the Hm genotype and when divided by BMI, 3 had a BMI ≤25 and only 2 a BMI > 25, thus making the groups too small for significant comparisons. For this AR gene polymorphism, the following significant differences were:

- BMI ≤25
    - Hm vs. Ht: HDL
    - Hm vs. N: -
    - Ht vs. N: -
- BMI > 25
    - Hm vs. Ht: DBP, Body fat %, OGTT60, OGTT120, INS60 and INS120, Trig
    - Hm vs. N: DPB, Body fat %, OGTT60, FFA30
    - Ht vs. N: OGTT60
- Entire group
    - Hm vs. Ht: DBP, Body fat %, OGTT60, OGTT90, FFA30, INS60
    - Hm vs. N: DBP, Body fat %, OGTT60, FFA30, INS60, INS90
    - Ht vs. N: OGTT60, OGTT90, INS90

When the **entire group** was **compared to the BMI divided groups,** it became clear that the only significant difference in HDL shown in the group with **BMI ≤25** disappeared and most of the associations in the group with **BMI > 25** persisted. Two additional parameters were shown to be significantly different when comparing Ht and N genotypes in the entire group. In most cases, one or more of the parameters measured over time were present (OGTT, INS, FFA), and more were shown to be significantly different when the BMI division was excluded.

The *β₂-AR Arg16Gly polymorphism* showed the following significant differences between parameters measured for the genotypes:

- BMI ≤25
  - Hm vs. Ht: FFA60, FFA90
  - Hm vs. N: FFA30, FFA60, FFA90
  - Ht vs. N: -
- BMI > 25
  - Hm vs. Ht: SBP, OGTT120, FFA90, FFA120
  - Hm vs. N: FFA60, FFA90, FFA120
  - Ht vs. N: INS0, HOMA-IR
- Entire group
  - Hm vs. Ht: FFA90, FFA120
  - Hm vs. N: FFA30, FFA60, FFA90, FFA120
  - Ht vs. N: -

Most of the parameters of the FFA differed significantly except for the FFA0 (free fatty acids present in the blood under fasting conditions). When the **entire group** was compared, all the significant differences observed for the group with **BMI ≤25** persisted, but only the FFA parameters for the comparison between genotypes of the group with **BMI > 25**.

For the *β₃-AR Trp64Arg polymorphism* the following differences in measured parameters were found:

- BMI ≤25
  - Hm vs. Ht: -
  - Hm vs. N: INS30
  - Ht vs. N: -
- BMI > 25
  - Hm vs. Ht: -
  - Hm vs. N: -
  - Ht vs. N: -
- Entire group
  - Hm vs. Ht: -
  - Hm vs. N: OGTT90, INS30
  - Ht vs. N: -

Note that there were only 2 individuals in the group with **BMI** ≤**25** with the Hm genotype. When comparing the three genotypes, only a single significant difference was found in INS30 between the Hm and the N genotypes. This difference persisted when the **entire group** irrespective of BMI genotypes Hm and N were compared and OGTT90 parameter appeared as significantly different between these two genotypes.

4.5.2 GROUP OF WHITE WOMEN

For the *β₁-AR Ser49Gly polymorphism* the following significant differences were observed:
- BMI ≤25
  - o Hm vs. Ht: BMI
- BMI > 25
  - o Hm vs. Ht: -
- Entire group
  - o Hm vs. Ht: INS0, INS30, INS120, HOMA-IR

Where the genotypes were compared in the **entire group**, the difference in BMI between the Hm and Ht genotypes found in the group with BMI ≤ 25 disappeared and three of the five INS parameters and the HOMA-IR showed significant difference between genotypes.

The *β₂-AR Gln27Glu polymorphism* showed the following significant differences:
- BMI ≤25
  - o Hm vs. Ht: Tc, HDL
  - o Hm vs. N: INS0, HOMA-IR
  - o Ht vs. N: -
- BMI > 25
  - o Hm vs. Ht: -
  - o Hm vs. N: OGTT0, OGTT120
  - o Ht vs. N: -
- Entire group
  - o Hm vs. Ht: -
  - o Hm vs. N: -
  - o Ht vs. N: -

When the entire group was used in the statistical analysis, no statistically significant differences persisted that were observed in the groups divided by BMI and no physiological parameters showed significant difference.

When the measured physiological parameters for the genotypes of the *β₂-AR Arg16Gly polymorphism* were compared, the following significant differences were observed:

- BMI ≤25
    - o   Hm vs. Ht: Age
    - o   Hm vs. N: DBP
    - o   Ht vs. N: Age, INS30, INS90, INS120
- BMI > 25
    - o   Hm vs. Ht: FFA30
    - o   Hm vs. N: -
    - o   Ht vs. N: -
- Entire group
    - o   Hm vs. Ht: FFA30, INS60
    - o   Hm vs. N: -
    - o   Ht vs. N: BMI, WC

When a covariance analysis for age was performed for the group with **BMI ≤25** no significant differences were found between the measured parameters for the genotypes of the AR genes. Only a single parameter differed significantly between the Hm and Ht genotypes of the group with **BMI > 25** namely FFA30. This was the only significant difference that persisted from comparison of genotypes for the BMI divided groups to the **entire group**.

In the case of the *β₃-AR Trp64Arg polymorphism* the following parameters were found to be different:

- BMI ≤25
    - o   Ht vs. N: -
- BMI > 25
    - o   Ht vs. N: SBP, OGTT0
- Entire group
    - o   Ht vs. N: OGTT30

The significantly differing physiological parameters observed for the group with **BMI > 25**, were not present for the entire group. Here the **OGTT30** appeared as significantly different.

The results obtained when the measured physiological parameters were compared between the two groups of women are listed and discussed below with regards to each of the investigated AR gene polymorphisms.

4.5.3 GROUPS COMPARED IRRESPECTIVE OF BMI

This discussion focusses on a comparison between the black women and the white women with reference to measured physiological parameters and the genotypes of the four investigated AR gene polymorphisms.

Table 4.5: Comparison of measured parameters for the genotypes of the β_1-AR Ser49Gly polymorphism

Parameter	Hm (n=71)		Ht (n=30)		P-values
	Mean	SD	Mean	SD	Hm vs. Ht
Black women					
OGTT30 (mmol/l)	7.00	1.37	7.60	1.49	**0.054**
White women					
INS0 (pmol/l)	89.75	32.59	115.23	32.78	**0.009**
INS30	575.59	349.84	813.62	500.76	**0.031**
INS120	360.04	240.02	576.00	441.79	**0.008**
HOMA-IR	2.94	1.29	3.75	1.15	**0.034**

Bold print shows significant statistical difference (P ≤0.05)
Hm: homozygote for the polymorphism, Ht: heterozyogte, N: homozygote for the wild type allele
OGTT: oral glucose tolerance test, INS: insulin, HOMA-IR: index of insulin resistance
SD: standard deviation

In table 4.5 only a single significant difference in the OGTT30 was observed between the Hm and the Ht genotypes of the β_1-AR Ser49Gly polymorphism in the black women. Significant differences were observed between only four of the physiological parameters measured between the Hm and Ht genotypes for the white women. Three of which were measured during the oral glucose tolerance test. All of these parameters are related to insulin activity.

Table 4.6: Comparison of measured parameters for the genotypes of the β_2-AR Gln27Glu polymorphism

Parameter	Hm (n=5) Mean	SD	Ht (n=23) Mean	SD	N (n=74) Mean	SD	P-values Hm vs. Ht	Hm vs. N	Ht Vs. N
Black women									
Body fat %	22.58	4.28	35.16	12.45	33.75	11.01	**0.037**	**0.027**	0.606
DBP (mmHg)	64.67	9.54	79.52	10.61	77.99	10.32	**0.008**	**0.006**	0.538
OGTT60 (mmol/l)	4.65	1.53	8.03	2.56	6.84	2.01	**0.009**	**0.020**	**0.025**
OGTT90	5.11	1.35	7.70	2.42	6.59	2.05	**0.031**	0.118	**0.036**
FFA30 (mmol/l)	0.08	0.07	0.35	0.21	0.30	0.20	**0.011**	**0.017**	0.333
INS60 (pmol/l)	222.00	132.04	749.00	458.89	580.10	377.50	**0.019**	**0.039**	0.089
INS90	179.40	52.06	636.19	553.43	452.41	301.54	0.082	**0.048**	**0.048**
White women									
None									

Bold print shows significant statistical difference (P ≤0.05)
Hm: homozygote for the polymorphism, Ht: heterozyogte, N: homozygote for the wild type allele
DBP: diastolic blood pressure, OGTT: oral glucose tolerance test, FFA: free fatty acids, INS: insulin
SD: standard deviation

For the β_2-AR Gln27Glu polymorphism, several significant differences were present (table 4.6 above) in the black women, most of which were one or more parameters measured during the oral glucose tolerance test. For the white women no significant differences were found between the three genotypes of this AR gene polymorphism.

Table 4.7: Comparison of measured parameters for the genotypes of the β_2-AR Arg16Gly polymorphism

Parameter	Hm (n=20) Mean	SD	Ht (n=61) Mean	SD	N (n=21) Mean	SD	P-values Hm vs. Ht	Hm vs. N	Ht vs. N
Black women									
FFA30 (mmol/l)	0.40	0.23	0.30	0.20	0.23	0.15	0.071	**0.008**	0.155
FFA60	0.13	0.09	0.08	0.10	0.05	0.03	0.066	**0.000**	0.147
FFA90	0.07	0.04	0.04	0.04	0.03	0.02	**0.008**	**0.004**	0.513
FFA120	0.04	0.03	0.03	0.01	0.02	0.01	**0.000**	**0.004**	0.628
White women									
BMI (kg/m^2)	27.01	6.00	30.12	7.69	26.22	6.13	0.062	0.634	**0.024**
WC (cm)	83.57	13.40	89.51	15.60	80.35	13.01	0.085	0.376	**0.010**
FFA30 (mmol/l)	0.28	0.16	0.38	0.21	0.32	0.23	**0.022**	0.400	0.254
INS60 (pmol/l)	465.32	316.18	636.64	356.87	563.68	400.84	**0.033**	0.323	0.413

Bold print shows significant statistical difference (P ≤0.05)
Hm: homozygote for the polymorphism, Ht: heterozyogte, N: homozygote for the wild type allele
FFA: free fatty acids, BMI: body mass index, WC: waist circumference, INS: insulin
SD: standard deviation

The differences observed for the FFA parameters measured during the oral glucose tolerance test for the black women, were between the Hm and Ht, and the Hm and N genotypes. All the FFA parameters differed significantly between the Hm and N genotypes except for the fasting value. For the white women, however, no significant differences were found between the Hm and N genotypes.

Table 4.8: Comparison of the measured physiological parameters for the genotypes of the β_3-AR Trp64Arg polymorphism

Parameter	Hm (n=5)		Ht (n=48)		N (n=49)		P-values		
	Mean	SD	Mean	SD	Mean	SD	Hm vs. Ht	Hm vs. N	Ht Vs. N
Black women									
OGTT90 (mmol/l)	5.22	0.83	6.55	2.28	7.11	2.11	0.204	**0.054**	0.227
INS30 (pmol/l)	942.60	743.42	712.91	489.61	579.55	344.09	0.348	**0.052**	0.126
White women									
OGTT30	*	*	8.33	1.70	7.64	1.23	*	*	**0.053**

Bold print shows significant statistical difference (P ≤0.05)
*insufficient number of subjects with specific genotype
Hm: homozygote for the polymorphism, Ht: heterozyogte, N: homozygote for the wild type allele
OGTT: oral glucose tolerance test, INS: insulin
SD: standard deviation

For the β_3-AR Trp64Arg polymorphism, there were only two significant differences observed in the entire group of black women (table 4.8). Both were measured during the oral glucose tolerance test. Only one significant difference was observed for the white women between the Ht and N genotypes of the β_3-AR Trp64Arg polymorphism.

4.5.4 GENOTYPES COMPARED IRRESPECTIVE OF BMI

The physiological parameters measured for both the groups were compared with respect to the same genotype of an AR gene polymorphism.

Table 4.9: Comparison of AR gene polymorphism genotypes between the two study cohorts

Parameter	β_1-AR Ser49Gly		β_2-AR Gln27Glu			β_2-AR Arg16Gly			β_3-AR Trp64Arg	
	Hm	Ht	Hm	Ht	N	Hm	Ht	N	Ht	N
SBP (mmHg)	**0.047**	0.932	0.265	**0.043**	0.310	**0.046**	0.500	0.443	0.538	0.181
DBP (mmHg)	**0.001**	0.390	0.067	**0.002**	**0.023**	0.200	**0.005**	**0.016**	0.141	**0.013**
Tc (mmol/l)	**0.000**	0.232	0.081	**0.008**	**0.000**	0.104	**0.000**	**0.007**	0.079	**0.000**
HDL (mmol/l)	0.804	**0.044**	**0.049**	0.934	0.893	0.377	0.392	0.912	0.073	0.807
LDL (mmol/l)	**0.012**	0.931	0.059	0.245	0.184	0.355	**0.051**	0.378	0.401	**0.021**
Trig (mmol/l)	**0.000**	**0.000**	0.069	**0.001**	**0.000**	0.022	**0.000**	**0.001**	**0.000**	**0.000**
OGTT0 (mmol/l)	0.197	0.601	**0.047**	0.271	0.398	**0.042**	0.602	0.581	0.713	0.420
OGTT30	**0.001**	0.367	**0.052**	0.979	**0.008**	0.646	**0.011**	0.061	**0.004**	0.106
OGTT60	**0.017**	0.519	**0.006**	0.412	**0.015**	0.746	0.058	**0.055**	**0.042**	0.421
OGTT90	0.285	0.825	**0.044**	0.267	0.303	0.850	0.474	0.216	0.091	0.555
FFA30 (mmol/l)	0.086	0.704	**0.013**	0.662	0.684	**0.039**	**0.028**	0.115	0.198	0.589
FFA120	0.073	0.157	0.444	0.213	0.013	0.392	0.114	0.110	**0.028**	0.178
INS0 (pmol/l)	0.349	**0.004**	0.502	0.652	0.611	0.866	0.284	0.220	0.524	0.497
INS90	0.971	0.073	**0.054**	0.105	0.197	0.520	0.423	0.947	0.311	0.532
HOMA-IR	0.303	**0.013**	0.315	0.410	0.705	0.512	0.359	0.239	0.553	0.440

Bold print shows significant statistical difference (P ≤0.05)
Hm: homozygote for the polymorphism, Ht: heterozyogte, N: homozygote for the wild type allele
SBP: systolic blood pressure, DBP: diastolic blood pressure, Tc: total blood cholesterol, HDL: high density lipoproteins, LDL: low density lipoproteins, Trig: triglycerides, OGTT: oral glucose tolerance test, FFA: free fatty acids, INS: insulin, HOMA-IR: index of insulin resistance

It was found that between the two groups the least amount of differences occurred for the FFA and INS parameters measured during the oral glucose tolerance test. Significant differences were found between the Hm genotype for the β_1-AR Ser49Gly in the black and the white women with respect to the blood pressure parameters. The same differences were observed for the Ht genotype of the β_2-AR Gln27Glu. For both groups the Tc differs between the majority of the AR gene polymorphism genotypes.

4.6 AREA UNDER THE CURVE (AUC) ANALYSIS

Area under the curve analyses were performed in order to compare the total response to the oral glucose tolerance test (OGTT) and effectiveness of the glucose-insulin system with FFA (free fatty acids) and INS (insulin) measured simultaneously. This was performed because the t-test only compares the values at a certain time in the OGTT, for example the glucose, insulin and free fatty acid concentration at 60 minutes after the OGTT has commenced. A difference may exist at one of the time points when calculated with the t-test, and be missed, while AUC analysis shows that there exists a difference in the overall test.
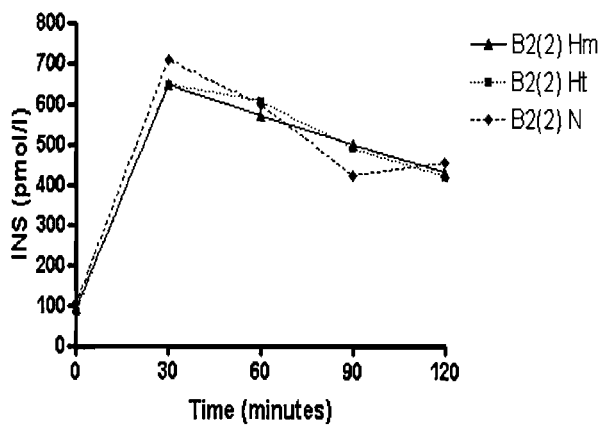
Figure 4.5a: AUC results for black women showing no significant difference
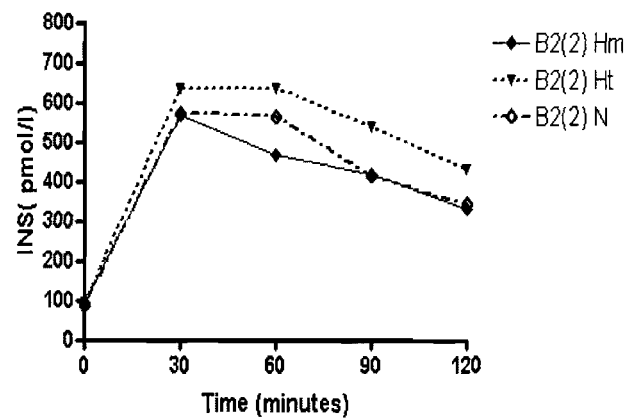
Figure 4.5b: AUC results for white women showing significant differences

Figure 4.5: Examples of area under the curve (AUC) analysis for the insulin levels (INS) in different genotypes of the β_2-AR Arg16Gly polymorphism

Figure 4.5 is an example of the results obtained from AUC analysis. Figure 4.5a was obtained by plotting the results of the INS for black women with respect to the β_2-Arg16Gly polymorphism. When inspected visually (and not statistically analyzed) one may speculate that there is a significant difference between the three genotypes, but after AUC analysis, it becomes clear that there is indeed no significant difference (see table 4.12). Figure 4.5b was obtained by plotting the INS levels over time for the white women for the same polymorphism. In this case, there exists a clear statistically significant difference between the AUC values for the INS parameters of all three genotypes.

Table 4.10: AUC analysis for OGTT

| Group | Polymorphism | AUC value | | | Percentage difference | | |
		Hm	Ht	N	Hm vs. Ht	Hm vs. N	Ht vs. N
Black women	β_1-Ser49Gly	791.6	831.7	*	4.8	*	*
	β_2-Gln27Glu	633.5	887.7	791.6	**28.6**	**20.0**	**10.9**
	β_2-Arg16Gly	809.9	813.9	772.4	0.5	4.6	5.1
	β_3-Trp64Arg	698.1	791.9	827.7	**11.8**	**15.7**	4.3
White women	β_1-Ser49Gly	844.5	860.8	*	1.9	*	*
	β_2-Gln27Glu	750.7	842.9	847.2	**10.9**	**11.4**	0.5
	β_2-Arg16Gly	809.9	862.4	849.9	**6.1**	4.7	1.4
	β_3-Trp64Arg	*	906.1	836.5	*	*	**7.7**

*indicates where there was insufficient number of subjects (or none) to be statistically useful in comparisons
Bold print indicates significant difference in AUC (> 5%)
Hm: homozygote for the polymorphism, Ht: heterozyogte, N: homozygote for the wild type allele

For the blood glucose concentration measured during the oral glucose tolerance test, the biggest differences in AUC were observed between the Hm and Ht, and the Hm and N genotypes of the β_2-Gln27Glu polymorphism in both groups. Differences for the same genotype comparisons were found for the β_3-Trp64Arg polymorphism in the black women. The Hm and Ht genotypes differed for the β_2-Arg16Gly polymorphism and the Ht and N genotypes for the β_3-Trp64Arg polymorphism in the white groups.

Table 4.11: AUC analysis for INS

| Group | Polymorphism | AUC value | | | Percentage difference | | |
		Hm	Ht	N	Hm vs. Ht	Hm vs. N	Ht vs. N
Black women	β_1-Ser49Gly	57992	59079	*	1.8	*	*
	β_2-Gln27Glu	32157	71874	58287	**55.3**	**44.8**	**18.9**
	β_2-Arg16Gly	59378	59922	60141	0.9	1.3	0.4
	β_3-Trp64Arg	63309	60984	58467	3.7	**7.4**	4.1
White women	β_1-Ser49Gly	54507	75468	*	**27.8**	*	*
	β_2-Gln27Glu	53601	55688	61026	3.7	**12.2**	**8.7**
	β_2-Arg16Gly	49782	62155	52948	**19.9**	**6.0**	**14.8**
	β_3-Trp64Arg	*	61418	56210	*	*	**8.5**

*indicates where there was insufficient number of subjects (or none) to be statistically useful in comparisons
Bold print indicates significant difference in AUC (> 5%)
Hm: homozygote for the polymorphism, Ht: heterozyogte, N: homozygote for the wild type allele

When the blood insulin levels were measured for the black women, all the genotypes for the β_2-Gln27Glu polymorphism yielded significant difference in results. In the case of the white women, all three genotypes for the β_2-Arg16Gly polymorphism differed significantly.

Table 4.12: AUC analysis for FFA

Group	Polymorphism	AUC value Hm	AUC value Ht	AUC value N	Percentage difference Hm vs. Ht	Percentage difference Hm vs. N	Percentage difference Ht vs. N
Black women	β_1 Ser49Gly	20.9	22.0	*	5.0	*	*
	β_2 Gln27Glu	12.5	23.7	21.5	**47.4**	**42.0**	**9.4**
	β_2 Arg16Gly	27.5	21.1	17.0	**23.3**	**38.4**	**19.7**
	β_3 Trp64Arg	16.9	21.6	22.0	**21.7**	**23.3**	2.1
White women	β_1 Ser49Gly	23.6	21.4	*	**9.7**	*	*
	β_2 Gln27Glu	21.4	25.4	22.2	**16.7**	3.4	**12.7**
	β_2 Arg16Gly	22.0	24.9	21.4	**12.1**	2.4	**14.1**
	β_3 Trp64Arg	*	25.0	23.1	*	*	**7.6**

*indicates where there was insufficient number of subjects (or none) to be statistically useful in comparisons
Bold print indicates significant difference in AUC (> 5%)
Hm: homozygote for the polymorphism, Ht: heterozyogte, N: homozygote for the wild type allele

For the free fatty acid levels measured in both cohorts, only in two instances did the genotypes of the AR gene polymorphisms not differ significantly.

Table 4.13: Comparison of the AUC between the two groups for each genotype of the investigated AR polymorphisms

Polymorphism	Parameter	Genotype Hm	Genotype Ht	Genotype N
β_1-Ser49Gly	OGTT	**6.3**	3.4	*
β_2-Gln27Glu		**15.6**	5.0	**6.6**
β_2-Arg16Gly		0.0	**5.6**	**9.1**
β_3-Trp64Arg		*	**12.6**	1.1
β_1-Ser49Gly	INS	**6.0**	**21.7**	*
β_2-Gln27Glu		**40.0**	**22.5**	4.5
β_2-Arg16Gly		**16.2**	3.6	**12.0**
β_3-Trp64Arg		*	0.7	3.9
β_1 Ser49Gly	FFA	**11.6**	3.0	*
β_2 Gln27Glu		**41.8**	**6.7**	3.1
β_2 Arg16Gly		**20.4**	**15.2**	**20.7**
β_3 Trp64Arg		*	**13.7**	4.6

*indicates where there was insufficient number of subjects (or none) to be statistically useful in comparisons
Bold print indicates significant difference in AUC
OGTT: oral glucose tolerance test, INS: insulin, FFA: free fatty acids
Hm: homozygote for the polymorphism, Ht: heterozyogte, N: homozygote for the wild type allele

From table 4.13 it is clear that when comparing the two groups in terms of the same genotype for the same AR gene polymorphism, in most cases the difference was significant. For the N genotype of the β_3-Trp64Arg polymorphism, the AUC is the same in the black and in the white women.

Table 4.14: Differences and similarities in results obtained from the t-test and the AUC analysis

Group	Parameter	Polymorphism	Hm vs. Ht T-test	Hm vs. Ht AUC	Hm vs. N T-test	Hm vs. N AUC	Ht vs. N T-test	Ht vs. N AUC
Black women	OGTT	β_1-AR Ser49Gly	OGTT30	x	-	-	-	-
		β_2-AR Gln27Glu	OGTT60 OGTT90	√	OGTT60	√	OGTT60 OGTT90	√
		β_2-AR Arg16Gly	x	x	x	x	x	x
		β_3-AR Trp64Arg	x	√	OGTT90	√	x	x
	INS	β_1-AR Ser49Gly	x	x	-	-	-	-
		β_2-AR Gln27Glu	INS60	√	INS60 INS90	√	INS90	√
		β_2-AR Arg16Gly	x	x	x	x	x	x
		β_3-AR Trp64Arg	x	x	INS30	√	x	x
	FFA	β_1-AR Ser49Gly	x	x	-	-	-	-
		β_2-AR Gln27Glu	FFA30 FFA90 FFA120	√	FFA30	√	x	√
		β_2-AR Arg16Gly	x	x	FFA30 FFA60 FFA90 FFA120	√	x	√
		β_3-AR Trp64Arg	x	√	x	√	x	x
White women	OGTT	β_1-AR Ser49Gly	x	x	-	-	-	-
		β_2-AR Gln27Glu	x	√	x	√	x	x
		β_2-AR Arg16Gly	x	√	x	x	x	x
		β_3-AR Trp64Arg	-	-	-	-	OGTT30	√
	INS	β_1-AR Ser49Gly	INS0 INS30 INS120	√	-	-	-	-
		β_2-AR Gln27Glu	x	x	x	√	x	√
		β_2-AR Arg16Gly	INS60	√	x	√	x	√
		β_3-AR Trp64Arg	-	-	-	-	x	√
	FFA	β_1-AR Ser49Gly	x	√	-	-	-	-
		β_2-AR Gln27Glu	x	√	x	x	x	√
		β_2-AR Arg16Gly	FFA30	√	x	x	x	√
		β_3-AR Trp64Arg	-	-	-	-	x	√

"-"indicates instances where there were not enough individuals with a particular genotype for the AR gene polymorphism to use in statistical analysis
√: difference obeserved
X: no difference observed
OGTT: oral glucose tolerance test, INS: insulin, FFA: free fatty acids

Table 4.14 illustrates that when considering the t-test results, even when no significant differences exist between the individual parameters measured during the oral glucose tolerance test, the AUC analysis between the different genotypes of a specific AR gene polymorphism may show significant difference. In some cases, (for example the significant difference between the Hm and Ht genotypes values for the OGTT of the β_2-AR Gln27Glu and Arg16Gly polymorphisms in the white women) significant differences between values of genotypes may become apparent only after AUC analysis (table 4.10).

Table 4.15: Comparison of the AUC between the two groups irrespective of genotype and polymorphism

	Parameter		
	OGTT	INS	FFA
Black vs. white (% difference)	4.9	4.8	7.8

OGTT: oral glucose tolerance test, INS: insulin, FFA: free fatty acids

The response of the black and white women are significantly different when the AUC analysis was performed on the means of the OGTT, INS and FFA parameters. The OGTT and INS show 5% difference while the FFA > 5%.

4.7   COMBINATIONS OF SNPs: DIPLOTYPES AND HAPLOTYPES

As discussed in paragraph 2.4.2, combinations of SNPs rather than individual ones, should be considered when searching for predictive power in genetic predisposition. The possible combinations and numbers of test subjects associated with each combination were determined for the genotypes of the β_2-AR Gln27Glu and Arg16Gly polymorphisms (diplotypes) as well as the four AR gene polymorphisms, i.e. the β_1-AR Ser49Gly, β_2-AR Gln27Glu, β_2-AR Arg16Gly and the β_3AR Trp64Arg polymorphisms. The physiological parameters were then incorporated in the comparisons between the groups containing more than five test subjects.

Tables F.18 and F.20 (Appendix F) contain the means and standard deviations of the diplotypes for the black and the white women respectively, and the p-values are reported in tables F.19 and F.21. Listed and discussed here are only those groups and parameters found to be significantly different.

4.7.1   DIPLOTYPE ANALYSIS

All the possible combinations of genotypes identified in the investigated β_2-AR gene polymorphisms tested as well as the group number used for comparison of the groups containing five and more female test subjects are listed in tables 4.16 and 4.17.

Table 4.16: β_2-AR gene polymorphism diplotypes observed in the group of black women

β_2-AR Gln27Glu	β_2-AR Arg16Gly N	β_2-AR Arg16Gly Ht	β_2-AR Arg16Gly Hm
Hm	1	3	1
N	14 [1]	47 [2]	13 [3]
Ht	5	12 [4]	6 [5]

Number assigned to group for comparison indicated in brackets
Hm: homozygote for the polymorphism, Ht: heterozyogte, N: homozygote for the wild type allele

The diplotype was present in 46% of the black women - group 2 with 47 individuals (shaded cell in table 4.16). Only 4 of the possible 9 genotype combinations (diplotypes) were represented in groups of 5 or less individuals. All possible genotype combinations were represented in the group of black women. The following table lists the identical analysis performed on the group of white test subjects.

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Table 4.17: β_2-AR gene polymorphism diplotypes observed in the group of white women

β_2-AR Gln27Glu	β_2-AR Arg16Gly N	β_2-AR Arg16Gly Ht	β_2-AR Arg16Gly Hm
Hm	5	13 [3]	10 [5]
N	8 [7]	17 [2]	13 [4]
Ht	13 [6]	31 [1]	5

Number assigned to group for comparison indicated in brackets
Hm: homozygote for the polymorphism, Ht: heterozyogte,
N: homozygote for the wild type allele

There were only 2 of the possible 9 genotype combinations (diplotypes) not represented in more than 5 individuals in the group of white women and each of the possible diplotypes were present. The biggest group (group 1 with 31 women, shaded cell in table 4.17) comprised of the diplotype Gln/Glu/Arg/Gly (this combination is present in 31 of the 115 subjects, thus 27%). A comparison of the prevalence of the diplotype combinations in the black women with that of the white women showed that for the white women.

Table 4.18: Comparison of the measured physiological parameters for the diplotypes observed in the group of black women

Parameter	P-values for comparison between groups							
	2 vs. 4	2 vs. 6	2 vs. 7	3 vs. 4	3 vs. 7	4 vs. 6	4 vs. 7	6 vs. 7
SBP (mmHg)	0.340	0.757	0.091	0.494	**0.044**	0.464	0.394	0.145
OGTT90 (mmol/l)	0.759	**0.055**	**0.020**	0.658	0.074	0.143	0.081	0.674
FFA0 (mmol/l)	0.738	0.732	**0.021**	0.377	**0.016**	0.529	**0.024**	**0.035**
FFA30	0.090	0.287	**0.005**	0.546	**0.014**	0.465	**0.053**	**0.019**
FFA60	**0.006**	0.121	**0.002**	0.128	0.223	0.278	0.908	0.278
FFA90	**0.011**	0.859	0.085	**0.013**	0.362	**0.018**	0.404	0.117
FFA120	**0.010**	0.762	0.063	**0.001**	0.076	**0.018**	0.538	0.094
INS60	0.956	0.230	**0.055**	0.320	0.382	0.350	0.145	0.456
INS120	0.345	0.569	0.434	0.504	0.120	**0.043**	0.130	0.627

Bold print indicates significant statistical difference (P ≤0.05)
Note: comparisons between groups yielding no significant difference were omitted from this table
Hm: homozygote for the polymorphism, Ht: heterozyogte, N: homozygote for the wild type allele
SBP: systolic blood pressure, OGTT: oral glucose tolerance test, FFA: free fatty acids, INS: insulin

After comparison of the diplotypes of the β_2-AR gene for the black women, a few significant differences between diplotypes were observed (bold in table 4.18). Between groups 3 and 7 there were significant difference in the measured SBP. The values of both groups were > 130 (cut-off

point). This was the only parameter not related to glucose, insulin and/or free fatty acid concentration to show a significant difference.

Table 4.19: Comparison of the measured parameters for the diplotypes of the β_2-AR gene polymorphisms observed in the white women

Parameter	P-values								
	1 vs. 5	2 vs. 4	2 vs. 5	2 vs. 6	3 vs. 4	3 vs. 5	4 vs. 5	4 vs. 6	4 vs. 7
Age (years)	0.338	0.252	0.365	0.335	0.308	0.547	0.075	**0.049**	0.528
BMI (kg/m^2)	0.517	**0.021**	0.098	0.173	0.337	0.719	0.528	0.324	0.529
WC (cm)	0.568	**0.039**	0.100	0.065	0.316	0.558	0.635	0.749	0.548
SBP (mmHg)	0.572	**0.048**	0.105	**0.027**	0.785	0.707	0.822	0.601	0.947
Tc (mmol/l)	0.312	0.432	0.428	0.694	**0.033**	0.753	0.157	0.213	0.066
LDL (mmol/l)	0.507	0.506	0.882	0.707	**0.033**	0.430	0.388	0.250	**0.049**
FFA60 (mmol/l)	0.104	0.058	0.306	0.579	0.298	0.159	**0.041**	0.255	**0.033**
FFA90	0.286	**0.050**	0.527	0.640	0.202	0.246	0.084	0.114	0.095
FFA120	0.381	0.098	**0.019**	0.357	0.110	0.286	0.123	0.290	0.352
INS0 (pmol/l)	0.108	0.623	**0.012**	0.230	0.642	0.168	0.147	0.633	0.583
INS60	0.061	0.149	**0.008**	0.154	0.646	0.098	0.199	1.000	0.941
INS90	**0.025**	0.423	**0.016**	0.111	0.873	**0.049**	0.150	0.532	0.641
HOMA-IR	0.109	0.487	**0.029**	0.203	0.864	0.186	0.214	0.679	0.708

Bold print indicates significant statistical difference (P ≤0.05)
Note: comparisons yielding no significant difference were omitted from this table
BMI: body mass index, WC: waist circumference, SBP: systolic blood pressure, Tc: total blood cholesterol, LDL: low density lipoproteins, FFA: free fatty acids, INS: insulin, HOMA-IR: index of insulin resistance

For the white cohort, a difference between groups 4 and 6 with regards to age was observed. Covariance analysis for age was performed but no significant differences were observed between the measured physiological parameters for the genotypes of the AR genes.

Considering the group of volunteers at large, significant differences were not only found in parameters not measured during the oral glucose tolerance test, i.e. when comparing groups 2 and 4, significant differences were found between BMI, WC and SBP; groups 2 and 6 showed difference in SBP, groups 3 and 4 in Tc and LDL, groups 4 and 6 differed in age and groups 4 and 6 showed significant difference in LDL levels. Group 4 and 6 were analyzed with age as the covariant and only LDL was found to differ significantly between the two diplotypes.

With regards to the measurements taken during the oral glucose tolerance test, none of the groups compared showed significant differences in glucose concentration. Group 2 compared to group 5 showed the most parameters differing significantly, one FFA parameter, three INS parameters and the HOMA-IR.

Table 4.20: Comparison of the AUC of the observed diplotypes

	Parameter	Comparison between AUC of groups										
Black women		1 vs. 2	1 vs. 3	1 vs. 4	1 vs. 5	2 vs. 3	2 vs. 4	2 vs. 5	3 vs. 4	3 vs. 5	4 vs. 5	
	OGTT	6	1*	14	21	5*	8	16	13	20	8	
	INS	5*	13	12	32	17	7	28	24	41	23	
	FFA	17	26	14	50	12	3*	40	14	33	42	
White women		1 vs. 2	1 vs. 3	1 vs. 4	1 vs. 5	1 vs. 6	1 vs. 7	2 vs. 3	2 vs. 4	2 vs. 5	2 vs. 6	2 vs. 7
	OGTT	1*	2*	3*	3*	0*	3*	1*	4*	4*	0*	3*
	INS	17	8	1*	32	9	13	9	18	44	24	28
	FFA	14	14	9	27	9	38	0*	6	16	6	28
		3 vs. 4	3 vs. 5	3 vs. 6	3 vs. 7	4 vs. 5	4 vs. 6	4 vs. 7	5 vs. 6	5 vs. 7	6 vs. 7	
	OGTT	4*	5*	1*	4*	1*	3*	0*	4*	1*	3*	
	INS	9	38	16	20	31	8	12	26	22	5*	
	FFA	6	16	6	28	20	0*	32	20	15	32	

*indicates *no* significant difference in AUC
Note: comparisons yielding no significant difference in AUC were omitted from this table
OGTT: oral glucose tolerance test, INS: insulin, FFA: free fatty acids

In most cases, a significant difference in AUC was found. Table 4.20 shows that in many cases all three of the measured parameters differed between the diplotypes in the black as well as the white women. The most prominent exception to this is the OGTT in the white women. For the black women there were only four instances where the AUC for the compared groups did not differ significantly. The response to the oral glucose tolerance test in terms of glucose concentration did not differ between any of the diplotypes observed for the group of white women.

4.7.2 HAPLOTYPE ANALYSIS

The following table lists all the possible combinations of haplotypes for the black women for the four AR gene polymorphisms investigated.

Table 4.21: Haplotypes observed in the group of black women

β_1-AR Ser49Gly	β_2-AR Gln27Glu	β_2-AR Arg16Gly	β_3-AR Trp64Arg N	β_3-AR Trp64Arg Ht	β_3-AR Trp64Arg Hm
Hm	Hm	N	1	0	0
Hm	Hm	Ht	2	1	0
Hm	Hm	Hm	0	1	0
Hm	N	N	5	2	0
Hm	N	Ht	13 [1]	17 [2]	3
Hm	N	Hm	6 [3]	6 [4]	0
Hm	Ht	N	0	2	0
Hm	Ht	Ht	4	4	0
Hm	Ht	Hm	3	1	0
Ht	Hm	N	0	0	0
Ht	Hm	Ht	0	0	0
Ht	Hm	Hm	0	0	0
Ht	N	N	3	4	0
Ht	N	Ht	6 [5]	6 [6]	2
Ht	N	Hm	0	1	0
Ht	Ht	N	2	1	0
Ht	Ht	Ht	2	2	0
Ht	Ht	Hm	1	0	0
N	Hm	N	0	0	0
N	Hm	Ht	0	0	0
N	Hm	Hm	0	0	0
N	N	N	0	0	0
N	N	Ht	0	0	0
N	N	Hm	0	0	0
N	Ht	N	0	0	0
N	Ht	Ht	0	0	0
N	Ht	Hm	1	0	0

Number assigned to group for comparison indicated in brackets
Hm: homozygote for the polymorphism, Ht: heterozygote, N: homozygote for the wild type allele

Of the 81 possible genotype combinations (haplotypes) for the four investigated AR polymorphisms, a number were prominant while others were not represented at all. Although there is a vast array op possible haplotypes, it is clear from the table above that clustering of individuals took place for only six of the haplotypes (shaded cells in table 4.21). The biggest number of individuals with the same haplotype was 17 for the haplotype Gly49/Gln27/Arg16Gly/Trp64Arg (15% of the group of black women).

Table 4.22: Haplotypes observed in the group of white women

β_1-AR Ser49Gly	β_2-AR Gln27Glu	β_2-AR Arg16Gly	β_3-AR Trp64Arg N	β_3-AR Trp64Arg Ht
Hm	Ht	Ht	24 [1]	4
Hm	Ht	Hm	2	1
Hm	Ht	N	10 [2]	2
Hm	N	Ht	16 [3]	0
Hm	N	Hm	13 [4]	0
Hm	N	N	5	3
Hm	Hm	Ht	10 [5]	1
Hm	Hm	Hm	5	2
Hm	Hm	N	3	1
Ht	Ht	Ht	2	1
Ht	Ht	Hm	2	0
Ht	Ht	N	1	0
Ht	N	Ht	1	0
Ht	N	Hm	0	0
Ht	N	N	0	0
Ht	Hm	Ht	2	0
Ht	Hm	Hm	3	0
Ht	Hm	N	0	1

Number given to group for comparison indicated in brackets
Hm: homozygote for the polymorphism, Ht: heterozyogte, N: homozygote for the wild type allele

This table differs from that of the black group because certain genotypes of AR gene polymorphisms were absent from the group of white female volunteers. More than 5 individuals presented with the same genotype combination (haplotype) in five haplotypes of a possible 36. The largest number of subjects with a single haplotype was 24 and thus 21 % of the total group (shaded cell in table 4.22).

When table 4.21 is compared to table 4.22 it becomes apparent that the distribution of individuals across the different possible haplotypes is more homogenous in the white women that the black women.

Table 4.23: Comparison of the measured physiological parameters for the different haplotypes observed in the group of black women

Parameter	1 vs. 2	1 vs. 3	1 vs. 4	1 vs. 5	1 vs. 6	2 vs. 4	2 vs. 5	3 vs. 5	4 vs. 6	5 vs. 6
	P-values for comparison between groups									
Tc (mmol/l)	**0.003**	**0.010**	0.420	0.189	**0.034**	0.172	0.147	0.071	0.322	0.274
LDL (mmol/l)	**0.005**	**0.016**	0.458	0.228	0.099	0.182	0.199	0.154	0.460	0.480
OGTT60 (mmol/l)	0.993	0.166	0.990	0.120	0.503	0.996	0.201	**0.052**	0.725	0.116
OGTT90	0.981	**0.032**	0.182	**0.046**	0.229	0.427	0.195	**0.023**	0.155	0.067
FFA0 (mmol/l)	0.147	0.342	0.434	0.141	0.689	0.737	0.804	**0.042**	0.734	0.354
FFA30	0.798	0.636	0.188	0.092	0.278	0.120	**0.052**	0.051	**0.011**	**0.016**
FFA60	0.684	0.337	**0.016**	**0.019**	0.648	0.034	**0.024**	0.171	0.131	0.144
FFA90	0.280	**0.012**	**0.002**	**0.015**	**0.041**	**0.037**	0.090	0.540	0.154	0.300
FFA120	0.222	**0.007**	**0.002**	**0.010**	0.107	**0.028**	0.204	0.812	0.140	0.393

Bold print indicates significant statistical difference (P ≤0.05)
Note: comparisons yielding no significant difference were omitted from this table
Tc: total blood cholesterol, LDL: low density lipoproteins, OGTT: oral glucose tolerance test, FFA: free fatty acids

For the black women most of the significant differences observed between the haplotypes centered around FFA parameters, a few differences presented in some of the OGTT parameters and no INS parameters differed significantly. When group 1 was compared to group 3 and group 6, Tc showed significant difference while LDL differed when group 1 was compared to group 2 and group 3.

Table 4.24: Comparison of the measured physiological parameters for the different haplotypes observed in the group of white women

Parameter	1 vs. 3	1 vs. 4	2 vs. 3	2 vs. 4	3 vs. 4	4 vs. 5
	P-values for comparison between groups					
Age (years)	0.963	0.305	0.192	**0.046**	0.317	0.062
BMI (kg/m^2)	0.214	0.247	**0.052**	0.855	**0.026**	0.433
WC (cm)	0.118	0.361	**0.024**	0.783	**0.038**	0.418
SBP (mmHg)	0.171	0.684	**0.008**	0.238	**0.028**	0.933
Tc (mmol/l)	0.893	0.583	0.819	0.326	0.469	**0.009**
LDL (mmol/l)	0.567	0.982	0.793	0.341	0.524	**0.015**
OGTT0 (mmol/l)	0.144	0.205	**0.015**	0.804	**0.010**	0.400
INS0 (pmol/l)	0.953	**0.040**	0.853	0.115	0.063	0.293
INS30	0.207	**0.033**	0.718	0.180	0.110	0.186
HOMA-IR	**0.022**	0.411	**0.042**	0.313	0.369	0.575

Bold print indicates significant statistical difference (P ≤0.05)
Note: comparisons yielding no significant difference were omitted from this table
BMI: body mass index, WC: waist circumference, SBP: systolic blood pressure, Tc: total blood cholesterol, LDL: low density lipoproteins, OGTT: oral glucose tolerance test, INS: insulin, HOMA-IR: index of insulin resistance

Haplotypes 2 and 4 in the white women showed significant difference in age. A covariance analysis was performed between group 2 and 4 for age and no significant differences were found in the measured parameters. The BMI, WC and SBP differed significantly between groups 2 and 3 and groups 3 and 4. When comparing groups 4 and 5 the Tc and LDL showed significant difference. Two of the FFA parameters proved significant different between haplotype group 1 and 4. One and two of the INS parameters respectively differed between groups 1 and 3 and groups 2 and 3.

Table 4.25: Comparison of the AUC of the observed haplotypes

	Parameter	Comparison between AUC of groups											
		1 vs. 2	1 vs. 3	1 vs. 4	1 vs. 5	2 vs. 3	2 vs. 4	2 vs. 5	3 vs. 4	3 vs. 5	4 vs. 5	4 vs. 6	5 vs. 6
Black women	OGTT	2*	10	6	16	12	4*	14	16	25	10	10	19
	INS	25	0*	5*	15	25	21	12	6	15	10	23	15
	FFA	11	4*	37	44	14	30	37	40	46	11	41	47
		1 vs. 2	1 vs. 3	1 vs. 4	1 vs. 5	2 vs. 3	2 vs. 4	2 vs. 5	3 vs. 4	3 vs. 5	4 vs. 5*		
White women	OGTT	2*	4*	0*	4*	2*	2*	2*	4*	0*	4*		
	INS	9	28	11	9	34	19	17	19	21	2*		
	FFA	10	3*	2*	1*	12	8	10	5*	3*	2*		

*indicates **no** significant difference in AUC
Note: comparisons yielding no significant difference in AUC were omitted from this table
OGTT: oral glucose tolerance test, INS: insulin, FFA: free fatty acids

In most of the comparisons, significant differences were found in AUC (table 4.25). For the black women there are only 5 exceptions to this. Again, as was the case with the β_2-AR gene diplotypes, none of the groups differed significantly in glucose concentration in response to the OGTT of the white women.

CHAPTER 5: DISCUSSION

In this study the four investigated AR gene polymorphisms were compared within each of the subject groups. Thereafter comparisons were made between the two subject groups. The results obtained can not, however, be extrapolated to the entire black and white populations in total.

5.1. HARDY-WEINBERG EQUILIBRIUM

As all four investigated AR gene polymorphisms were found to be in HWE, this proves that in these cohorts the alleles were inherited over generations in the assumed ratios (paragraph 4.3).

5.2. MINOR ALLELE FREQUENCIES

Since the number of subjects in both groups is comparable (102 black and 115 white women) it is not expected that it will play a role in causing the differences observed in the allele frequency between the two groups. The differences observed in minor allele frequencies might be a result of the test group being volunteers (as discussed in paragraph 3.2) or can be attributed to the fact that the two groups are of different ethnicities with results from studies around the world supporting this observation (Leineweber *et al.*, 2004). Differences in ancestral descent have also been introduced as one of the possible causes (Small *et al.*, 2003; Leinweber *et al.*, 2004). The fact that the study groups were volunteers impacts upon the validity of the calculated minor allele frequencies as being representative of that in the rest of these respective populations. This is because some genotypes were not present in the study groups (see table 4.2) which could affect the calculation of the minor allele frequencies. The observed allele frequency might be a true reflection of the prevalence of the polymorphic alleles in these population groups, or not, as a result of the study groups consisting of selected volunteers.

When the minor allele frequencies calculated for the subjects studied in this investigation were compared to those from published studies, significant differences, but also resemblances, became apparent (paragraph 2.5.1). The minor allele frequencies of both the groups are much higher than the others at the β_1-AR Ser49Gly locus (figure 5.1). For the remaining three investigated polymorphisms, the minor allele frequencies observed are fairly similar to those in other ethnicities and population groups. Noteworthy is the fact that the white female group had the lowest minor allele frequency for the β_3-AR Trp64Arg polymorphism (7%).
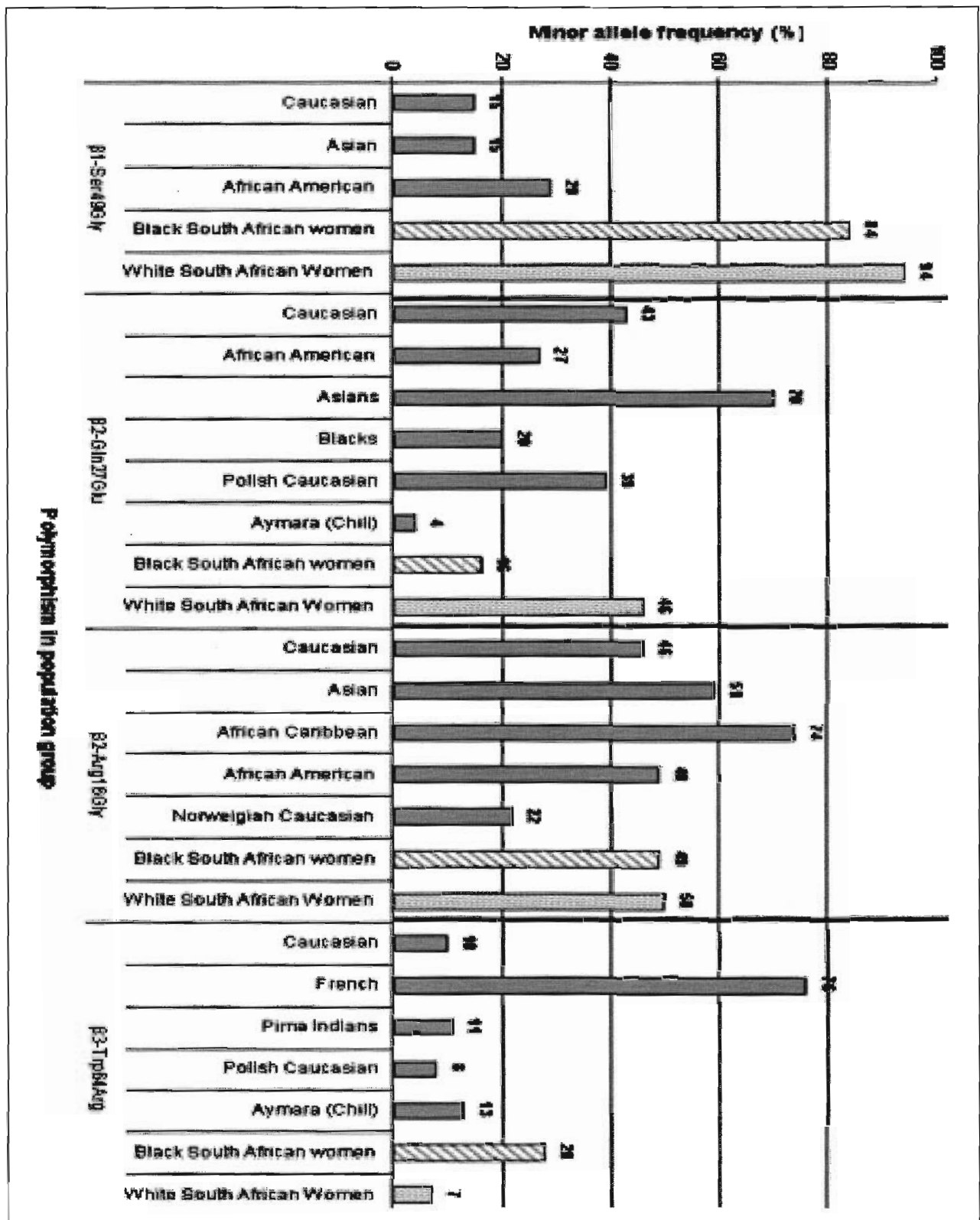
Figure 5.1: Global comparison of the AR minor allele frequencies
(Compiled from the results of this study and recent published studies: Buscher et al., 1999;
Johnson & Terra, 2002; Santos et al., 2002; Malczewska-Malec et al., 2003 and Small et al., 2003)

5.3   COMPARISON OF MEASURED PHYSIOLOGICAL PARAMETERS WITH GENOTYPES

The cut-off points for the measured physiological parameters are provided in paragraph 2.2.2, table 2.1. In order to prove association with either obesity or elevated blood pressure, enough measured parameters must differ AND be above the cut-off point.

5.3.1 β_1-AR SER49GLY

The parameter (OGTT30) differed significantly the Hm and Ht genotypes of the black women (table 4.5), was insufficient to support the notion of possible association with obesity or high blood pressure. The insulin parameters of the white women of the Ht genotype were significantly higher than those of the Hm, but the HOMA-IR was still below the cut-off point of 2.5 for insulin resistance. Thus also no associations were demonstrated for the white women cohort.

5.3.2 β_2-AR GLN27GLU

Although the body fat % of the Ht and N genotypes showed a significant difference in the black women, the values were still within the normal ranges (table 4.6). This was the case with the SBP as well. For the white women there were no significant differences found between the genotypes. In both groups, no association was found between this AR gene polymorphism and obesity or high blood pressure.

5.3.3 β_2-AR ARG16GLY

From table 4.7 it was clear that all the FFA parameters differed significantly between the Hm and other two genotypes in the black women except the fasting value. This might be explained by the fact that differences only come to light when the system is challenged – in this case with the oral glucose tolerance test. The difference observed might be a result of over- or hyperactivity of the adrenergic receptors as a result of the polymorphism (paragraph 2.4.2). The other parameters found to be significantly different between the genotypes were all within the normal ranges, thus no associations were found for this AR gene polymorphism in the black women with either obesity or high blood pressure.

The BMI of the Ht genotype was significantly different than that of the N genotype, but the BMI of all three genotypes still fell into the overweight category (BMI between 25 and 30). The WC of the Ht genotype was higher than that of N and above the cut-off value of 88cm but those of the Hm and N genotypes were still within the normal range. No association with obesity or high blood pressure was therefore established in the group of white women.

5.3.4 β_3-AR TRP64ARG

The three parameters found to differ significantly (table 4.8) were insufficient to prove possible associations between this AR gene polymorphism and obesity or high blood pressure in either of the subject groups.

5.3.5 GENOTYPES COMPARED IRRESPECTIVE OF BMI

This comparison was performed to determine whether values of parameters differed between the black and the white women for the same genotype of the same AR gene polymorphism. The significant differences in measured parameters observed between the two groups (table 4.9) suggest that similar genotypes do not necessarily mean that the values obtained will be similar.

Evans *et al.* (2001) stated that there exists interethnic differences in responses to medication. In one of their studies it was found that morphine caused a significantly higher respiratory depression in Caucasians than in Chinese at the same plasma morphine concentrations. The studies listed in this paper, focused mainly on bronchodilation and constriction, which in no way says that statements about other β-AR functions would not follow the same trend. The differences observed could therefore be attributed to the fact that the two study groups are of different ethnicities.

5.3.6 AREA UNDER THE CURVE (AUC) ANALYSIS

The initial (fasting) concentration of the measured parameters (at time 0 minutes) in most cases did not differ significantly, thus the base value of operation for the ARs can be said to be more or less the same for all four polymorphic ARs. It is only when the system is put under stress / challenged with the loading test, that the differences in response (under- of over-activity) become noticeable.

This could possible be the result of altered ARs by these polymorphisms (also discussed in paragraph 2.4.2). The AUC analyses of the parameters measured during the oral glucose tolerance test are represented in figure 4.10 to 4.12. Although many significant differences came to light, the parameters were still within normal ranges. This suggests that the adrenergic response between different genotypes differ, but not necessarily to the extent of being abnormal (under- or over-activity).

From Table 4.13 it is clear that when the same genotype for a specific AR gene polymorphism was compared between the two groups of women, most was found to differ significantly. This again shows that there was a difference in adrenergic response between the black and the white women, because the parameters were still within the normal ranges (paragraph 5.2.5 also ties in here).

Table 4.14 shows that while the t-test may not show any significant differences in parameters measured over time (such as those during the oral glucose tolerance test), the AUC could. This may be because the t-test only compares the values (means) for a single time point, e.g. the insulin concentration at 30 minutes into the oral glucose tolerance test, whereas the AUC analysis takes into account the change in glucose concentration over time (as was done with INS levels to obtain figure 4.5). This serves as an indicator of insulin secretion (changes in insulin concentration, INS, absorption of glucose, changes in glucose concentration, OGTT, and changes in free fatty acid concentration, FFA; all of these measured in 30 minute intervals over a period of two hours).

The two groups of women differed significantly with regards to the three sets of parameters measured during the oral glucose tolerance test (table 4.15). This difference could possibly be attributed to differences in the prevalence of the polymorphic allele in the two different groups (adrenergic response between different groups varying in ethnicity). It is unlikely that the size of the study group impacted upon this difference, as both groups comprised of a fairly equal number of subjects.

5.4   DIPLOTYPE ANALYSIS

Studies have shown that the Arg16 (N) genotype rarely occurs with Glu27 (Hm). The haplotype Arg16Glu27 occurs in less than 1% of the population in a study by Bruck *et al.* (2005). In our study this combination was present in 1% of the black women and 4% of the white women (discussed in paragraph 2.5.2).

Table 4.16 shows the diplotypes observed in the black women along with the groups for comparison. The P-values are reported in table 4.18. The SBP was found to differ significantly between groups 3 and 7 and both were somewhat elevated. There was no association found with DBP however. Although some of the parameters measured during the oral glucose tolerance test showed significant differences between some of the diplotypes, all the values were still within the normal ranges. No association was therefore found between the observed β_2-AR diplotypes for the black women.

Tables 4.17 and 4.19 report the results obtained from diplotype analysis for the white women. In all except one comparison, parameters were found to be significant different, yet still within the normal ranges and therefore no possible associations were found. The HOMA-IR of diplotype 2 and 5 was compared and found to differ significantly. For diplotype 2 the HOMA-IR value was found to be 3.43 and over the cut-off point, while that of diplotype 5 was normal. The INS parameters measured for diplotype 2 was also significantly higher than that of diplotype 5. This could possibly link this diplotype to the onset and progression of insulin resistance along with the effects thereof such as hyperinsulinemia, (further) weight gain and increases in blood cholesterol (paragraph 2.1 and 2.5.2).

Table 4.20 noting the results of the AUC analyses for the diplotypes observed in both the subject groups showed that two diplotypes differed in adrenergic response, but not necessarily in an abnormal direction such as under- or over-activity.

5.5   HAPLOTYPE ANALYSIS

In the white women clustering of individuals into certain haplotypes was more frequent than in the black women (multitude of 0's in table 4.21). This may be explained by the fact that the group of black women were not of homogenous descent (not all Zulu or Xhosa, for instance).

Differences observed between different haplotypes for the black women (tables 4.21 and 4.23) are not enough to prove possible associations of any of these haplotypes with obesity or high blood pressure.

Although the HOMA-IR differed significantly between haplotypes 1,2 and 3 of the white women (tables 4.22 and 4.24) and the mean of this parameter exceeded the cut-off point, the fasting plasma glucose as well as the other parameters measured during the oral glucose tolerance test were not significantly different between these 3 haplotypes. No other parameters found to be

significantly different were outside normal ranges, thus showing no possible associations between haplotypes observed for the white women with obesity or high blood pressure.

Table 4.25 notes the differences in AUC analyses for the parameters measured during the oral glucose tolerance test between the different haplotypes observed in both study groups. As was the case for the diplotypes in the white women, no differences were observed between the haplotypes for the glucose concentration (OGTT) during the oral glucose tolerance test. The haplotypes of both groups only differed in adrenergic response, but parameters remain within normal ranges.

5.6   SUMMARY

Insel *et al.* (2006) stated that most associations found between AR gene polymorphisms and disease states (such as the metabolic syndrome) were too weak to allow meaningful prediction of onset, progression or drug response in disease. The findings of our study therefore support the notion that there are no association between these AR genes and the metabolic syndrome (paragraphs 2.4.3 to 2.4.5 and 2.5.2), but at the same time indications arise that these four investigated polymorphisms, diplotypes and haplotypes may in some cases in the two study groups, act not as risk factors themselves, but as contributors to risk factors for onset and progression of obesity, high blood pressure and the metabolic syndrome.

CHAPTER 6: CONCLUSION

For both the black and the white groups of women the conclusions reached are the same. The amount of measured physiological parameters found to be significantly different in the comparisons was insufficient to indicate possible association between the investigated AR gene polymorphisms, diplotypes or haplotypes and the two characteristics of the metabolic syndrome, namely obesity and hypertension (high blood pressure).

Although no associations could be demonstrated, there are indications that these AR gene polymorphisms, diplotypes and haplotypes investigated may act as contributors to the risk factors for development and progression of the metabolic syndrome.

APPENDIX A: OLIGONUCLEOTIDES USED IN THIS STUDY

INTRODUCTION

For successful amplification of the desired gene fragments, the effective design of oligonucleotide primers is essential. Factors to be taken into account are length, melting temperature, GC content, and self-complementarity. Well designed oligonucleotides will ensure the success of the polymorphic analysis. Although the oligonucleotides for this investigation were taken from published studies, every set was tested.

EVALUATION OF THE OLIGONUCLEOTIDES

Published oligonucleotide sequences used in this study were from the following sources:

β_1-Ser49Gly: Maqbool et al., 1999

β_2-Gln27Glu: Santos et al., 2002

β_2-Arg16Gly: Kim et al., 2002

β_3-Trp64Arg: Yuan et al., 1997

The sequences of the oligonucleotides are given in Appendix D. They were synthesized by Inqaba Biotec.

Annealing of the oligonucleotide primer sets to their respective gene sequences was tested *in silico* with Vector NTI 10.0.1 (2005) from Invitrogen and is displayed in Appendix B. The GenBank accession numbers for the AR gene sequences used are:

β_1-AR: NM_000684

β_2-AR: NM_000024

β_3-AR: NM_000025

A BLAST search was also carried out to verify the specific binding of the oligonucleotides to their respective target gene fragments (http://www.ensembl.org).

APPENDIX B: ANNEALING OF THE OLIGONUCLEOTIDES TO THE AR GENE SEQUENCES

β_1-AR Ser49Gly

```
                              1                                                                                                    100
ADRB1                   (1)   TGCTACCCGCGCCCGGGCTTCTGGGGTGTTCCCCAACCACGGCCCAGCCCTGCCACACCCCCCGCCCCCGGCCTCCGCAGCTCGGCATGGGCGCGGGGGT
pr B1 Ser49 Gly FOR     (1)   -----------CCGGGCTTCTGGGGTGTTCC-------------------------------------------------------------------------
pr B1 Ser49 Gly REV rc  (1)   ------------------------------------------------------------------------------------------------------
                              101                                                                                                  200
ADRB1                 (101)   GCTCGTCCTGGGCGCCTCCGAGCCCGGTAACCTGTCGTCGGCCGCACCGCTCCCCGACGGCGCGGCCACCGCGGCGCGGCTGCTGGTGCCCGCGTCGCCG
pr B1 Ser49 Gly FOR    (21)   ------------------------------------------------------------------------------------------------------
pr B1 Ser49 Gly REV rc  (1)   ------------------------------------------------------------------------------------------------------
                              201                                                                                                  300
ADRB1                 (201)   CCCGCCTCGTTGCTGCCTCCCGCCAGCGAAAGCCCCGAGCCGCTGTCTCAGCAGTGGACAGCGGGCATGGGTCTGCTGATGGCGCTCATCGTGCTGCTCA
pr B1 Ser49 Gly FOR    (21)   ------------------------------------------------------------------------------------------------------
pr B1 Ser49 Gly REV rc  (1)   ------------------------------------------------------------------------------------------------------
                              301                                                                                                  400
ADRB1                 (301)   TCGTGGCGGGCAATGTGCTGGTGATCGTGGCCATCGCCAAGACGCGCGGCTGCAGACGCTCACCAACCTCTTCATCATGTCCCTGGCCAGCGCCGACCT
pr B1 Ser49 Gly FOR    (21)   ------------------------------------------------------------------------------------------------------
pr B1 Ser49 Gly REV rc  (1)   ------------------------------------------------------------------------------------------------------
                              401                                                                                                  500
ADRB1                 (401)   GGTCATGGGGCTGCTGGTGGTGCCGTTCGGGGCCACCATCGTGGTGTGGGGCCGCTGGGAGTACGGCTCCTTCTTCTGCGAGCTGTGGACCTCAGTGGAC
pr B1 Ser49 Gly FOR    (21)   ------------------------------------------------------------------------------------------------------
pr B1 Ser49 Gly REV rc  (1)   ------------------------------------------------------------------------------------------------------
                              501                                                                                                  600
ADRB1                 (501)   GTGCTGTGCGTGACGGCCAGCATCGAGACCCTGTGTGTGTCATTGCCCTGGACCGCTACCTCGCCATCACCTCGCCCTTCCGCTACCAGAGCCTGCTGACGC
pr B1 Ser49 Gly FOR    (21)   ------------------------------------------------------------------------------------------------------
pr B1 Ser49 Gly REV rc  (1)   ----------------------------------------------GCTACCTCGCCATCACCTCGCC--------------------------------
                              601                                                                                                  700
ADRB1                 (601)   GCGCGCGGGCGCGGGGCCTCGTGTGCACCGTGTGGGCCATCTCGGCCCTGGTGTCCTTCCTGCCCATCCTCATGCACTGGTGGCGGGCGGAGAGCGACGA
pr B1 Ser49 Gly FOR    (21)   ------------------------------------------------------------------------------------------------------
pr B1 Ser49 Gly REV rc  (23)  ------------------------------------------------------------------------------------------------------
                              701                                                                                                  800
ADRB1                 (701)   GGCGCGCCGCTGCTACAACGACCCCAAGTGCTGCGACTTCGTCACCAACCGGGCCTACGCCATCGCCTCGTCCGTAGTCTCCTTCTACGTGCCCCTGTGC
pr B1 Ser49 Gly FOR    (21)   ------------------------------------------------------------------------------------------------------
pr B1 Ser49 Gly REV rc  (23)  ------------------------------------------------------------------------------------------------------
                              801                                                                                                  900
ADRB1                 (801)   ATCATGGCCTTCGTGTACCTGCGGGTGTTCCGCGAGGCCCAGAAGCAGGTGAAGAAGATCGACAGCTGCGAGCGCCGTTTCCTCGGCGGCCCAGCGCGGC
pr B1 Ser49 Gly FOR    (21)   ------------------------------------------------------------------------------------------------------
pr B1 Ser49 Gly REV rc  (23)  ------------------------------------------------------------------------------------------------------
                              901                                                                                                  1000
```

β_2-AR Gln27Glu

```
                              1                                                                                                    100
ADBR2                   (1)   ACTGCGAAGCGGCTTCTTCAGAGCACGGGCTGGAACTGGCAGGCACCGCGAGCCCCTAGCACCCGACAAGCTGAGTGTGCAGGACGAGTCCCCACCACAC
pr B2 Gln27 Glu FOR     (1)   ------------------------------------------------------------------------------------------------------
pr B2 Gln27 Glu REV     (1)   ------------------------------------------------------------------------------------------------------
                              101                                                                                                  200
ADBR2                 (101)   CCACACCACAGCCGCTGAATGAGGCTTCCAGGCGTCCGCTCGCGGCCCGCAGAGCCCCGCCGTGGGTCCGCCCGCTGAGGCGCCCCCAGCCAGTGCGCTT
pr B2 Gln27 Glu FOR     (1)   ------------------------------------------------------------------------------------------------------
pr B2 Gln27 Glu REV     (1)   ------------------------------------------------------------------------------------------------------
                              201                                                                                                  300
ADBR2                 (201)   ACCTGCCAGACTGCGCGCCATGGGGCAACCCGGGAACGGCAGCGCCTTCTTGCTGGCACCCAATAGAAGCCATGCGCCGGACCCACGACGTCACGCAGCA
pr B2 Gln27 Glu FOR     (1)   ----------------------------------------------------------------------------GGCGGACCCACGCGCGG---------
pr B2 Gln27 Glu REV     (1)   ------------------------------------------------------------------------------------------------------
                              301                                                                                                  400
ADBR2                 (300)   AAGGGACGAGGTGTGGGTGGTGGGCATGGGCATCGTCATGTCTCTCATCGTCCTGGCCATCGTGTTTGGCAATGTGCTGGTCATCACAGCCATTGCCAAG
pr B2 Gln27 Glu FOR    (17)   ------------------------------------------------------------------------------------------------------
pr B2 Gln27 Glu REV     (1)   ------------------------------------------------------------------------------------------------------
                              401                                                                                                  500
ADBR2                 (400)   TTCGAGCGTCTGCAGACGGTCACCAACTACTTCATCACTTCACTGGCCTGTGCTGATCTGGTCATGGGCCTGGCAGTGGTGCCCTTTGGGGCCGCCCATA
pr B2 Gln27 Glu FOR    (17)   ------------------------------------------------------------------------------------------------------
pr B2 Gln27 Glu REV     (1)   ----------------------------------------------GTGCTGATCTGGTCATGG--------------------------------------
                              501                                                                                                  600
ADBR2                 (500)   TTCTTTATGAAAATGTGGACTTTTGGCAACTTCTGGTGCGAGTTTTGGACTTCCATTGATGTGCTGTGCGTCACGGCCAGCATTGAGACCCTGTGCGTGAT
pr B2 Gln27 Glu FOR    (17)   ------------------------------------------------------------------------------------------------------
pr B2 Gln27 Glu REV    (19)   ------------------------------------------------------------------------------------------------------
                              601                                                                                                  700
ADBR2                 (600)   CGCAGTGGATCGCTACTTTGCCATTACTTCACCTTTCAAGTACCAGAGCCTGCTGACCAAGAATAAGGCCCGGGTGATCATTCTGATGGTGTGGATTGTG
pr B2 Gln27 Glu FOR    (17)   ------------------------------------------------------------------------------------------------------
pr B2 Gln27 Glu REV    (19)   ------------------------------------------------------------------------------------------------------
                              701                                                                                                  800
ADBR2                 (700)   TCAGGCCTTACCTCCTTCTTGCCCATTCAGATGCACTGGTACCGGGCCACCCACCAGGAAGCCATCAACTGCTATGCCAATGAGACCTGCTGTGACTTCT
pr B2 Gln27 Glu FOR    (17)   ------------------------------------------------------------------------------------------------------
pr B2 Gln27 Glu REV    (19)   ------------------------------------------------------------------------------------------------------
```

β_2-AR Arg16Gly

```
                          1                                                                                        100
        ADBR2        (1)  ACTGCGAAGCGGCTTCTTCAGAGCACGGGCTGGAACTGGCAGGCACCGCGAGCCCCTAGCACCCGACAAGCTGAGTGTGCAGGACGAGTCCCCACCACAC
pr B2 Arg16 Gly FOR  (1)  ------------------------------------------------------------------------------------------------------
pr B2 Arg16 Gly REV rc (1) -----------------------------------------------------------------------------------------------------
                          101                                                                                      200
        ADBR2      (101)  CCACACCACAGCCGCTGAATGAGGCTTCCAGGCGTCCGCTCGCGGCCCGCAGAGCCCCGCCGTGGGTCCGCCCGCTGAGGCGCCCCCAGCCAGTGCGCTT
pr B2 Arg16 Gly FOR  (1)
pr B2 Arg16 Gly REV rc (1)
                          201                                                                                      300
        ADBR2      (201)  ACCTGCCAGACTGCGCGCCATGGGGCAACCCGGGAACGGCAGCGCCTTCTTGCTGGCACCCAATAGAAGCCATGCGCCGGACCACGACGTCACGCAGCAA
pr B2 Arg16 Gly FOR  (1)                                                       CTTCTTGCTGGCACCCAAT
pr B2 Arg16 Gly REV rc (1)
                          301                                                                                      400
        ADBR2      (301)  AGGGACGAGGTGTGGGTGGTGGGCATGGGCATCGTCATGTCTCTCATCGTCCTGGCCATCGTGTTTGGCAATGTGCTGGTCATCACAGCCATTGCCAAGT
pr B2 Arg16 Gly FOR (20)
pr B2 Arg16 Gly REV rc (1)
                          401                                                                                      500
        ADBR2      (401)  TCGAGCGTCTGCAGACGGTCACCAACTACTTCATCACTTCACTGGCCTGTGCTGATCTGGTCATGGGCCTGGCAGTGGTGCCCTTTGGGGGCCGCCCATAT
pr B2 Arg16 Gly FOR (20)
pr B2 Arg16 Gly REV rc (1)                      CGAACTACTTCATCACTTCACTGG
                          501                                                                                      600
        ADBR2      (501)  TCTTATGAAAATGTGGCACTTTTGGCAACTTCTGGTGCGAGTTTTGGACTTCCATTGATGTGCTGTGCGTCACGGCCAGCATTGAGACCCTGTGCGTGATC
pr B2 Arg16 Gly FOR (20)
pr B2 Arg16 Gly REV rc (25)
                          601                                                                                      700
        ADBR2      (601)  GCAGTGGATCGCTACTTTGCCATTACTTCACCTTTCAAGTACCAGAGCCTGCTGACCAAGAATAAGGCCCGGGTGATCATTCTGATGGTGTGGATTGTGT
pr B2 Arg16 Gly FOR (20)
pr B2 Arg16 Gly REV rc (25)
                          701                                                                                      800
        ADBR2      (701)  CAGGCCTTACCTCCTTCTTGCCCATTCAGATGCACTGGTACCGGGCCCACCCACCAGGAAGCCATCAACTGCTATGCCAATGAGACCTGCTGTGACTTCTT
pr B2 Arg16 Gly FOR (20)
pr B2 Arg16 Gly REV rc (25)
                          801                                                                                      900
        ADBR2      (801)  CACGAACCAAGCCTATGCCATTGCCTCTTCCATCGTGTCCTTCTACGTTCCCCTGGTGATCATGGTCTTCGTCTACTCCAGGGTCTTTCAGGAGGCCAAA
pr B2 Arg16 Gly FOR (20)
pr B2 Arg16 Gly REV rc (25)
                          901                                                                                      1000
```

β_3-AR Trp64Arg

```
                          1                                                                                        100
        ADBR3        (1)  GCTACTCCTCCCCCAAGAGCGGTGGCACCGAGGGAGTTGGGGTGGGGGGGAGGCTGAGCGCTCTGGCTGGGACAGCTAGAGAAGATGGCCCAGGCTGGGGA
pr B3 Trp64 Arg FOR  (1)
pr B3 Trp64 Arg REV  (1)
                          101                                                                                      200
        ADBR3      (101)  AGTCGCTCTCATGCCTTGCTGTCCCCTCCCCTGAGCCAGGTGATTTGGGAGACCCCCTCCTTCCTTCTTTCCCTACCGCCCCACGCGCGCGACCCGGGGATG
pr B3 Trp64 Arg FOR  (1)
pr B3 Trp64 Arg REV  (1)
                          201                                                                                      300
        ADBR3      (201)  GCTCCGTGGCCTCACGGAGAACAGCTCTCTTGCCCCATGGCCGGACCTCCCCACCCTGGCGCCCAATACCGCCAACACCAGTGGGCTGCCAGGGGTTCCGT
pr B3 Trp64 Arg FOR  (1)
pr B3 Trp64 Arg REV  (1)
                          301                                                                                      400
        ADBR3      (301)  GGGAGGCGGCCCTAGCCGGGGCCCTGCTGGCGCTGGCGGTGCTGGCCCACGGTGGGAGGCAACCTGCTGGTCATCGTGGCCATCGCCTGGACTCCGAGACT
pr B3 Trp64 Arg FOR  (1)
pr B3 Trp64 Arg REV  (1)
                          401                                                                                      500
        ADBR3      (401)  CCAGACCATGACCAACGTGTTCGTGACTTCGCTGGCCGCAGCCGACCTGGTGATGGGACTCCTGGTGGTGCCGCCGGCGGCCACCTTGGCGCTGACTGGC
pr B3 Trp64 Arg FOR  (1)
pr B3 Trp64 Arg REV  (1)                                         GGTGATGGGACTCCTGGTG
                          501                                                                                      600
        ADBR3      (501)  CACTGGCCGTTGGGCGCCACTGGCTGCGAGCTGTGGACCTCGGTGGACGTGCTGTGTGTGACCGCCAGCATCGAAACCCTGTGCGCCCCTGGCCGTGGACC
pr B3 Trp64 Arg FOR  (1)
pr B3 Trp64 Arg REV (20)
                          601                                                                                      700
        ADBR3      (601)  GCTACCTGGCTGTGACCAACCCGCTGCGTTACGGCGCACTGGTCACCAAGCGCTGCGCCCGGACAGCTGTGGTGCTGGTGTGGGTCGTGTCGGCCGCGGT
pr B3 Trp64 Arg FOR  (1)
pr B3 Trp64 Arg REV (20)
                          701                                                                                      800
        ADBR3      (701)  GTCGTTTGCGCCCATCATGAGCCAGTGGTGGCGCGTAGGGGCCGACGCCGAGGCGCAGCGCTGCCACTCCAAGCCGGGCTGCTGTGCCTTCGCCTCCAAC
pr B3 Trp64 Arg FOR  (1)                                   GTGTTGGCG GTATTGGGCG
pr B3 Trp64 Arg REV (20)
                          801                                                                                      900
        ADBR3      (801)  ATGCCCTACGTGCTGCTGTCCTCCTCCGTCTCCTTCTACCTTCCTCTTCTCGTGATGCTCTTCGTCTACGCGCGGGTTTTCGTGGTGGCTAGGGGCCAGC
pr B3 Trp64 Arg FOR (20)
pr B3 Trp64 Arg REV (20)
                          901                                                                                      1000
        ADBR3      (901)  TGCGCTTGCTGCGCGGGGAGCTGGGCCGCTTTCCGCCCGAGGAGTCTCCGCCGGCGCCGTCGCGCTCTCTGGCCCCGGCCCCGGTGGGGACGTGCGCGCTCC
pr B3 Trp64 Arg FOR (20)
pr B3 Trp64 Arg REV (20)
                          1001                                                                                     1100
        ADBR3     (1001)  GCCCGAAGGGGTGCCCGGCCTGCGGCCGGCGGCCGCGCGCCTCCTGCCTCTCCGGGAACACCGGGCCCTGTGCACCTTGGGTCTCATCATGGGCCACTTC
pr B3 Trp64 Arg FOR (20)
pr B3 Trp64 Arg REV (20)
```

APPENDIX C: PROTEIN SEQUENCES OF DIFFERENT ADRENERGIC RECEPTORS SHOWING THE POSITION OF THE RESPECTIVE INVESTIGATED POLYMORPHISMS

β_1-AR Ser49Gly

MGAGVLVLGASEPGNLSSAAPLPDGAATAARLLVPASPPASLLPPASE[S→G]PEPLSQQWTAGMGLLMALIV
LLIVAGNVLVIVAIAKTPRLQTLTNLFIMSLASADLVMGLLVVPFGATIVVWGRWEYGSFFCELWTSVDVLCVT
ASIETLCVIALDRYLAITSPFRYQSLLTRARARGLVCTVWAISALVSFLPILMHWWRAESDEARRCYNDPKCCD
FVTNRAYAIASSVVSFYVPLCIMAFVYLRVFREAQKQVKKIDSCERRFLGGPARPPSPSPSPVPAPAPPPGPP
RPAAAAATAPLANGRAGKRRPSRLVALREQKALKTLGIIMGVFTLCWLPFFLANVVKAFHRELVPDRLFVFFN
WLGYANSAFNPIIYCRSPDFRKAFQGLLCCARRAARRRHATHGDRPRASGCLARPGPPPSPGAASDDDDDD
VVGATPPARLLEPWAGCNGGAAADSDSSLDEPCRPGFASESKV

β_2-AR Gln27Glu

MGQPGNGSAFLLAPNRSHAPDHDVTQ[Q→E]RDEVWVVGMGIVMSLIVLAIVFGNVLVITAIAKFERLQTVTNY
FITSLACADLVMGLAVVPFGAAHILMKMWTFGNFWCEFWTSIDVLCVTASIETLCVIAVDRYFAITSPFKYQSLL
TKNKARVIILMVWIVSGLTSFLPIQMHWYRATHQEAINCYANETCCDFFTNQAYAIASSIVSFYVPLVIMVFVYS
RVFQEAKRQLQKIDKSEGRFHVQNLSQVEQDGRTGHGLRRSSKFCLKEHKALKTLGIIMGTFTLCWLPFFIVN
IVHVIQDNLIRKEVYILLNWIGYVNSGFNPLIYCRSPDFRIAFQELLCLRRSSLKAYGNGYSSNGNTGEQSGYH
VEQEKENKLLCEDLPGTEDFVGHQGTVPSDNIDSQGRNCSTNDSLL

β_2-AR Arg16Gly

MGQPGNGSAFLLAPN[R→G]SHAPDHDVTQQRDEVWVVGMGIVMSLIVLAIVFGNVLVITAIAKFERLQTVTNY
FITSLACADLVMGLAVVPFGAAHILMKMWTFGNFWCEFWTSIDVLCVTASIETLCVIAVDRYFAITSPFKYQSLL
TKNKARVIILMVWIVSGLTSFLPIQMHWYRATHQEAINCYANETCCDFFTNQAYAIASSIVSFYVPLVIMVFVYS
RVFQEAKRQLQKIDKSEGRFHVQNLSQVEQDGRTGHGLRRSSKFCLKEHKALKTLGIIMGTFTLCWLPFFIVN
IVHVIQDNLIRKEVYILLNWIGYVNSGFNPLIYCRSPDFRIAFQELLCLRRSSLKAYGNGYSSNGNTGEQSGYH
VEQEKENKLLCEDLPGTEDFVGHQGTVPSDNIDSQGRNCSTNDSLL

β_3-AR Trp64Arg

MAPWPHENSSLAPWPDLPTLAPNTANTSGLPGVPWEAALAGALLALAVLATVGGNLLVIVAIA[W→R]TPRLQT
MTNVFVTSLAAADLVMGLLVVPPAATLALTGHWPLGATGCELWTSVDVLCVTASIETLCALAVDRYLAVTNPL
RYGALVTKRCARTAVVLVWVVSAAVSFAPIMSQWWRVGADAEAQRCHSNPRCCAFASNMPYVLLSSSVSF
YLPLLVMLFVYARVFVVATRQLRLLRGELGRFPPEESPPAPSRSLAPAPVGTCAPPEGVPACGRRPARLLPL
REHRALCTLGLIMGTFTLCWLPFFLANVLRALGGPSLVPGPAFLALNWLGYANSAFNPLIYCRSPDFRSAFRR
LLCRCGRRLPPEPCAAARPALFPSGVPAARSSPAQPRLCQRLDGASWGVS

APPENDIX D: DETAILS OF THE VARIOUS AR GENE POLYMORPHISMS

Table D.1: Details for the analysis of the four investigated AR gene polymorphisms

β_1-AR Ser49Gly	β_2-AR Gln27Glu
GenBank NM_000684	**GenBank** NM_000024
Chromosome 10q24-q26	**Chromosome** 5q31-q32
Amino acid 49	**Amino acid** 27
Reference: 49	**Reference:** 5
Forward primer	**Forward primer**
5'-CCGGGCTTCTGGGGTGTTCC-3'	5'-CCGCCGTGGGTCCGCC-'3
Reverse primer	**Reverse primer**
5'-GGCGAGGTGATGGCGAGGTAGC-3'	5'-CCATGACCAGATCAGCAC -'3
Amplicon length: 564	**Amplicon length:** 310 bp
Restriction enzyme: *Eco* 0109I (Fermentas, ER0261)	**Restriction enzyme:** *SatI* (Fermentas, ER1641)
Fragments after digestion:	**Fragments after digestion:**
Ser: 564 bp	Gln27: 171, 84, 55 bp
Gly: 345, 219 bp	Glu27: 226, 84 bp
β_2-AR Arg16Gly	β_3-AR Trp64Arg
GenBank NM_000024	**GenBank** NM_000025
Chromosome 5q31-q32	**Chromosome** 8p12-p11.2
Amino acid 16	**Amino acid** 64
Reference: 7	**Reference:** 5
Forward primer	**Forward primer**
5'-CTTCTTGCTGGCACCCAAT-'3	5'-CGCCCAATACCGCCAACAC-'3
Reverse primer	**Reverse primer**
5'-CCAGTGAAGTGATGAAGTAGTTGG-'3	5'-CCACCAGGAGTCCCATCACC-'3
Amplicon length: 201 bp	**Amplicon length:** 210 bp
Restriction enzyme: *BsrDI* (Fermentas, ER1262)	**Restriction enzyme:** *MvaI* (Fermentas, ER0551)
Fragments after digestion:	**Fragments after digestion:**
Arg16: 131, 56, 14 bp	Trp64: 97, 61, 31, 15, 6 bp
Gly: 108, 56, 23, 14 bp	Arg64: 158, 31, 15, 6 bp

APPENDIX E: EXPECTED DNA FRAGMENTS AFTER RESTRICTION ENZYME DIGESTION
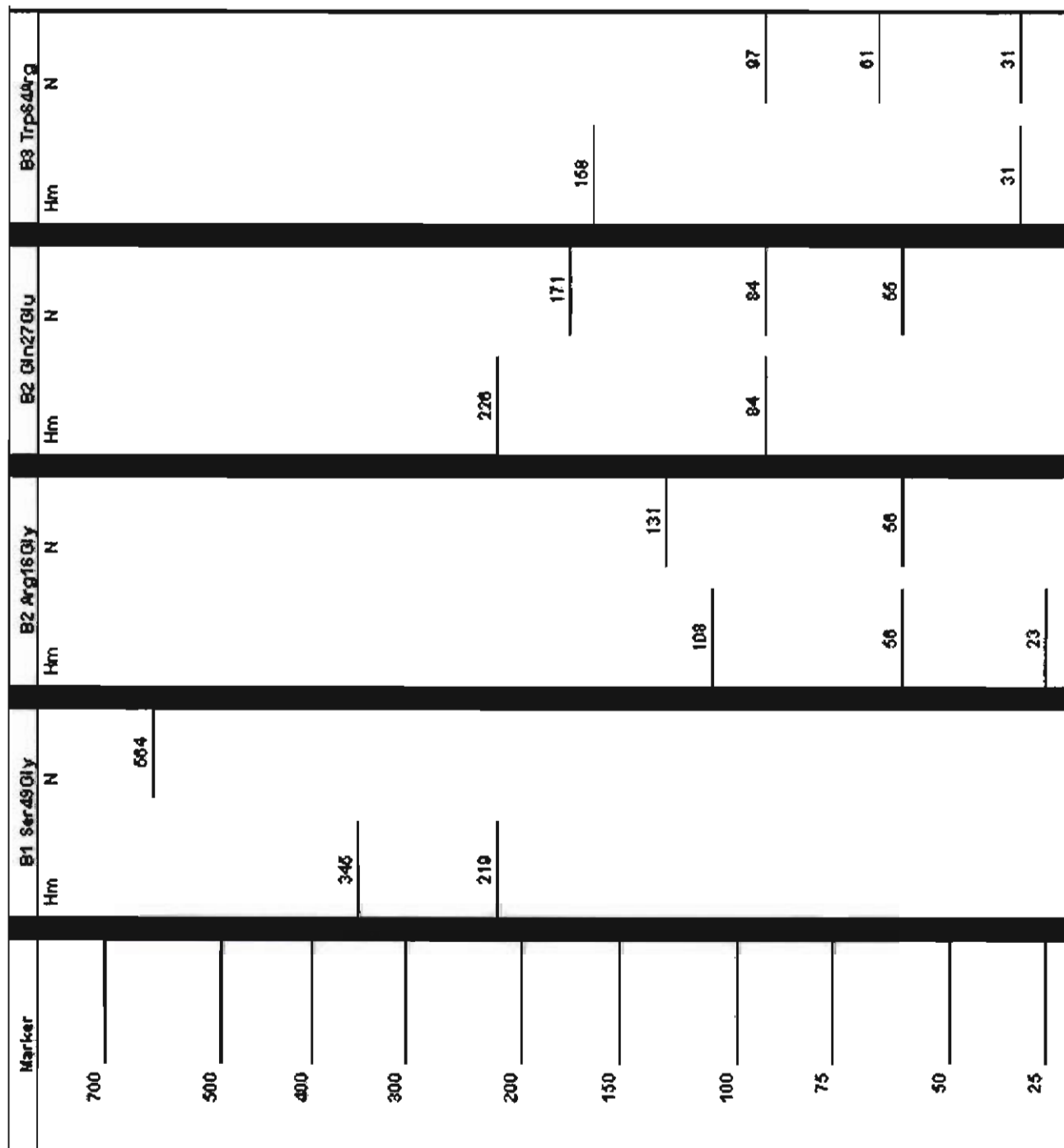
Figure E.1: Expected DNA fragments for the genotypes of the polymorphisms

Note: Only fragments from about 50 bp are clearly distinguishable on the general purpose agarose gel (cost efficient and yield results of sufficient resolution at standardized concentration). Smaller fragments tend to diffuse too much in the agarose gel. Better resolution can be achieved with polyacrylamide gels, but this was not necessary, because the smaller fragments were not needed for distinguishing between variants of the four polymorphisms investigated.

APPENDIX F: EXTENDED TABLES OF RESULTS

Some of the results of statistical analysis included in the tables below, was not done in this study, but obtained from the POWIRS I study on black female volunteers: results pertaining to the β_2-AR Gln27Glu and β_3-AR Trp64Arg polymorphisms. These results were not kept in the original tables as it was generated from the POWIRS I study, but was included in the same format as the other results for the sake of uniformity.

In the POWIRS I study (black women, β_2-AR Gln27Glu and β_3-AR Trp64Arg polymorphisms), body fat % was measured as well as WC, but in the tables of results received from that study, only the body fat % was reported. In the white women, the body fat % was not measured, but the WC was. The WC is indicative of abdominal obesity and is one of the criteria used to diagnose the metabolic syndrome (see table 2.1).

The entire group of volunteers were compared to gain statistical power, seeing as the test group was small to begin with, the division into 2 groups with respect to BMI, further deducts from the statistical power of analysis.

Table F.1: Parameters measured for the four investigated AR gene polymorphisms in the black women with BMI ≤ 25

B1-AR Ser49Gly	Age	SBP	DBP	BMI	Body fat %	Tc	HDL	LDL	Trig	OGTT0	OGTT30	OGTT60	OGTT90	OGTT120	INS0	INS30	INS60	INS90	INS120	FFA0	FFA30	FFA60	FFA90	FFA120	HOMA-IR
Mean Hm (n = 29)	29.833	124.724	72.890	21.957	23.089	4.143	1.316	2.718	0.545	4.870	6.759	6.193	5.872	6.224	75.207	628.483	543.103	395.000	311.690	0.497	0.280	0.073	0.040	0.027	2.355
SD Hm	7.916	10.472	6.879	1.719	4.451	0.815	0.334	0.763	0.225	0.376	1.111	1.495	1.253	2.225	26.780	425.707	381.012	286.927	182.485	0.237	0.208	0.060	0.037	0.018	0.898
Mean Ht (n = 10)	25.600	117.783	72.314	22.311	23.258	4.319	1.298	2.896	0.627	4.829	7.233	6.243	5.843	5.684	78.600	679.800	572.500	408.500	389.100	0.498	0.216	0.055	0.028	0.023	2.424
SD Ht	4.526	6.301	5.189	1.391	3.944	0.934	0.343	0.749	0.403	0.331	1.068	2.013	1.638	1.337	24.391	282.143	249.790	212.624	163.406	0.192	0.154	0.049	0.018	0.007	0.771
B2-AR Gln27Glu																									
Mean Hm* (n = 3)	25.667	123.000	70.000	21.700	20.900	4.410	1.670	2.582	0.773	4.630	8.140	5.183	4.887	6.147	80.000	575.000	331.000	198.000	259.000	0.547	0.123	0.047	0.017	0.020	1.670
SD Hm*	4.933	15.000	7.200	0.970	1.300	0.800	0.090	0.710	0.150	0.370	1.090	1.840	1.800	0.480	3.610	334.600	59.400	84.100	0.340	0.060	0.030	0.005	0.010	0.580
Mean Ht (n = 8)	27.667	125.000	72.000	21.100	20.300	3.930	1.220	2.630	0.463	4.670	6.770	6.320	6.244	5.984	72.300	693.600	598.500	596.100	471.600	0.694	0.349	0.051	0.023	0.018	1.750
SD Ht	4.472	9.700	6.800	1.600	3.600	0.450	0.190	0.550	0.240	0.360	1.250	0.960	1.460	1.240	19.400	530.000	498.000	840.000	610.000	0.380	0.230	0.060	0.020	0.010	0.710
Mean N (n = 28)	29.241	123.000	74.000	22.100	23.400	4.130	1.270	2.740	0.567	4.890	7.010	6.038	5.892	77.960	686.900	603.300	449.100	357.600	0.466	0.279	0.078	0.043	0.028	2.140
SD N	8.271	10.600	8.700	1.700	3.800	0.810	0.350	0.730	0.290	0.380	1.080	1.700	1.350	1.170	28.800	415.000	343.000	298.000	173.000	0.230	0.210	0.080	0.040	0.020	1.000
B2-AR Arg16Gly																									
Mean Hm (n = 11)	27.818	126.299	70.638	21.342	24.232	4.312	1.358	2.839	0.574	4.889	6.422	5.455	5.717	5.799	83.818	730.000	601.545	584.364	488.818	0.591	0.385	0.124	0.059	0.035	2.634
SD Hm	5.528	10.840	7.094	2.026	4.080	1.119	0.488	0.960	0.232	0.392	1.172	1.681	1.798	1.144	25.218	459.560	403.484	719.519	510.199	0.418	0.234	0.092	0.045	0.027	0.885
Mean Ht (n = 18)	28.000	121.525	73.828	22.483	21.935	4.153	1.340	2.689	0.621	4.827	7.034	6.539	6.209	6.468	73.500	864.778	597.278	455.611	338.278	0.493	0.301	0.061	0.030	0.022	2.278
SD Ht	5.696	11.342	8.276	1.617	3.910	0.806	0.289	0.769	0.324	0.391	1.051	1.389	1.374	2.788	24.062	505.840	404.352	335.126	181.381	0.216	0.211	0.071	0.027	0.008	0.777
Mean N (n = 11)	30.545	123.751	73.829	21.735	22.904	4.072	1.191	2.789	0.461	4.820	7.181	6.500	5.760	5.945	73.909	619.909	514.455	324.636	329.727	0.505	0.165	0.037	0.027	0.021	2.270
SD N	11.067	8.167	6.780	1.300	4.222	0.554	0.259	0.472	0.208	0.351	1.048	1.725	1.138	1.124	29.463	217.003	270.254	165.821	158.288	0.191	0.099	0.025	0.018	0.003	0.939
B3-AR Trp64Arg																									
Mean Hm* (n = 2)	29.500	120.000	76.000	23.800	27.300	3.680	1.230	2.350	0.510	4.850	7.700	6.000	4.800	5.100	101.000	1354.000	922.000	449.000	427.000	0.355	0.150	0.020	0.020	0.025	3.110
SD Hm*	6.364	11.000	5.000	0.600	4.100	0.600	0.200	0.400	0.100	0.400	0.700	1.000	1.000	1.800	21.200	1273.000	640.000	139.000	186.000	0.250	0.030	0.000	0.000	0.010	0.430
Mean Ht (n = 18)	27.167	123.000	73.000	21.500	21.200	4.090	1.290	2.690	0.590	4.760	6.770	6.090	5.750	5.890	73.900	717.300	543.200	389.800	321.200	0.501	0.291	0.075	0.042	0.025	2.270
SD Ht	4.091	9.000	7.000	1.600	3.300	0.860	0.310	0.750	0.360	0.300	1.000	1.420	1.440	1.010	28.900	319.000	306.000	249.000	169.000	0.230	0.190	0.080	0.040	0.020	0.950
Mean N (n = 21)	29.810	124.000	72.000	22.000	23.300	4.170	1.300	2.780	0.540	4.860	6.920	6.440	6.320	6.040	77.200	680.300	570.800	520.800	412.500	0.550	0.287	0.072	0.034	0.025	2.400
SD N	9.421	12.000	10.000	1.700	3.800	0.850	0.370	0.650	0.210	0.460	1.230	1.790	1.350	1.180	23.200	362.000	398.000	586.000	396.000	0.320	0.230	0.070	0.020	0.020	0.780

*signifies a very number of test subjects deducting from statistical power

Table F.2: Comparison of measured parameters for the four investigated AR gene polymorphisms in the black women with BMI ≤ 25

B1-AR Ser49Gly	Age	SBP	DBP	BMI	WC	Body fat %	Tc	HDL	LDL	Trig	OGTT0	OGTT30	OGTT60	OGTT90	OGTT120	INS0	INS30	INS60	INS90	INS120	FFA0	FFA30	FFA60	FFA90	FFA120	HOMA-IR
Hm vs Ht	0.119	0.075	0.914	0.690	0.911	0.917	0.742	0.861	0.697	0.489	0.763	0.502	0.755	0.653	0.364	0.705	0.663	0.808	0.969	0.291	0.908	0.312	0.527	0.348	0.452	0.830
B2-AR Gln27Glu																										
Hm vs Ht*	0.528	0.941	0.748	0.880	0.871	0.808	0.685	**0.004**	0.639	0.128	0.874	0.363	0.295	0.264	0.696	0.474	0.824	0.339	0.405	0.514	0.703	0.130	0.929	0.481	0.816	0.568
Hm vs N	0.472	0.988	0.476	0.709	0.874	0.261	0.570	0.061	0.718	0.239	0.262	0.195	0.266	0.181	0.715	0.905	0.656	0.158	0.162	0.343	0.581	0.209	0.518	0.233	0.481	0.906
Ht vs N	0.590	0.934	0.463	0.338	0.642	0.215	0.933	0.711	0.925	0.555	0.098	0.614	0.228	0.110	0.508	0.601	0.881	0.970	0.351	0.259	0.106	0.458	0.312	0.113	0.356	0.427
B2-AR Arg16Gly																										
Hm vs Ht	0.933	0.216	0.368	0.073	0.867	0.291	0.889	0.914	0.883	0.591	0.680	0.109	0.071	0.265	0.306	0.257	0.647	0.960	0.547	0.384	0.347	0.426	**0.040**	**0.032**	0.119	0.265
Hm vs N	0.473	0.540	0.360	0.595	0.465	0.481	0.531	0.312	0.878	0.244	0.668	0.135	0.165	0.948	0.785	0.407	0.481	0.559	0.257	0.398	0.540	**0.017**	**0.007**	**0.037**	0.087	0.351
Ht vs N	0.410	0.484	0.890	0.144	0.410	0.796	0.542	0.133	0.999	0.121	0.963	0.754	0.947	0.372	0.561	0.988	0.784	0.553	0.239	0.898	0.889	0.057	0.295	0.783	0.611	0.982
B3-AR Trp64Arg																										
Hm vs Ht*	0.471	0.626	0.515	0.106	0.088	0.092	0.469	0.721	0.466	0.720	0.881	0.218	0.981	0.435	0.538	0.195	0.057	0.131	0.716	0.401	0.365	0.301	0.348	0.446	0.927	0.231
Hm vs N*	0.985	0.714	0.521	0.201	0.561	0.264	0.313	0.768	0.374	0.751	0.971	0.615	0.654	0.251	0.384	0.162	**0.027**	0.259	0.849	0.992	0.451	0.422	0.339	0.449	0.898	0.224
Ht vs N	0.277	0.854	0.748	0.326	0.082	0.166	0.766	0.904	0.757	0.575	0.724	0.424	0.238	0.099	0.971	0.705	0.234	0.779	0.397	0.383	0.601	0.947	0.901	0.482	0.957	0.856

Significant difference indicated by bold print and shaded blocks

Table F.3: Parameters measured for the four investigated AR gene polymorphisms in the black women with BMI > 25

B1-AR Ser49Gly	Age	SBP	DBP	BMI	Body fat %	Tc	HDL	LDL	Trig	OGTT0	OGTT30	OGTT60	OGTT90	OGTT120	INS0	INS30	INS60	INS90	INS120	FFA0	FFA30	FFA60	FFA90	FFA120	HOMA-IR
Mean Hm* (n = 35)	32.884	131.271	79.034	31.823	40.059	4.071	1.161	2.757	0.782	5.049	6.979	6.740	6.010	5.513	105.600	648.200	656.200	513.086	448.114	0.534	0.292	0.086	0.043	0.028	3.484
SD Hm*	0.757	20.258	11.990	5.631	9.838	0.678	0.314	0.844	0.405	0.573	1.284	1.058	1.624	1.692	53.919	382.450	466.355	984.413	311.480	0.255	0.203	0.083	0.037	0.017	1.837
Mean Ht (n = 20)	33.350	138.452	82.883	31.872	41.565	4.466	1.312	3.007	0.733	5.328	7.781	7.463	7.791	7.200	91.400	664.700	577.600	471.700	493.903	0.583	0.350	0.112	0.052	0.031	3.113
SD Ht	8.826	28.853	9.635	4.128	6.751	1.032	0.333	1.072	0.343	0.618	1.658	2.307	1.938	25.987	623.828	378.995	257.183	409.292	0.256	0.214	0.135	0.047	0.017	0.990	
B2-AR Gln27Glu																									
Mean Hm* (n = 2)	28.500	110.000	57.000	28.600	25.1	3.200	1.060	2.070	0.380	4.700	6.350	5.450	3.850	4.800	68.000	459.000	88.500	152.000	134.000	0.390	0.020	0.090	0.020	0.020	1.500
SD Hm*	2.121	8.200	8.300	1.300	6.900	0.810	0.320	0.510	0.130	0.140	1.770	0.640	0.640	1.130	12.700	401.000	17.700	34.000	11.300	0.520	0.000	0.100	0.000	0.000	0.710
Mean Ht (n = 14)	37.215	138.000	84.000	32.300	43.100	4.510	1.180	3.140	0.930	5.510	8.100	8.530	9.000	8.050	113.800	672.000	842.000	661.000	616.800	0.523	0.354	0.120	0.050	0.033	3.360
SD Ht	8.746	30.400	10.200	5.000	6.700	1.060	0.280	0.930	0.320	0.980	1.830	2.500	2.690	2.030	40.600	320.000	426.000	312.000	224.000	0.250	0.220	0.080	0.030	0.020	0.840
Mean N (n = 45)	31.911	133.000	81.000	31.800	40.000	4.330	1.250	2.820	0.790	5.410	7.180	6.920	7.100	6.800	101.000	648.000	568.000	454.000	435.000	0.574	0.319	0.090	0.050	0.031	2.710
SD N	9.000	20.000	10.400	5.200	8.000	1.010	0.360	0.980	0.500	1.580	1.380	2.280	2.080	2.260	48.300	503.000	400.000	307.000	353.000	0.260	0.200	0.110	0.040	0.020	1.160
B2-AR Arg16Gly																									
Mean Hm (n = 7)	37.860	157.496	88.972	32.570	43.884	4.586	1.146	3.254	0.931	5.730	8.323	8.414	8.798	7.940	99.857	511.714	574.143	398.143	399.143	0.623	0.404	0.131	0.071	0.046	3.601
SD Hm	10.203	51.255	17.842	3.904	5.783	0.950	0.188	0.877	0.377	0.987	2.143	2.734	2.898	2.387	41.503	201.258	380.542	144.583	71.404	0.258	0.211	0.096	0.047	0.024	1.344
Mean Ht (n = 40)	32.233	130.138	78.599	31.300	38.810	4.133	1.212	2.779	0.708	5.016	7.087	6.810	6.944	6.593	92.425	658.000	619.400	505.575	450.575	0.555	0.298	0.090	0.043	0.028	3.003
SD Ht	8.978	15.828	10.141	4.830	8.545	0.896	0.332	0.972	0.380	0.482	1.358	1.785	1.834	1.608	32.210	498.521	425.079	329.793	337.823	0.258	0.209	0.114	0.042	0.015	1.177
Mean N (n = 8)	31.860	131.940	82.136	33.407	41.852	4.285	1.296	2.837	0.811	5.318	7.269	6.734	7.303	8.584	141.875	759.875	715.500	547.750	593.125	0.471	0.306	0.083	0.040	0.028	4.772
SD N	7.373	7.551	5.996	7.927	8.822	0.658	0.405	0.767	0.475	0.406	0.939	1.514	1.639	1.599	82.128	503.452	568.693	436.602	508.540	0.256	0.194	0.034	0.021	0.012	2.577
B3-AR Trp64Arg																									
Mean Hm* (n = 3)	27.667	120.000	77.000	23.300	33.100	4.080	1.050	2.920	0.530	4.570	6.630	5.500	5.770	5.900	62.700	668.700	352.700	334.300	277.300	0.603	0.337	0.017	0.020	0.020	1.880
SD Hm*	33.467	11.000	7.000	4.800	13.000	0.970	0.090	0.860	0.300	0.500	0.750	0.780	1.100	0.260	26.800	114.300	52.800	148.800	88.000	0.220	0.250	0.010	0.000	0.000	0.850
Mean Ht (n = 28)	33.467	137.000	83.000	31.100	40.700	4.540	1.280	3.080	0.860	5.650	7.330	7.020	7.160	6.810	100.600	710.300	653.200	492.600	469.100	0.585	0.292	0.099	0.046	0.030	3.500
SD Ht	9.051	22.000	12.000	3.900	7.400	1.100	0.370	1.100	0.500	1.900	2.500	2.500	2.200	2.500	35.200	572.000	491.000	299.000	336.000	0.290	0.200	0.090	0.020	0.020	1.500
Mean N (n = 30)	33.107	131.000	79.000	32.700	40.500	4.150	1.190	2.800	0.790	5.230	7.470	7.650	7.870	7.350	109.600	579.100	591.800	502.000	479.600	0.520	0.341	0.103	0.051	0.032	3.740
SD N	9.134	24.000	10.000	6.100	10.000	0.800	0.320	0.800	0.400	0.700	1.800	2.400	2.600	2.000	55.700	338.000	363.000	353.000	352.000	0.240	0.200	0.110	0.040	0.020	1.900

*signifies a very low number of test subjects excluding from statistical power

Table F.4: Comparison of measured parameters for the four investigated AR gene polymorphisms in the black women with BMI > 25

B1-AR Ser49Gly	Age	SBP	DBP	BMI	WC	Body fat %	Tc	HDL	LDL	Trig	OGTT0	OGTT30	OGTT60	OGTT90	OGTT120	INS0	INS30	INS60	INS90	INS120	FFA0	FFA30	FFA60	FFA90	FFA120	HOMA-IR
Hm vs Ht	0.842	0.365	0.354	0.743	0.952	0.540	0.288	0.127	0.488	0.367	0.721	0.084	0.302	0.514	0.191	0.128	0.994	0.642	0.761	0.631	0.624	0.425	0.508	0.430	0.506	0.156
B2-AR Gln27Glu																										
Hm vs Ht	0.194	0.203	0.001	0.098	0.090	0.001	0.129	0.581	0.155	0.035	0.281	0.281	0.010	0.019	0.019	0.117	0.320	0.031	0.055	0.018	0.475	0.055	0.546	0.169	0.359	0.121
Hm vs N	0.599	0.100	0.693	0.153	0.137	0.026	0.125	0.483	0.244	0.234	0.530	0.415	0.034	0.355	0.149	0.332	0.595	0.103	0.171	0.240	0.360	0.043	0.991	0.388	0.437	0.258
Ht vs N	0.058	0.373	0.204	0.599	0.394	0.107	0.701	0.496	0.584	0.333	0.875	0.163	0.049	0.142	0.053	0.287	0.748	0.030	0.055	0.112	0.719	0.621	0.255	0.006	0.958	0.204
B2-AR Arg16Gly																										
Hm vs Ht	0.099	0.024	0.075	0.831	0.749	0.148	0.117	0.477	0.198	0.248	0.493	0.103	0.077	0.102	0.019	0.294	0.531	0.618	0.335	0.524	0.810	0.077	0.263	0.000	0.041	0.140
Hm vs N	0.172	0.247	0.544	0.778	0.828	0.501	0.172	0.853	0.190	0.437	0.162	0.198	0.209	0.112	0.141	0.241	0.152	0.471	0.322	0.198	0.208	0.175	0.028	0.054	0.018	0.309
Ht vs N	0.915	0.795	0.270	0.414	0.880	0.453	0.904	0.542	0.901	0.809	0.895	0.912	0.938	0.725	0.948	0.043	0.376	0.597	0.750	0.249	0.323	0.974	0.445	0.872	0.772	0.069
B3-AR Trp64Arg																										
Hm vs Ht	0.300	0.183	0.416	0.403	0.897	0.105	0.550	0.320	0.867	0.285	0.335	0.394	0.270	0.277	0.547	0.058	0.870	0.298	0.357	0.334	0.901	0.728	0.141	0.262	0.401	0.068
Hm vs N	0.337	0.407	0.636	0.314	0.782	0.228	0.896	0.511	0.700	0.337	0.111	0.462	0.179	0.117	0.232	0.155	0.684	0.276	0.453	0.357	0.622	0.057	0.195	0.236	0.234	0.111
Ht vs N	0.881	0.308	0.241	0.215	0.626	0.921	0.144	0.290	0.256	0.546	0.280	0.726	0.265	0.329	0.379	0.460	0.292	0.594	0.914	0.909	0.351	0.374	0.898	0.695	0.671	0.604

Significant difference indicated by bold print and shaded effects

Table F.5: Parameters measured for the four investigated AR gene polymorphisms in the white women with BMI ≤ 25

B1-AR Ser49Gly	Age	SBP	DBP	BMI	WC	Tc	HDL	LDL	Trig	OGTT0	OGTT30	OGTT60	OGTT90	OGTT120	INS0	INS30	INS60	INS90	INS120	FFA0	FFA30	FFA60	FFA90	FFA120	HOMA-IR
Mean Hm (n = 45)	27.891	120.889	67.880	21.907	71.571	4.858	1.39?	3.016	0.995	4.816	7.331	7.351	6.511	6.287	72.422	508.511	463.800	350.556	276.178	0.676	0.313	0.061	0.033	0.028	2.245
SD Hm	8.351	9.533	8.504	1.941	6.829	0.920	0.309	0.813	0.443	0.316	1.185	1.598	1.804	1.523	17.857	328.638	265.221	164.812	163.975	0.209	0.190	0.053	0.029	0.025	0.618
Mean Ht* (n = 2)	32.000	116.008	66.876	25.309	78.400	4.110	1.105	2.448	1.230	4.800	6.700	5.550	5.850	5.550	77.000	577.000	405.000	429.000	351.500	0.335	0.110	0.035	0.035	0.030	2.385
SD Ht*	4.243	3.456	0.813	0.043	5.233	1.160	0.290	0.471	0.877	0.283	1.980	2.899	1.768	2.051	0.000	161.220	386.080	342.240	292.035	0.035	0.071	0.007	0.021	0.014	0.139
B2-AR Gln27Glu																									
Mean Hm (n =13)	27.067	112.923	71.846	22.717	75.331	4.482	1.213	2.661	0.895	4.862	7.146	7.362	6.646	6.192	64.692	488.154	468.769	358.462	280.231	0.555	0.340	0.056	0.025	0.022	2.023
SD Hm	6.563	9.785	6.950	1.895	9.665	0.637	0.249	0.659	0.304	0.362	1.246	2.034	2.548	2.006	10.873	308.746	291.252	189.728	204.329	0.197	0.196	0.046	0.013	0.007	0.412
Mean Ht (n = 16)	25.625	110.188	68.250	21.663	70.269	0.790	1.496	3.130	1.000	0.323	1.477	1.794	1.399	1.503	71.313	485.813	439.875	356.813	241.875	0.573	0.321	0.057	0.034	0.024	2.174
SD Ht	4.603	7.574	8.820	2.146	4.620	4.850	0.311	0.744	0.405	4.861	7.544	7.228	6.411	6.217	20.058	302.641	288.727	155.421	123.852	0.249	0.177	0.042	0.023	0.007	0.681
Mean N (n = 18)	31.235	112.444	70.833	21.739	70.772	5.081	1.393	2.962	1.089	4.725	7.163	7.256	6.406	6.350	79.500	552.444	476.389	349.444	312.111	0.565	0.264	0.065	0.038	0.036	2.481
SD N	11.144	8.389	9.432	1.996	5.473	1.176	0.314	0.985	0.576	0.255	0.951	1.231	1.243	1.071	17.016	356.888	241.050	175.954	172.578	0.194	0.202	0.068	0.039	0.036	0.594
B2-AR Arg16Gly																									
Mean Hm (n =13)	32.846	121.584	72.320	21.571	71.185	4.863	1.421	3.005	0.982	4.762	6.908	8.962	8.762	6.515	73.077	440.077	399.077	340.154	264.154	0.588	0.283	0.077	0.043	0.035	2.225
SD Hm	9.521	8.117	9.704	2.113	6.090	0.958	0.342	0.595	0.480	0.283	1.091	1.872	1.455	1.396	13.232	237.837	187.757	135.649	108.276	0.241	0.191	0.074	0.048	0.044	0.410
Mean Ht (n = 18)	23.737	120.978	66.772	22.331	73.079	4.838	1.367	2.976	1.089	4.847	7.542	7.289	6.132	6.028	70.632	651.388	540.474	418.474	340.579	0.568	0.321	0.053	0.032	0.025	2.196
SD Ht	2.491	11.251	7.798	2.181	8.893	0.914	0.275	0.628	0.442	0.259	1.329	1.182	1.557	1.178	19.743	411.640	317.493	198.747	194.969	0.194	0.202	0.040	0.018	0.008	0.826
Mean N (n = 15)	29.313	119.525	65.303	22.115	70.907	4.780	1.355	3.000	0.935	4.820	7.347	7.527	6.660	6.320	74.733	396.000	414.933	284.000	215.067	0.545	0.303	0.054	0.025	0.025	2.340
SD N	9.350	8.257	6.440	1.777	4.248	0.987	0.343	0.974	0.463	0.416	1.110	2.166	2.312	2.017	18.534	160.906	240.772	129.395	150.697	0.215	0.187	0.044	0.018	0.011	0.730
B3-AR Trp64Arg																									
Mean Ht (n =6)	31.000	111.732	69.878	21.954	71.787	4.157	1.401	3.058	1.024	4.883	7.417	7.200	6.917	6.500	73.951	538.146	479.024	381.902	287.976	0.580	0.310	0.064	0.034	0.029	2.288
SD Ht	10.954	8.929	8.793	2.027	2.569	4.925	0.309	0.794	0.467	4.805	7.288	7.285	6.410	6.220	18.120	332.086	275.228	178.236	175.673	0.218	0.198	0.055	0.030	0.026	0.826
Mean N (n = 41)	27.643	112.333	72.667	22.721	71.878	0.952	1.220	2.537	0.878	0.349	0.818	1.226	1.577	0.672	63.500	342.500	340.187	299.187	220.667	0.483	0.272	0.032	0.025	0.018	1.991
SD N	7.655	7.633	6.772	2.026	7.307	0.895	0.300	0.805	0.370	0.311	1.258	1.725	1.830	1.617	8.550	156.156	154.789	77.288	64.893	0.119	0.141	0.016	0.014	0.004	0.345

*signifies a very number of test subjects deducting from statistical power

Table F.6: Comparison of measured parameters for the four investigated AR gene polymorphisms in the white women with BMI ≤ 25

B1-AR Ser49Gly	Age	SBP	DBP	BMI	WC	Tc	HDL	LDL	Trig	OGTT0	OGTT30	OGTT60	OGTT90	OGTT120	INS0	INS30	INS60	INS90	INS120	FFA0	FFA30	FFA60	FFA90	FFA120	HOMA-IR
Hm vs Ht	0.496	0.478	0.870	0.018	0.171	0.269	0.207	0.334	0.481	0.946	0.474	0.135	0.512	0.510	0.721	0.771	0.783	0.528	0.538	0.114	0.142	0.501	0.912	0.902	0.785
B2-AR Gln27Glu																									
Hm vs Ht	0.482	0.403	0.242	0.272	0.074	0.032	0.013	0.319	0.448	0.299	0.974	0.885	0.764	0.816	0.295	0.998	0.805	0.996	0.538	0.838	0.788	0.965	0.217	0.294	0.492
Hm vs N	0.215	0.885	0.748	0.180	0.106	0.336	0.098	0.747	0.280	0.997	0.368	0.807	0.626	0.958	0.018	0.594	0.921	0.916	0.642	0.881	0.308	0.660	0.258	0.200	0.024
Ht vs N	0.071	0.419	0.417	0.863	0.775	0.490	0.342	0.578	0.611	0.210	0.318	0.960	0.994	0.808	0.207	0.564	0.689	0.898	0.187	0.927	0.392	0.675	0.723	0.255	0.170
B2-AR Arg16Gly																									
Hm vs Ht	0.000	0.869	0.084	0.335	0.510	0.942	0.628	0.914	0.446	0.388	0.185	0.520	0.258	0.293	0.700	0.106	0.181	0.227	0.210	0.776	0.602	0.236	0.328	0.338	0.885
Hm vs N	0.324	0.513	0.033	0.466	0.888	0.823	0.619	0.986	0.881	0.666	0.303	0.452	0.892	0.772	0.791	0.566	0.849	0.273	0.338	0.615	0.779	0.319	0.179	0.373	0.618
Ht vs N	0.017	0.879	0.561	0.757	0.391	0.859	0.910	0.939	0.330	0.816	0.651	0.686	0.432	0.599	0.541	0.030	0.214	0.030	0.048	0.760	0.801	0.925	0.319	0.656	0.539
B3-AR Trp64Arg																									
Ht vs N	0.356	0.872	0.462	0.392	0.972	0.058	0.185	0.141	9.471	0.573	0.809	0.908	0.523	0.879	0.174	0.170	0.236	0.403	0.361	0.208	0.655	0.162	0.483	0.312	0.265

Significant difference indicated by bold print and shaded blocks

Table F.7: Parameters measured for the four investigated AR gene polymorphisms in the white women with BMI > 25

B1-AR Ser49Gly	Age	SBP	DBP	BMI	WC	Tc	HDL	LDL	Trig	OGTT0	OGTT30	OGTT60	OGTT90	OGTT120	INS0	INS30	INS60	INS90	INS120	FFA0	FFA30	FFA60	FFA90	FFA120	HOMA-IR
Mean Hm (n = 52)	33.478	129.125	76.062	41.073	108.559	5.081	1.078	3.278	1.594	5.353	6.312	8.735	8.124	7.429	115.529	663.235	640.765	543.000	400.588	0.626	0.491	0.115	0.057	0.056	4.046
SD Hm	6.557	9.798	8.023	6.325	11.212	0.970	0.252	0.854	0.812	0.415	1.157	2.073	2.043	1.815	35.144	361.068	373.150	386.683	272.862	0.182	0.201	0.070	0.053	0.075	1.438
Mean Ht (n = 11)	33.091	133.609	78.427	32.153	95.109	4.936	1.083	3.096	1.871	5.116	6.335	8.145	7.327	6.691	122.182	856.636	785.182	668.182	616.818	0.500	0.307	0.110	0.098	0.064	3.999
SD Ht	12.251	18.577	8.029	4.325	8.526	0.619	0.306	0.869	0.733	0.368	1.808	1.578	1.034	1.487	30.727	533.933	494.154	400.793	462.355	0.167	0.280	0.142	0.101	0.125	1.063
B2-AR Gln27Glu																									
Mean Hm (n = 17)	35.000	125.824	82.000	34.009	99.400	5.262	1.070	3.417	1.704	5.371	8.618	8.053	7.852	7.371	110.882	683.528	648.647	569.000	411.708	0.559	0.360	0.090	0.049	0.033	3.899
SD Hm	11.321	4.994	14.349	6.892	10.383	1.035	0.255	0.709	1.135	0.369	1.078	1.915	1.936	1.919	41.708	378.823	432.478	450.227	254.034	0.150	0.191	0.055	0.034	0.018	1.798
Mean Ht (n = 30)	33.212	124.467	78.467	32.746	94.557	4.806	1.054	3.106	1.423	5.031	7.838	7.544	8.844	6.219	106.800	644.867	638.633	539.300	473.833	0.590	0.409	0.117	0.058	0.053	3.554
SD Ht	8.908	8.295	10.725	7.080	10.176	0.818	0.237	0.940	0.708	0.495	1.669	2.455	2.049	1.958	36.429	388.178	349.045	308.756	335.459	0.215	0.262	0.108	0.069	0.084	1.356
Mean N (n = 15)	33.819	121.875	77.500	33.079	96.775	4.806	1.145	3.048	1.374	5.157	8.000	7.823	7.430	6.907	104.188	733.563	754.625	652.375	509.063	0.488	0.314	0.079	0.066	0.078	3.370
SD N	8.441	6.711	9.394	9.675	11.181	0.700	0.307	0.688	0.460	0.298	1.074	1.420	1.574	0.846	24.139	462.028	392.260	481.733	350.316	0.117	0.124	0.061	0.077	0.120	0.875
B2-AR Arg16Gly																									
Mean Hm (n = 14)	31.467	125.027	74.585	31.882	95.079	5.133	1.044	3.483	1.378	5.066	7.929	7.438	8.871	6.421	112.143	705.857	552.571	497.786	394.357	0.493	0.270	0.098	0.070	0.074	3.678
SD Hm	8.026	11.201	7.403	3.673	8.543	1.341	0.251	1.144	0.880	0.301	1.314	1.990	1.690	1.637	45.845	491.478	390.531	437.390	256.488	0.118	0.139	0.067	0.081	0.129	1.691
Mean Ht (n = 39)	34.500	128.104	74.824	33.783	97.356	4.887	1.103	3.123	1.454	5.215	8.038	8.046	7.713	7.095	105.333	627.256	663.487	595.154	475.154	0.563	0.413	0.109	0.056	0.044	3.574
SD Ht	9.716	8.543	7.978	6.832	12.171	0.857	0.247	0.696	0.742	0.445	1.108	2.071	2.045	1.852	32.106	345.814	369.331	355.325	285.344	0.196	0.208	0.095	0.063	0.068	1.366
Mean N (n = 10)	33.600	133.490	79.524	32.591	94.690	4.828	1.046	2.974	1.769	5.190	8.740	8.460	7.110	6.540	107.200	835.800	786.800	611.000	530.700	0.535	0.353	0.080	0.047	0.067	3.595
SD N	8.553	18.850	8.874	5.069	8.059	0.700	0.331	0.719	0.911	0.381	1.755	1.614	1.472	1.452	28.421	457.268	490.084	464.731	491.225	0.175	0.297	0.075	0.028	0.088	1.091
B3-AR Trp64Arg																									
Mean Ht (n = 10)	34.700	123.148	78.815	33.351	92.250	5.394	1.090	3.132	1.437	5.410	8.880	8.620	8.090	8.390	104.741	845.389	843.741	548.833	445.352	0.548	0.360	0.096	0.058	0.053	3.491
SD Ht	9.551	8.975	7.499	8.225	8.053	4.875	0.285	0.852	0.817	5.143	7.950	7.794	7.278	6.756	33.888	381.493	367.239	386.108	296.832	0.163	0.203	0.080	0.006	0.088	1.350
Mean N (n = 54)	33.509	131.600	81.800	31.835	96.901	4.945	1.050	3.565	1.714	5.296	8.892	8.674	8.844	8.385	118.300	809.700	760.300	692.500	559.200	0.673	0.439	0.139	0.094	0.078	4.133
SD N	9.167	19.109	9.449	4.493	10.862	0.957	0.231	0.869	0.630	0.408	1.112	1.672	1.472	1.855	38.520	491.002	467.909	388.160	390.026	0.268	0.290	0.121	0.101	0.090	1.457

Table F.8: Comparison of measured parameters for the four investigated AR gene polymorphisms in the white women with BMI > 25

B1-AR Ser49Gly	Age	SBP	DBP	BMI	WC	Tc	HDL	LDL	Trig	OGTT0	OGTT30	OGTT60	OGTT90	OGTT120	INS0	INS30	INS60	INS90	INS120	FFA0	FFA30	FFA60	FFA90	FFA120	HOMA-IR
Hm vs Ht	0.815	0.030	0.193	0.541	0.651	0.982	0.084	0.726	0.403	0.566	0.551	0.758	0.845	0.732	0.118	0.103	0.295	0.401	0.083	0.264	0.281	0.445	0.546	0.680	0.296
B2-AR Gln27Glu																									
Hm vs Ht	0.573	0.526	0.104	0.482	0.127	0.147	0.026	0.242	0.300	0.099	0.130	0.205	0.382	0.433	0.715	0.742	0.935	0.790	0.512	0.593	0.503	0.342	0.634	0.337	0.460
Hm vs N	0.687	0.253	0.132	0.653	0.490	0.148	0.450	0.134	0.288	0.024	0.123	0.125	0.085	0.038	0.560	0.736	0.499	0.603	0.398	0.147	0.419	0.560	0.438	0.135	0.296
Ht vs N	0.668	0.420	0.700	0.808	0.500	0.969	0.288	0.828	0.806	0.249	0.828	0.611	0.304	0.181	0.813	0.493	0.309	0.327	0.740	0.085	0.178	0.199	0.733	0.411	0.628
B2-AR Arg16Gly																									
Hm vs Ht	0.284	0.293	0.923	0.331	0.510	0.435	0.451	0.198	0.749	0.319	0.783	0.344	0.174	0.235	0.551	0.519	0.288	0.412	0.356	0.112	0.021	0.436	0.510	0.276	0.520
Hm vs N	0.532	0.182	0.144	0.701	0.698	0.517	0.975	0.246	0.299	0.462	0.208	0.194	0.722	0.858	0.770	0.518	0.209	0.549	0.384	0.487	0.367	0.790	0.399	0.885	0.834
Ht vs N	0.789	0.184	0.104	0.809	0.518	0.838	0.561	0.550	0.258	0.870	0.122	0.561	0.387	0.384	0.868	0.118	0.466	0.907	0.042	0.482	0.462	0.357	0.669	0.335	0.984
B3-AR Trp64Arg																									
Ht vs N	0.707	0.030	0.271	0.468	0.197	0.120	0.654	0.134	0.314	0.033	0.035	0.237	0.216	0.292	0.259	0.236	0.401	0.284	0.298	0.693	0.317	0.154	0.138	0.418	0.177

Significant difference indicated by bold print and shaded blocks

Table F.9: Comparison of measured parameters for the genotypes of the β_1-AR Ser49Gly polymorphism in the entire group of black women

| Parameter | Hm (n=71) | | Ht (n=30) | | P-values |
	Mean	SD	Mean	SD	Hm vs. Ht
Age (years)	31.58	8.49	30.77	9.13	0.669
BMI (kg/m^2)	27.87	6.58	28.55	5.65	0.623
WC (cm)	86.83	14.85	88.59	14.64	0.587
Body fat %	32.89	11.61	35.46	10.57	0.299
SBP (mmHg)	128.89	16.66	131.56	25.61	0.535
DBP (mmHg)	76.93	11.12	79.36	9.74	0.301
Tc (mmol/l)	4.19	0.90	4.42	0.99	0.261
HDL (mmol/l)	1.23	0.34	1.31	0.33	0.317
LDL (mmol/l)	2.81	0.84	2.97	0.96	0.401
Trig (mmol/l)	0.73	0.44	0.70	0.36	0.763
OGTT0 (mmol/l)	5.22	1.38	5.16	0.58	0.816
OGTT30	7.00	1.37	7.60	1.49	**0.054**
OGTT60	6.86	2.22	7.28	2.28	0.401
OGTT90	6.65	2.16	6.92	2.22	0.576
OGTT120	6.52	2.04	6.69	1.88	0.698
FFA0 (mmol/l)	0.52	0.26	0.55	0.24	0.556
FFA30	0.30	0.21	0.31	0.20	0.855
FFA60	0.08	0.08	0.09	0.12	0.587
FFA90	0.04	0.04	0.04	0.04	0.829
FFA120	0.03	0.02	0.03	0.01	0.799
INS0 (pmol/l)	95.46	46.81	87.13	25.78	0.363
INS30	638.00	387.24	669.73	525.69	0.738
INS60	593.49	414.91	575.90	336.86	0.839
INS90	457.41	327.81	450.63	241.41	0.919
INS120	392.90	260.35	458.97	344.91	0.299
HOMA-IR	3.19	1.76	2.88	0.93	0.377

Bold print shows significant statistical difference (P ≤0.05)
BMI: body mass index; WC: waist circumference, SBP: Systolic blood pressure, DBP: diastolic blood pressure, Tc: total blood cholesterol, HDL: high density lipoproteins, LDL: low density lipoproteins, Trig: triglycerides, OGTT: oral glucose tolerance test, FFA: free fatty acids, INS: insulin, HOMA-IR: index of insulin resistance

Table F.10: Comparison of measured parameters for the genotypes of the β_2-AR Gln27Glu polymorphism in the entire group of black women

Parameter	Hm (n=5) Mean	SD	Ht (n=23) Mean	SD	N (n=74) Mean	SD	P-values Hm vs. Ht	Hm vs. N	Ht Vs. N
Age (years)	26.80	3.96	33.48	8.67	30.86	8.76	0.108	0.308	0.214
BMI (kg/m^2)	23.71	2.84	28.39	6.79	28.15	6.31	0.147	0.124	0.876
WC (cm)	76.87	6.60	89.74	15.59	87.08	14.72	0.085	0.129	0.458
Body fat %	22.58	4.28	35.16	12.45	33.75	11.01	**0.037**	**0.027**	0.606
SBP (mmHg)	117.73	13.40	133.45	25.65	129.50	17.58	0.199	0.147	0.404
DBP (mmHg)	64.67	9.54	79.52	10.61	77.99	10.32	**0.008**	**0.006**	0.538
Tc (mmol/l)	3.93	0.88	4.31	0.92	4.25	0.94	0.403	0.453	0.796
HDL (mmol/l)	1.43	0.38	1.20	0.23	1.26	0.36	0.088	0.315	0.439
LDL (mmol/l)	2.38	0.63	2.96	0.84	2.85	0.89	0.158	0.247	0.610
Trig (mmol/l)	0.61	0.26	0.77	0.37	0.71	0.44	0.373	0.627	0.553
OGTT0 (mmol/l)	4.66	0.28	5.20	0.90	5.23	1.30	0.200	0.332	0.923
OGTT30	6.22	1.18	7.62	1.80	7.12	1.27	0.115	0.130	0.154
OGTT60	4.65	1.53	8.03	2.56	6.84	2.01	**0.009**	**0.020**	**0.025**
OGTT90	5.11	1.35	7.70	2.42	6.59	2.05	**0.031**	0.118	**0.036**
OGTT120	5.61	0.99	7.30	2.03	6.45	1.98	0.084	0.354	0.081
FFA0 (mmol/l)	0.48	0.37	0.58	0.30	0.53	0.25	0.529	0.683	0.437
FFA30	0.08	0.07	0.35	0.21	0.30	0.20	**0.011**	**0.017**	0.333
FFA60	0.06	0.06	0.10	0.08	0.09	0.10	0.411	0.631	0.677
FFA90	0.02	0.00	0.04	0.03	0.05	0.04	0.096	0.133	0.431
FFA120	0.02	0.01	0.03	0.02	0.03	0.02	0.320	0.245	0.590
INS0 (pmol/l)	75.20	9.50	99.35	39.75	92.14	43.18	0.195	0.387	0.479
INS30	528.40	316.68	680.14	391.38	661.64	468.64	0.428	0.534	0.867
INS60	222.00	132.04	749.00	458.89	580.10	377.50	**0.019**	**0.039**	0.089
INS90	179.40	52.06	636.19	553.43	452.41	301.54	0.082	**0.048**	**0.048**
INS120	209.00	90.87	561.57	406.82	405.36	298.24	0.070	0.149	0.055
HOMA-IR	2.24	0.29	3.41	1.72	3.05	1.54	0.145	0.246	0.346

Bold print shows significant statistical difference (P ≤0.05)
BMI: body mass index; WC: waist circumference, SBP: Systolic blood pressure, DBP: diastolic blood pressure, Tc: total blood cholesterol, HDL: high density lipoproteins, LDL: low density lipoproteins, Trig: triglycerides, OGTT: oral glucose tolerance test, FFA: free fatty acids, INS: insulin, HOMA-IR: index of insulin resistance

Table F.11: Comparison of measured parameters for the genotypes of the β_2-AR Arg16Gly polymorphism in the entire group of black women

Parameter	Hm (n=20) Mean	SD	Ht (n=61) Mean	SD	N (n=21) Mean	SD	Hm vs. Ht	Hm vs. N	Ht Vs. N
Age (years)	32.35	9.29	30.94	8.30	31.15	9.37	0.522	0.687	0.923
BMI (kg/m^2)	26.33	6.25	28.86	5.83	26.90	7.65	0.101	0.798	0.230
WC (cm)	84.97	12.98	89.04	14.34	83.65	17.34	0.263	0.786	0.169
Body fat %	33.26	11.15	34.25	11.43	31.52	11.58	0.734	0.631	0.356
SBP (mmHg)	137.52	33.45	128.01	15.45	127.71	8.81	0.084	0.212	0.935
DBP (mmHg)	77.95	14.39	77.47	9.96	78.07	9.04	0.869	0.975	0.813
Tc (mmol/l)	4.51	1.04	4.20	0.98	4.14	0.59	0.223	0.167	0.786
HDL (mmol/l)	1.32	0.40	1.24	0.32	1.23	0.32	0.340	0.403	0.881
LDL (mmol/l)	3.04	0.90	2.82	0.94	2.79	0.59	0.352	0.301	0.898
Trig (mmol/l)	0.76	0.38	0.73	0.44	0.62	0.37	0.826	0.264	0.321
OGTT0 (mmol/l)	5.29	0.79	5.21	1.45	5.04	0.43	0.826	0.218	0.591
OGTT30	7.19	1.79	7.20	1.43	7.14	1.00	0.976	0.919	0.865
OGTT60	6.90	2.81	7.08	2.20	6.81	1.65	0.762	0.905	0.612
OGTT90	6.89	2.52	6.91	2.27	6.17	1.36	0.980	0.277	0.184
OGTT120	6.75	1.95	6.67	2.19	6.22	1.31	0.886	0.320	0.389
FFA0 (mmol/l)	0.60	0.34	0.54	0.26	0.49	0.21	0.388	0.209	0.419
FFA30	0.40	0.23	0.30	0.20	0.23	0.15	0.071	**0.008**	0.155
FFA60	0.13	0.09	0.08	0.10	0.05	0.03	0.066	**0.000**	0.147
FFA90	0.07	0.04	0.04	0.04	0.03	0.02	**0.008**	**0.004**	0.513
FFA120	0.04	0.03	0.03	0.01	0.02	0.01	**0.000**	**0.004**	0.628
INS0 (pmol/l)	95.05	35.52	88.07	32.16	105.70	65.34	0.414	0.526	0.112
INS30	645.65	371.73	647.83	491.06	706.10	371.22	0.986	0.610	0.629
INS60	571.95	381.72	606.20	410.19	597.35	409.38	0.748	0.842	0.934
INS90	499.79	552.84	489.30	326.18	421.45	311.45	0.919	0.586	0.418
INS120	428.68	388.19	420.10	297.46	453.90	359.02	0.919	0.834	0.677
HOMA-IR	3.25	1.42	2.92	1.35	3.43	2.16	0.346	0.759	0.213

Bold print shows significant statistical difference (P ≤0.05)
BMI: body mass index; WC: waist circumference, SBP: Systolic blood pressure, DBP: diastolic blood pressure, Tc: total blood cholesterol, HDL: high density lipoproteins, LDL: low density lipoproteins, Trig: triglycerides, OGTT: oral glucose tolerance test, FFA: free fatty acids, INS: insulin, HOMA-IR: index of insulin resistance

Table F.12: Comparison of measured parameters for the genotypes of the β_3-AR Trp64Arg polymorphism in the entire group of black women

Parameter	Hm (n=5) Mean	Hm (n=5) SD	Ht (n=48) Mean	Ht (n=48) SD	N (n=49) Mean	N (n=49) SD	P-values Hm vs. Ht	P-values Hm vs. N	P-values Ht Vs. N
Age (years)	28.40	7.57	31.10	8.13	31.69	9.31	0.480	0.448	0.741
BMI (kg/m^2)	27.11	4.57	27.69	5.66	28.36	7.14	0.828	0.705	0.609
WC (cm)	88.18	12.92	86.34	13.94	87.90	15.91	0.779	0.970	0.610
Body fat %	120.01	9.22	132.40	19.52	128.28	20.21	0.170	0.372	0.881
SBP (mmHg)	76.63	5.58	79.20	11.35	76.30	10.36	0.621	0.946	0.311
DBP (mmHg)	30.79	9.94	33.84	11.29	33.49	11.70	0.564	0.621	0.192
Tc (mmol/l)	3.92	0.78	4.38	1.05	4.16	0.80	0.346	0.529	0.238
HDL (mmol/l)	1.12	0.14	1.29	0.34	1.23	0.34	0.306	0.478	0.470
LDL (mmol/l)	2.69	0.78	2.94	1.00	2.78	0.74	0.588	0.789	0.376
Trig (mmol/l)	0.52	0.21	0.77	0.48	0.69	0.37	0.266	0.336	0.358
OGTT0 (mmol/l)	4.68	0.43	5.36	1.60	5.08	0.62	0.351	0.167	0.257
OGTT30	7.06	0.86	7.13	1.27	7.25	1.60	0.902	0.799	0.700
OGTT60	5.86	0.93	6.80	2.09	7.29	2.41	0.329	0.198	0.294
OGTT90	5.22	0.83	6.55	2.28	7.11	2.11	0.204	**0.054**	0.227
OGTT120	5.58	1.04	6.46	2.18	6.82	1.85	0.378	0.150	0.402
FFA0 (mmol/l)	0.50	0.24	0.56	0.27	0.53	0.28	0.681	0.825	0.681
FFA30	0.26	0.20	0.29	0.19	0.32	0.22	0.745	0.587	0.531
FFA60	0.02	0.00	0.09	0.09	0.09	0.10	0.075	0.110	0.991
FFA90	0.02	0.00	0.04	0.04	0.04	0.04	0.167	0.170	0.900
FFA120	0.02	0.00	0.03	0.02	0.03	0.02	0.447	0.334	0.796
INS0 (pmol/l)	78.00	30.19	90.96	35.19	96.37	47.75	0.433	0.405	0.530
INS30	942.60	743.42	712.91	489.61	579.55	344.09	0.348	**0.052**	0.126
INS60	580.20	448.15	612.59	431.06	585.54	373.81	0.874	0.976	0.746
INS90	380.00	140.70	454.61	282.72	509.81	459.05	0.566	0.535	0.487
INS120	337.00	132.78	414.46	293.69	451.65	368.16	0.565	0.496	0.591
HOMA-IR	2.36	0.94	3.07	1.47	3.18	1.68	0.298	0.292	0.738

Bold print shows significant statistical difference (P ≤0.05)
BMI: body mass index; WC: waist circumference, SBP: Systolic blood pressure, DBP: diastolic blood pressure, Tc: total blood cholesterol, HDL: high density lipoproteins, LDL: low density lipoproteins, Trig: triglycerides, OGTT: oral glucose tolerance test, FFA: free fatty acids, INS: insulin, HOMA-IR: index of insulin resistance

Table F.13: Comparison of measured parameters for the genotypes of the β_1-AR Ser49Gly polymorphism in the entire group of white women

| Parameter | Hm (n=102) | | Ht (n=13) | | P-values |
	Mean	SD	Mean	SD	Hm vs. Ht
Age (years)	31.14	8.93	32.92	11.26	0.511
BMI (kg/m^2)	28.15	7.35	31.10	4.71	0.162
WC (cm)	85.16	15.38	92.54	8.78	0.094
SBP (mmHg)	124.71	10.84	130.90	16.54	0.072
DBP (mmHg)	71.94	8.98	76.65	8.52	0.076
Tc (mmol/l)	4.95	1.05	4.81	0.96	0.640
HDL (mmol/l)	1.22	0.32	1.09	0.29	0.148
LDL (mmol/l)	3.17	0.96	3.00	0.67	0.530
Trig (mmol/l)	1.24	0.69	1.60	0.73	0.080
OGTT0 (mmol/l)	5.03	0.42	5.07	0.36	0.776
OGTT30	7.69	1.25	8.08	1.85	0.317
OGTT60	7.64	1.90	7.75	1.93	0.844
OGTT90	7.00	1.98	7.07	1.24	0.907
OGTT120	6.61	1.69	6.52	1.53	0.853
FFA0 (mmol/l)	0.57	0.19	0.47	0.17	0.099
FFA30	0.35	0.20	0.28	0.27	0.223
FFA60	0.08	0.07	0.11	0.13	0.301
FFA90	0.05	0.05	0.06	0.09	0.401
FFA120	0.04	0.06	0.06	0.12	0.454
INS0 (pmol/l)	89.75	32.59	115.23	32.78	**0.009**
INS30	575.59	349.84	813.62	500.76	**0.031**
INS60	557.13	339.94	726.69	486.10	0.112
INS90	459.28	319.17	629.69	389.30	0.081
INS120	360.04	240.02	576.00	441.79	**0.008**
HOMA-IR	2.94	1.29	3.75	1.15	**0.034**

Bold print shows significant statistical difference (P ≤0.05)
BMI: body mass index; WC: waist circumference, SBP: Systolic blood pressure, DBP: diastolic blood pressure, Tc: total blood cholesterol, HDL: high density lipoproteins, LDL: low density lipoproteins, Trig: triglycerides, OGTT: oral glucose tolerance test, FFA: free fatty acids, INS: insulin, HOMA-IR: index of insulin resistance

Table F.14: Comparison of measured parameters for the genotypes of the β_2-AR Gln27Glu polymorphism in the entire group of white women

Parameter	Hm (n=28) Mean	SD	Ht (n=49) Mean	SD	N (n=38) Mean	SD	P-values Hm vs. Ht	Hm vs. N	Ht Vs. N
Age (years)	30.75	9.77	30.73	8.51	32.55	9.68	0.994	0.459	0.355
BMI (kg/m^2)	27.33	6.56	29.11	7.19	28.52	7.57	0.283	0.506	0.710
WC (cm)	84.78	14.33	86.32	14.12	86.47	16.66	0.649	0.668	0.964
SBP (mmHg)	125.64	14.48	124.54	11.27	126.35	10.08	0.712	0.815	0.438
DBP (mmHg)	72.55	8.40	71.74	9.09	73.36	9.49	0.701	0.720	0.421
Tc (mmol/l)	4.69	0.87	5.05	1.13	4.98	1.01	0.147	0.233	0.745
HDL (mmol/l)	1.15	0.26	1.20	0.33	1.25	0.34	0.473	0.204	0.526
LDL (mmol/l)	3.03	0.70	3.27	1.11	3.09	0.85	0.322	0.789	0.413
Trig (mmol/l)	1.11	0.58	1.28	0.65	1.41	0.84	0.240	0.111	0.440
OGTT0 (mmol/l)	5.05	0.41	5.03	0.42	5.04	0.42	0.813	0.959	0.842
OGTT30	7.86	1.72	7.63	1.24	7.79	1.11	0.492	0.842	0.522
OGTT60	7.62	2.14	7.56	1.98	7.78	1.61	0.903	0.740	0.591
OGTT90	6.98	1.89	7.05	2.18	6.99	1.55	0.897	0.991	0.887
OGTT120	6.79	1.96	6.70	1.91	6.32	0.94	0.848	0.211	0.272
FFA0 (mmol/l)	0.56	0.18	0.58	0.23	0.53	0.16	0.717	0.463	0.260
FFA30	0.31	0.19	0.38	0.24	0.32	0.17	0.238	0.909	0.213
FFA60	0.07	0.05	0.10	0.10	0.08	0.06	0.128	0.579	0.216
FFA90	0.04	0.03	0.06	0.07	0.05	0.06	0.237	0.356	0.677
FFA120	0.02	0.01	0.05	0.08	0.06	0.08	0.122	0.065	0.647
INS0 (pmol/l)	83.19	25.64	95.08	36.00	96.26	34.54	0.134	0.101	0.878
INS30	627.07	434.15	582.21	364.87	613.24	352.24	0.636	0.889	0.695
INS60	537.26	377.08	559.62	342.30	628.05	377.66	0.795	0.346	0.387
INS90	404.22	246.68	471.49	276.43	543.81	429.05	0.299	0.135	0.352
INS120	353.11	225.44	390.79	298.06	401.92	289.54	0.571	0.469	0.864
HOMA-IR	2.74	1.08	3.10	1.32	3.16	1.39	0.224	0.191	0.843

Bold print shows significant statistical difference (P ≤0.05)
BMI: body mass index; WC: waist circumference, SBP: Systolic blood pressure, DBP: diastolic blood pressure, Tc: total blood cholesterol, HDL: high density lipoproteins, LDL: low density lipoproteins, Trig: triglycerides, OGTT: oral glucose tolerance test, FFA: free fatty acids, INS: insulin, HOMA-IR: index of insulin resistance

Table F.15: Comparison of measured parameters for the genotypes of the β_2-AR Arg16Gly polymorphism in the entire group of white women

Parameter	Hm (n=28) Mean	SD	Ht (n=61) Mean	SD	N (n=26) Mean	SD	P-values Hm vs. Ht	Hm vs. N	Ht Vs. N
Age (years)	32.11	8.61	31.15	9.57	30.96	9.13	0.652	0.637	0.933
BMI (kg/m^2)	27.01	6.00	30.12	7.69	26.22	6.13	0.062	0.634	**0.024**
WC (cm)	83.57	13.40	89.51	15.60	80.35	13.01	0.085	0.376	**0.010**
SBP (mmHg)	123.85	9.96	126.36	11.11	124.84	14.66	0.310	0.771	0.598
DBP (mmHg)	73.66	8.38	72.61	8.87	70.85	10.08	0.600	0.270	0.418
Tc (mmol/l)	5.06	1.17	4.95	1.05	4.78	0.86	0.680	0.323	0.447
HDL (mmol/l)	1.23	0.34	1.19	0.28	1.22	0.37	0.617	0.904	0.754
LDL (mmol/l)	3.30	0.96	3.15	0.96	2.98	0.85	0.520	0.214	0.439
Trig (mmol/l)	1.17	0.72	1.34	0.67	1.27	0.77	0.304	0.646	0.675
OGTT0 (mmol/l)	4.94	0.32	5.11	0.43	4.97	0.43	0.068	0.827	0.149
OGTT30	7.39	1.29	7.83	1.24	7.90	1.54	0.132	0.195	0.818
OGTT60	7.11	1.86	7.80	1.85	7.90	1.98	0.113	0.143	0.823
OGTT90	6.78	1.53	7.19	2.03	6.84	1.99	0.344	0.906	0.465
OGTT120	6.48	1.47	6.73	1.71	6.41	1.78	0.493	0.881	0.433
FFA0 (mmol/l)	0.53	0.19	0.58	0.19	0.54	0.20	0.300	0.861	0.430
FFA30	0.28	0.16	0.38	0.21	0.32	0.23	**0.022**	0.400	0.254
FFA60	0.09	0.08	0.09	0.09	0.06	0.06	0.919	0.196	0.160
FFA90	0.07	0.09	0.05	0.05	0.03	0.02	0.195	0.065	0.221
FFA120	0.06	0.10	0.04	0.06	0.04	0.05	0.151	0.359	0.756
INS0 (pmol/l)	93.18	38.94	94.44	33.28	87.72	27.69	0.875	0.563	0.376
INS30	566.00	403.78	635.16	365.19	571.92	376.66	0.429	0.956	0.475
INS60	465.32	316.18	636.64	356.87	563.68	400.84	**0.033**	0.323	0.413
INS90	416.54	327.54	537.28	321.93	414.80	342.77	0.109	0.985	0.123
INS120	329.89	202.99	431.07	265.23	341.32	358.67	0.079	0.886	0.209
HOMA-IR	2.98	1.41	3.14	1.33	2.84	1.07	0.600	0.694	0.319

Bold print shows significant statistical difference (P ≤0.05)
BMI: body mass index; WC: waist circumference, SBP: Systolic blood pressure, DBP: diastolic blood pressure, Tc: total blood cholesterol, HDL: high density lipoproteins, LDL: low density lipoproteins, Trig: triglycerides, OGTT: oral glucose tolerance test, FFA: free fatty acids, INS: insulin, HOMA-IR: index of insulin resistance

Table F.16: Comparison of measured parameters for the genotypes of the β_3-AR Trp64Arg polymorphism in the entire group of white women

Parameter	Ht (n=16)		N (n=99)		P-values
	Mean	SD	Mean	SD	Ht vs. N
Age (years)	33.31	9.88	31.02	9.07	0.356
BMI (kg/m^2)	28.42	5.85	28.49	7.36	0.969
WC (cm)	84.57	12.08	86.23	15.39	0.682
SBP (mmHg)	129.12	14.07	124.81	11.23	0.172
DBP (mmHg)	74.59	8.40	72.13	9.10	0.313
Tc (mmol/l)	4.93	1.11	4.94	1.03	0.974
HDL (mmol/l)	1.11	0.26	1.22	0.32	0.211
LDL (mmol/l)	3.18	0.87	3.15	0.95	0.892
Trig (mmol/l)	1.40	0.68	1.26	0.71	0.466
OGTT0 (mmol/l)	5.21	0.40	5.01	0.41	0.069
OGTT30	8.33	1.70	7.64	1.23	**0.053**
OGTT60	8.09	2.30	7.57	1.82	0.318
OGTT90	7.65	1.88	6.90	1.89	0.147
OGTT120	7.06	1.66	6.52	1.66	0.234
FFA0 (mmol/l)	0.53	0.23	0.56	0.19	0.565
FFA30	0.37	0.25	0.34	0.20	0.563
FFA60	0.10	0.11	0.08	0.07	0.426
FFA90	0.07	0.09	0.05	0.05	0.190
FFA120	0.06	0.08	0.04	0.07	0.504
INS0 (pmol/l)	97.75	40.81	91.83	32.29	0.514
INS30	634.50	455.35	598.24	363.24	0.723
INS60	602.75	428.34	572.65	351.44	0.760
INS90	545.00	362.04	468.16	325.94	0.392
INS120	432.25	353.71	377.43	263.93	0.467
HOMA-IR	3.33	1.57	2.99	1.24	0.330

Bold print shows significant statistical difference (P ≤0.05)
BMI: body mass index; WC: waist circumference, SBP: Systolic blood pressure, DBP: diastolic blood pressure, Tc: total blood cholesterol, HDL: high density lipoproteins, LDL: low density lipoproteins, Trig: triglycerides, OGTT: oral glucose tolerance test, FFA: free fatty acids, INS: insulin, HOMA-IR: index of insulin resistance

Table F.17: Comparison of measured parameters for the genotypes of the investigated AR gene polymorphisms between the two groups

B1-AR Ser49Gly	Age	SBP	DBP	BMI	Tc	HDL	LDL	Trig	OGTT	OGTT0	OGTT30	OGTT60	OGTT90	OGTT120	INS0	INS30	INS60	INS90	INS120	FFA0	FFA30	FFA60	FFA90	FFA120	HOMA-IR
Hm	0.745	0.047	0.001	0.800	0.477	0.000	0.804	0.012	0.000	0.197	0.001	0.017	0.285	0.775	0.349	0.279	0.537	0.971	0.403	0.183	0.086	0.972	0.443	0.073	0.303
Ht	0.511	0.932	0.390	0.162	0.373	0.232	0.044	0.931	0.000	0.601	0.367	0.519	0.825	0.764	0.004	0.408	0.247	0.073	0.354	0.276	0.704	0.760	0.352	0.157	0.013
N*																									
Entire group	0.998	0.043	0.000	0.588	0.558	0.000	0.287	0.017	0.000	0.190	0.004	0.021	0.381	0.990	0.956	0.326	0.692	0.973	0.300	0.621	0.167	0.858	0.328	0.026	0.791
B2-AR Gln27Glu																									
Hm	0.385	0.265	0.067	0.239	0.239	0.081	0.049	0.059	0.069	0.047	0.052	0.006	0.044	0.203	0.502	0.633	0.078	0.054	0.174	0.465	0.013	0.863	0.150	0.444	0.315
Ht	0.209	0.043	0.002	0.685	0.358	0.006	0.934	0.245	0.001	0.271	0.979	0.412	0.267	0.234	0.652	0.314	0.063	0.105	0.056	0.952	0.662	0.846	0.281	0.213	0.410
N	0.354	0.310	0.023	0.782	0.842	0.060	0.893	0.184	0.008	0.398	0.008	0.015	0.303	0.720	0.611	0.581	0.530	0.197	0.954	0.929	0.684	0.623	0.677	0.013	0.705
Entire group	0.945	0.043	0.000	0.588	0.558	0.000	0.287	0.017	0.000	0.190	0.004	0.021	0.381	0.990	0.956	0.326	0.692	0.973	0.300	0.621	0.167	0.858	0.328	0.026	0.791
B2-AR Arg16Gly																									
Hm	0.926	0.046	0.200	0.707	0.720	0.104	0.377	0.355	0.022	0.042	0.646	0.746	0.850	0.583	0.866	0.490	0.302	0.520	0.260	0.362	0.039	0.081	0.930	0.392	0.512
Ht	0.896	0.500	0.005	0.307	0.860	0.000	0.392	0.051	0.090	0.602	0.011	0.058	0.474	0.856	0.284	0.874	0.668	0.423	0.833	0.369	0.028	0.684	0.271	0.114	0.359
N	0.946	0.443	0.015	0.738	0.466	0.007	0.912	0.378	0.001	0.581	0.061	0.055	0.216	0.693	0.220	0.239	0.783	0.947	0.301	0.388	0.115	0.327	0.841	0.110	0.239
Entire group	0.945	0.043	0.000	0.588	0.558	0.000	0.287	0.017	0.000	0.190	0.004	0.021	0.381	0.990	0.956	0.326	0.692	0.973	0.300	0.621	0.167	0.858	0.328	0.026	0.791
B3-AR Trp64Arg																									
Ht	0.376	0.538	0.141	0.660	0.651	0.079	0.073	0.401	0.000	0.713	0.004	0.042	0.091	0.328	0.524	0.577	0.937	0.311	0.844	0.753	0.198	0.754	0.147	0.028	0.553
N	0.674	0.181	0.013	0.917	0.539	0.000	0.807	0.021	0.000	0.420	0.106	0.421	0.555	0.332	0.497	0.766	0.840	0.532	0.168	0.452	0.589	0.578	0.727	0.178	0.440
Entire group	0.945	0.043	0.000	0.588	0.558	0.000	0.287	0.017	0.000	0.190	0.004	0.021	0.381	0.990	0.956	0.326	0.692	0.973	0.300	0.621	0.167	0.858	0.328	0.026	0.791

Significant difference indicated by bold print and shaded blocks

Table F.18: Parameters measured for the β_2-AR gene polymorphism diplotypes observed in the black women

Parameter	Mean 2	SD 2	Mean 3	SD 3	Mean 4	SD 4	Mean 6	SD 6	Mean 7	SD 7
Age (years)	31.79	11.09	30.62	8.40	30.77	7.90	33.50	8.22	36.50	12.14
BMI (kg/m²)	27.39	8.49	28.96	5.44	26.02	6.53	29.50	7.58	27.86	6.17
WC (cm)	84.07	19.54	88.73	13.39	84.38	13.69	93.46	17.57	87.78	12.77
SBP (mmHg)	126.02	8.49	129.42	16.18	133.55	27.66	127.20	10.78	148.42	46.85
DBP (mmHg)	76.67	9.80	78.60	9.44	77.22	14.07	77.21	9.12	81.98	15.72
Tc (mmol/l)	4.10	0.70	4.24	0.97	4.46	1.07	4.14	1.00	4.72	1.12
HDL (mmol/l)	1.16	0.33	1.27	0.34	1.34	0.46	1.14	0.24	1.22	0.24
LDL (mmol/l)	2.82	0.64	2.83	0.96	2.98	0.92	2.85	0.94	3.32	0.88
Trig (mmol/l)	0.61	0.42	0.74	0.48	0.68	0.30	0.75	0.32	0.90	0.53
Body fat %	32.50	12.09	34.58	10.90	32.11	10.76	35.70	13.62	37.58	12.03
OGTT0 (mmol/l)	5.05	0.44	5.29	1.59	5.21	0.56	5.04	0.91	5.54	1.23
OGTT30	6.99	1.09	7.22	1.30	6.91	1.42	7.32	1.97	8.18	2.21
OGTT60	6.53	1.79	7.07	1.98	6.35	2.35	7.80	2.88	8.72	3.02
OGTT90	6.20	1.46	6.74	2.19	6.43	2.11	7.94	2.64	8.50	2.48
OGTT120	6.20	1.47	6.54	2.13	6.37	2.01	7.52	2.46	7.69	1.77
FFA0 (mmol/l)	0.51	0.21	0.55	0.26	0.48	0.26	0.54	0.19	0.86	0.41
FFA30	0.24	0.17	0.31	0.22	0.35	0.17	0.30	0.13	0.56	0.27
FFA60	0.05	0.03	0.08	0.11	0.13	0.10	0.09	0.08	0.14	0.08
FFA90	0.04	0.02	0.04	0.04	0.07	0.05	0.03	0.03	0.06	0.03
FFA120	0.03	0.01	0.03	0.01	0.05	0.03	0.02	0.01	0.04	0.02
INS0 (pmol/l)	110.43	72.32	85.54	31.21	95.77	36.04	101.42	36.71	96.50	40.10
INS30	720.64	413.68	672.41	526.66	560.00	276.63	608.55	369.63	779.50	530.66
INS60	494.71	221.85	628.46	417.64	500.92	351.25	641.45	369.29	802.60	435.26
INS90	423.00	335.65	479.37	316.61	388.69	198.51	614.55	360.51	862.40	1009.28
INS120	460.50	402.18	404.93	297.75	347.46	137.18	544.27	296.27	667.80	723.78
HOMA-IR	3.61	2.40	2.83	1.25	3.21	1.27	3.47	1.78	3.49	1.90

BMI: body mass index; WC: waist circumference, SBP: Systolic blood pressure, DBP: diastolic blood pressure, Tc: total blood cholesterol, HDL: high density lipoproteins, LDL: low density lipoproteins, Trig: triglycerides, OGTT: oral glucose tolerance test, FFA: free fatty acids, INS: insulin, HOMA-IR: index of insulin resistance

Table F.19: Comparison of measured parameters for the diplotypes of the β_2-AR gene polymorphisms observed in the black women

Parameter	P-values for comparison between groups							
	2 vs. 4	2 vs. 6	2 vs. 7	3 vs. 4	3 vs. 7	4 vs. 6	4 vs. 7	6 vs. 7
Age (years)	0.788	0.663	0.408	0.954	0.131	0.406	0.231	0.542
BMI (kg/m^2)	0.644	0.513	0.905	0.104	0.647	0.230	0.570	0.652
WC (cm)	0.962	0.213	0.676	0.306	0.870	0.161	0.615	0.494
SBP (mmHg)	0.340	0.757	0.091	0.494	**0.044**	0.464	0.394	0.145
DBP (mmHg)	0.906	0.885	0.367	0.679	0.450	0.999	0.518	0.423
Tc (mmol/l)	0.314	0.920	0.150	0.488	0.272	0.447	0.634	0.280
HDL (mmol/l)	0.256	0.836	0.709	0.525	0.741	0.186	0.557	0.509
LDL (mmol/l)	0.597	0.927	0.172	0.599	0.238	0.722	0.465	0.322
Trig (mmol/l)	0.617	0.338	0.210	0.641	0.476	0.556	0.264	0.482
Body fat %	0.931	0.530	0.400	0.471	0.533	0.470	0.334	0.779
OGTT0 (mmol/l)	0.433	0.954	0.206	0.863	0.715	0.580	0.430	0.356
OGTT30	0.867	0.599	0.119	0.450	0.127	0.558	0.147	0.425
OGTT60	0.818	0.190	0.057	0.272	0.076	0.187	0.078	0.545
OGTT90	0.759	**0.055**	**0.020**	0.658	0.074	0.143	0.081	0.674
OGTT120	0.799	0.109	0.066	0.800	0.214	0.223	0.188	0.882
FFA0 (mmol/l)	0.738	0.732	**0.021**	0.377	**0.016**	0.529	**0.024**	**0.035**
FFA30	0.090	0.287	**0.005**	0.546	**0.014**	0.465	**0.053**	**0.019**
FFA60	**0.006**	0.121	**0.002**	0.128	0.223	0.278	0.908	0.278
FFA90	**0.011**	0.859	0.085	**0.013**	0.362	**0.018**	0.404	0.117
FFA120	**0.010**	0.762	0.063	**0.001**	0.076	**0.018**	0.538	0.094
INS0 (pmol/l)	0.516	0.700	0.666	0.317	0.437	0.702	0.969	0.798
INS30	0.251	0.488	0.791	0.463	0.642	0.717	0.246	0.446
INS60	0.956	0.230	**0.055**	0.320	0.382	0.350	0.145	0.456
INS90	0.752	0.184	0.158	0.333	0.058	0.065	0.111	0.471
INS120	0.345	0.569	0.434	0.504	0.120	**0.043**	0.130	0.627
HOMA-IR	0.600	0.874	0.914	0.340	0.263	0.684	0.712	0.985

Bold print indicates significant statistical difference (P ≤0.05)
Note: comparisons yielding no significant difference were omitted from this table
BMI: body mass index; WC: waist circumference, SBP: Systolic blood pressure, DBP: diastolic blood pressure, Tc: total blood cholesterol, HDL: high density lipoproteins, LDL: low density lipoproteins, Trig: triglycerides, OGTT: oral glucose tolerance test, FFA: free fatty acids, INS: insulin, HOMA-IR: index of insulin resistance

Table F.20: Parameters measured for the β_2-AR gene polymorphism diplotypes observed in the white women

Parameter	Mean 1	SD 1	Mean 2	SD 2	Mean 3	SD 3	Mean 4	SD 4	Mean 5	SD 5	Mean 7	SD 7
Age (years)	31.45	9.82	31.00	8.38	30.62	11.09	35.00	10.36	28.30	5.01	31.88	11.53
BMI (kg/m^2)	29.41	7.70	32.49	7.64	28.73	7.65	25.97	6.71	27.67	5.76	24.24	4.54
WC (cm)	87.39	14.70	94.42	16.16	88.15	16.78	81.57	16.02	84.46	11.48	77.55	11.91
SBP (mmHg)	125.20	12.05	129.92	9.70	124.49	10.20	123.58	6.03	122.68	12.46	123.28	14.27
DBP (mmHg)	71.45	9.09	74.26	9.54	73.22	7.59	73.21	9.28	73.83	6.79	71.68	10.74
Tc (mmol/l)	5.10	1.16	5.00	0.99	4.55	0.74	5.28	0.90	4.67	1.10	4.42	1.11
HDL (mmol/l)	1.18	0.29	1.21	0.33	1.19	0.21	1.32	0.39	1.17	0.27	1.21	0.28
LDL (mmol/l)	3.31	1.08	3.11	0.97	2.83	0.52	3.32	0.58	3.06	0.84	2.65	0.88
Trig (mmol/l)	1.33	0.55	1.48	0.83	1.17	0.74	1.41	0.96	0.97	0.41	1.24	0.73
OGTT0 (mmol/l)	5.10	0.39	5.15	0.51	5.09	0.47	4.91	0.31	4.99	0.29	5.04	0.31
OGTT30	7.74	1.18	7.86	0.95	7.99	1.69	7.64	1.55	7.39	1.11	7.90	0.43
OGTT60	7.68	1.82	8.06	1.70	7.74	2.19	7.44	1.68	7.35	2.01	7.76	1.38
OGTT90	7.17	2.20	7.21	1.52	7.23	2.31	6.98	1.52	6.97	1.56	6.54	1.74
OGTT120	6.85	1.69	6.22	0.82	7.09	2.43	6.51	1.18	6.80	1.61	6.23	0.80
FFA0 (mmol/l)	0.61	0.24	0.55	0.11	0.55	0.17	0.54	0.23	0.58	0.16	0.47	0.09
FFA30	0.40	0.23	0.37	0.17	0.35	0.21	0.31	0.19	0.27	0.15	0.23	0.15
FFA60	0.11	0.11	0.07	0.04	0.08	0.06	0.11	0.08	0.05	0.03	0.04	0.02
FFA90	0.06	0.07	0.04	0.02	0.05	0.04	0.08	0.09	0.03	0.01	0.03	0.02
FFA120	0.04	0.08	0.03	0.01	0.03	0.02	0.09	0.13	0.02	0.01	0.04	0.04
INS0 (pmol/l)	92.87	35.81	102.00	31.14	88.31	30.19	95.23	43.55	73.70	12.77	85.75	25.05
INS30	572.21	343.17	664.69	285.44	739.23	484.12	618.69	477.92	466.70	249.48	501.50	227.10
INS60	586.93	326.50	746.88	374.09	611.85	398.01	542.31	362.52	374.90	187.89	529.75	389.06
INS90	505.24	280.68	638.06	411.60	484.69	278.72	508.23	446.32	293.10	87.62	413.13	447.23
INS120	426.34	285.63	430.56	232.53	442.23	275.69	383.46	258.00	271.70	132.89	374.63	444.74
HOMA-IR	3.07	1.33	3.43	1.40	2.94	1.27	3.04	1.61	2.36	0.47	2.80	0.95

BMI: body mass index; WC: waist circumference, SBP: Systolic blood pressure, DBP: diastolic blood pressure, Tc: total blood cholesterol, HDL: high density lipoproteins, LDL: low density lipoproteins, Trig: triglycerides, OGTT: oral glucose tolerance test, FFA: free fatty acids, INS: insulin, HOMA-IR: index of insulin resistance

Table F.21: Comparison of measured parameters for the diplotypes of the β_2-AR gene polymorphisms observed in the white women

Parameter	P-values for comparison between groups								
	1 vs. 5	2 vs. 4	2 vs. 5	2 vs. 6	3 vs. 4	3 vs. 5	4 vs. 5	4 vs. 6	4 vs. 7
Age (years)	0.338	0.252	0.365	0.335	0.308	0.547	0.075	**0.049**	0.528
BMI (kg/m^2)	0.517	**0.021**	0.098	0.173	0.337	0.719	0.528	0.324	0.529
WC (cm)	0.568	**0.039**	0.100	0.065	0.316	0.558	0.635	0.749	0.548
SBP (mmHg)	0.572	**0.048**	0.105	**0.027**	0.785	0.707	0.822	0.601	0.947
DBP (mmHg)	0.452	0.764	0.902	0.408	0.997	0.844	0.860	0.614	0.733
Tc (mmol/l)	0.312	0.432	0.428	0.694	**0.033**	0.753	0.157	0.213	0.066
HDL (mmol/l)	0.904	0.425	0.721	0.563	0.283	0.881	0.312	0.867	0.475
LDL (mmol/l)	0.507	0.506	0.882	0.707	**0.033**	0.430	0.388	0.250	**0.049**
Trig (mmol/l)	0.067	0.837	0.082	0.576	0.472	0.455	0.188	0.760	0.665
OGTT0 (mmol/l)	0.416	0.138	0.364	0.153	0.253	0.555	0.529	0.886	0.366
OGTT30	0.410	0.636	0.257	0.695	0.584	0.342	0.673	0.939	0.650
OGTT60	0.634	0.331	0.341	0.906	0.699	0.667	0.909	0.487	0.653
OGTT90	0.794	0.691	0.699	0.988	0.751	0.762	0.982	0.761	0.542
OGTT120	0.931	0.444	0.234	0.441	0.443	0.746	0.621	0.781	0.559
FFA0 (mmol/l)	0.725	0.914	0.616	0.603	0.922	0.703	0.687	0.649	0.388
FFA30	0.087	0.388	0.113	0.907	0.650	0.290	0.510	0.458	0.293
FFA60	0.104	0.058	0.306	0.579	0.298	0.159	**0.041**	0.255	**0.033**
FFA90	0.286	**0.050**	0.527	0.640	0.202	0.246	0.084	0.114	0.095
FFA120	0.381	0.098	**0.019**	0.357	0.110	0.286	0.123	0.290	0.352
INS0 (pmol/l)	0.108	0.623	**0.012**	0.230	0.642	0.168	0.147	0.633	0.583
INS30	0.379	0.750	0.084	0.544	0.529	0.121	0.372	0.856	0.526
INS60	0.061	0.149	**0.008**	0.154	0.646	0.098	0.199	1.000	0.941
INS90	**0.025**	0.423	**0.016**	0.111	0.873	**0.049**	0.150	0.532	0.641
INS120	0.109	0.610	0.062	0.445	0.580	0.087	0.227	0.752	0.954
HOMA-IR	0.109	0.487	**0.029**	0.203	0.864	0.186	0.214	0.679	0.708

Bold print indicates significant statistical difference (P ≤0.05)
Note: comparisons between groups yielding no significant difference were omitted from this table
BMI: body mass index; WC: waist circumference, SBP: Systolic blood pressure, DBP: diastolic blood pressure, Tc: total blood cholesterol, HDL: high density lipoproteins, LDL: low density lipoproteins, Trig: triglycerides, OGTT: oral glucose tolerance test, FFA: free fatty acids, INS: insulin, HOMA-IR: index of insulin resistance

Table F.22: Parameters measured for the haplotypes observed in the black women

Parameter	Mean 1	SD 1	Mean 2	SD 2	Mean 3	SD 3	Mean 4	SD 4	Mean 5	SD 5	Mean 6	SD 6
Age (years)	28.00	9.00	31.88	7.33	29.00	7.16	32.83	9.43	34.00	6.39	31.17	12.38
BMI (kg/m^2)	29.14	6.43	29.31	5.67	24.50	4.05	27.71	8.91	30.01	5.06	28.06	4.70
WC (cm)	85.42	11.78	88.45	14.57	81.46	8.53	88.46	18.37	94.13	13.95	91.74	15.33
SBP (mmHg)	127.24	12.70	131.11	16.23	119.26	8.67	149.01	34.92	131.80	17.27	134.81	25.72
DBP (mmHg)	76.95	8.24	79.77	11.13	71.49	9.53	83.21	17.36	78.45	6.93	80.64	12.94
Tc (mmol/l)	3.65	0.69	4.65	0.91	4.56	0.48	4.00	1.13	4.06	0.35	4.81	1.53
HDL (mmol/l)	1.24	0.32	1.28	0.37	1.33	0.55	1.28	0.40	1.26	0.43	1.42	0.34
LDL (mmol/l)	2.29	0.68	3.20	0.89	3.08	0.36	2.59	1.03	2.68	0.52	3.19	1.63
Trig (mmol/l)	0.64	0.39	0.85	0.60	0.73	0.16	0.63	0.43	0.62	0.16	0.96	0.59
Body fat %	32.76	11.73	35.28	11.63	29.64	7.98	34.63	14.12	37.74	9.99	36.54	10.51
OGTT0 (mmol/l)	5.10	0.52	5.66	2.57	5.05	0.38	5.40	0.73	5.37	0.54	5.05	0.21
OGTT30	6.95	1.21	7.23	1.38	6.91	1.38	6.95	1.71	7.81	2.01	7.34	0.81
OGTT60	7.07	1.53	7.07	2.33	5.98	1.54	7.06	3.04	8.56	2.42	6.56	1.50
OGTT90	6.79	1.08	6.77	2.82	5.59	0.97	7.91	2.41	8.54	2.53	6.01	1.66
OGTT120	6.41	1.19	6.55	2.98	5.59	0.77	7.45	2.53	7.86	2.50	6.29	1.26
FFA0 (mmol/l)	0.47	0.24	0.62	0.30	0.36	0.17	0.57	0.31	0.65	0.26	0.51	0.24
FFA30	0.31	0.22	0.29	0.22	0.27	0.14	0.45	0.16	0.51	0.23	0.21	0.11
FFA60	0.06	0.07	0.07	0.08	0.09	0.06	0.18	0.12	0.22	0.20	0.08	0.08
FFA90	0.03	0.02	0.04	0.04	0.06	0.04	0.09	0.06	0.08	0.07	0.05	0.03
FFA120	0.02	0.00	0.03	0.02	0.04	0.02	0.05	0.03	0.04	0.02	0.03	0.02
INS0 (pmol/l)	78.69	30.82	89.63	33.40	96.83	46.05	101.17	25.68	99.67	23.56	81.67	36.93
INS30	504.54	325.33	695.56	333.83	520.00	189.91	641.83	353.61	504.50	255.46	917.17	1070.91
INS60	516.62	388.87	762.31	415.61	589.50	420.49	426.83	319.03	554.00	310.25	628.50	577.01
INS90	447.77	363.42	511.19	346.32	331.50	151.09	467.00	239.27	586.33	340.68	438.83	249.12
INS120	317.23	169.02	412.56	214.07	349.67	107.24	351.50	182.63	516.17	347.76	520.00	633.45
HOMA-IR	2.58	1.13	3.01	1.47	3.15	1.56	3.50	0.97	3.48	1.07	2.66	1.27

BMI: body mass index; WC: waist circumference, SBP: Systolic blood pressure, DBP: diastolic blood pressure, Tc: total blood cholesterol, HDL: high density lipoproteins, LDL: low density lipoproteins, Trig: triglycerides, OGTT: oral glucose tolerance test, FFA: free fatty acids, INS: insulin, HOMA-IR: index of insulin resistance

Table F.23: Comparison of measured parameters between different haplotypes observed in the black women

Parameter	P-values for comparison between groups									
	1 vs. 2	1 vs. 3	1 vs. 4	1 vs. 5	1 vs. 6	2 vs. 4	2 vs. 5	3 vs. 5	4 vs. 6	5 vs. 6
Age (years)	0.203	0.814	0.298	0.162	0.534	0.802	0.538	0.230	0.798	0.629
BMI (kg/m^2)	0.941	0.125	0.694	0.774	0.718	0.615	0.790	0.064	0.933	0.504
WC (cm)	0.546	0.472	0.667	0.175	0.336	0.999	0.417	0.087	0.744	0.783
SBP (mmHg)	0.485	0.184	0.058	0.524	0.395	0.104	0.930	0.143	0.441	0.817
DBP (mmHg)	0.449	0.218	0.293	0.704	0.458	0.581	0.789	0.179	0.778	0.722
Tc (mmol/l)	**0.003**	**0.010**	0.420	0.189	**0.034**	0.172	0.147	0.071	0.322	0.274
HDL (mmol/l)	0.740	0.644	0.787	0.897	0.263	0.985	0.914	0.811	0.531	0.484
LDL (mmol/l)	**0.005**	**0.016**	0.458	0.228	0.099	0.182	0.199	0.154	0.460	0.480
Trig (mmol/l)	0.282	0.617	0.948	0.869	0.176	0.414	0.354	0.240	0.290	0.195
Body fat %	0.561	0.565	0.765	0.382	0.510	0.912	0.651	0.152	0.796	0.843
OGTT0 (mmol/l)	0.443	0.833	0.316	0.312	0.834	0.808	0.786	0.257	0.288	0.207
OGTT30	0.564	0.945	0.998	0.261	0.489	0.688	0.454	0.389	0.627	0.608
OGTT60	0.993	0.166	0.990	0.120	0.503	0.996	0.201	**0.052**	0.725	0.116
OGTT90	0.981	**0.032**	0.182	**0.046**	0.229	0.427	0.195	**0.023**	0.155	0.067
OGTT120	0.873	0.142	0.230	0.100	0.844	0.523	0.356	0.059	0.337	0.201
FFA0 (mmol/l)	0.147	0.342	0.434	0.141	0.689	0.737	0.804	**0.042**	0.734	0.354
FFA30	0.798	0.636	0.188	0.092	0.278	0.120	**0.052**	**0.051**	**0.011**	**0.016**
FFA60	0.684	0.337	**0.016**	**0.019**	0.648	0.034	**0.024**	0.171	0.131	0.144
FFA90	0.280	**0.012**	**0.002**	**0.015**	**0.041**	**0.037**	0.090	0.540	0.154	0.300
FFA120	0.222	**0.007**	**0.002**	**0.010**	0.107	**0.028**	0.204	0.812	0.140	0.393
INS0 (pmol/l)	0.372	0.321	0.140	0.159	0.856	0.455	0.510	0.896	0.313	0.338
INS30	0.133	0.916	0.416	1.000	0.210	0.744	0.221	0.907	0.563	0.380
INS60	0.115	0.715	0.629	0.839	0.623	0.090	0.280	0.871	0.471	0.786
INS90	0.635	0.466	0.908	0.442	0.957	0.778	0.654	0.125	0.846	0.412
INS120	0.202	0.674	0.693	0.106	0.284	0.544	0.405	0.289	0.545	0.990
HOMA-IR	0.397	0.377	0.105	0.122	0.895	0.458	0.485	0.681	0.225	0.255

Bold print indicates significant statistical difference (P ≤0.05)
Note: comparisons between groups yielding no significant difference were omitted from this table
BMI: body mass index; WC: waist circumference, SBP: Systolic blood pressure, DBP: diastolic blood pressure, Tc: total blood cholesterol, HDL: high density lipoproteins, LDL: low density lipoproteins, Trig: triglycerides, OGTT: oral glucose tolerance test, FFA: free fatty acids, INS: insulin, HOMA-IR: index of insulin resistance

Table F.24: Parameters measured for the haplotypes observed in the white women

Parameter	Mean 1	SD 1	Mean 2	SD 2	Mean 3	SD 3	Mean 4	SD 4	Mean 5	SD 5
Age (years)	31.29	10.34	27.50	4.70	31.44	8.45	35.00	10.36	27.20	8.01
BMI (kg/m^2)	29.15	8.39	26.47	6.22	32.49	7.89	25.97	6.71	28.48	8.38
WC (cm)	86.62	15.72	79.80	13.74	94.90	16.56	81.57	16.02	87.47	18.18
SBP (mmHg)	125.20	13.48	119.94	8.36	130.64	9.53	123.58	6.03	123.27	11.37
DBP (mmHg)	70.40	9.75	68.52	8.18	74.49	9.80	73.21	9.28	71.00	5.77
Tc (mmol/l)	5.07	1.23	4.93	0.74	5.02	1.02	5.28	0.90	4.33	0.60
HDL (mmol/l)	1.20	0.31	1.36	0.45	1.22	0.34	1.32	0.39	1.19	0.19
LDL (mmol/l)	3.33	1.20	3.01	0.93	3.12	1.00	3.32	0.58	2.72	0.48
Trig (mmol/l)	1.18	0.41	1.22	1.03	1.50	0.85	1.41	0.96	0.92	0.33
OGTT0 (mmol/l)	5.05	0.39	4.80	0.48	5.15	0.52	4.91	0.31	5.07	0.53
OGTT30	7.62	1.15	7.28	1.36	7.91	0.96	7.64	1.55	7.92	1.90
OGTT60	7.45	1.63	8.07	2.40	8.09	1.75	7.44	1.68	7.74	2.46
OGTT90	6.82	2.23	7.21	2.51	7.27	1.56	6.98	1.52	7.19	2.60
OGTT120	6.67	1.67	6.64	2.54	6.25	0.84	6.51	1.18	7.12	2.69
FFA0 (mmol/l)	0.59	0.23	0.62	0.24	0.54	0.11	0.54	0.23	0.56	0.18
FFA30	0.37	0.20	0.43	0.28	0.38	0.17	0.31	0.19	0.37	0.22
FFA60	0.08	0.06	0.09	0.08	0.07	0.04	0.11	0.08	0.08	0.07
FFA90	0.04	0.04	0.04	0.02	0.04	0.02	0.08	0.09	0.05	0.05
FFA120	0.03	0.02	0.03	0.02	0.03	0.01	0.09	0.13	0.03	0.02
INS0 (pmol/l)	84.92	28.90	79.30	26.60	102.00	32.16	95.23	43.55	85.20	30.61
INS30	517.68	267.80	513.30	419.98	671.73	294.02	618.69	477.92	684.00	474.46
INS60	535.73	299.67	525.70	397.64	749.87	387.02	542.31	362.52	533.10	334.99
INS90	420.23	172.89	351.70	192.96	657.73	418.19	508.23	446.32	419.80	229.91
INS120	363.86	195.73	230.00	170.86	438.33	238.53	383.46	258.00	375.40	215.09
HOMA-IR	2.77	1.06	2.49	1.03	3.43	1.44	3.04	1.61	2.85	1.38

BMI: body mass index; WC: waist circumference, SBP: Systolic blood pressure, DBP: diastolic blood pressure, Tc: total blood cholesterol, HDL: high density lipoproteins, LDL: low density lipoproteins, Trig: triglycerides, OGTT: oral glucose tolerance test, FFA: free fatty acids, INS: insulin, HOMA-IR: index of insulin resistance

Table F.25: Comparison of measured parameters between different haplotypes observed in the white women

Parameter	P-values for comparison between groups					
	1 vs. 3	1 vs. 4	2 vs. 3	2 vs. 4	3 vs. 4	4 vs. 5
Age (years)	0.963	0.305	0.192	**0.046**	0.317	0.062
BMI (kg/m^2)	0.214	0.247	**0.052**	0.855	**0.026**	0.433
WC (cm)	0.118	0.361	**0.024**	0.783	**0.038**	0.418
SBP (mmHg)	0.171	0.684	**0.008**	0.238	**0.028**	0.933
DBP (mmHg)	0.202	0.401	0.121	0.220	0.722	0.517
Tc (mmol/l)	0.893	0.583	0.819	0.326	0.469	**0.009**
HDL (mmol/l)	0.889	0.319	0.352	0.811	0.452	0.342
LDL (mmol/l)	0.567	0.982	0.793	0.341	0.524	**0.015**
Trig (mmol/l)	0.119	0.307	0.452	0.640	0.797	0.136
OGTT0 (mmol/l)	0.144	0.205	**0.015**	0.804	**0.010**	0.400
OGTT30	0.076	0.200	0.083	0.214	0.833	0.591
OGTT60	0.477	0.283	0.101	0.525	0.155	0.370
OGTT90	0.433	0.971	0.189	0.569	0.582	0.699
OGTT120	0.261	0.984	0.978	0.465	0.324	0.730
FFA0 (mmol/l)	0.498	0.813	0.938	0.792	0.625	0.814
FFA30	0.366	0.754	0.581	0.869	0.502	0.469
FFA60	0.459	0.544	0.321	0.462	0.988	0.806
FFA90	0.939	0.404	0.556	0.246	0.366	0.534
FFA120	0.482	0.223	0.426	0.466	0.082	0.397
INS0 (pmol/l)	0.953	**0.040**	0.853	0.115	0.063	0.293
INS30	0.207	**0.033**	0.718	0.180	0.110	0.186
INS60	0.088	0.393	0.074	0.321	0.634	0.543
INS90	0.108	0.427	0.277	0.587	0.722	0.748
INS120	0.066	0.954	0.174	0.918	0.157	0.951
HOMA-IR	**0.022**	0.411	**0.042**	0.313	0.369	0.575

Bold print indicates significant statistical difference (P ≤0.05)
Note: comparisons between groups yielding no significant difference were omitted from this table
BMI: body mass index; WC: waist circumference, SBP: Systolic blood pressure, DBP: diastolic blood pressure, Tc: total blood cholesterol, HDL: high density lipoproteins, LDL: low density lipoproteins, Trig: triglycerides, OGTT: oral glucose tolerance test, FFA: free fatty acids, INS: insulin, HOMA-IR: index of insulin resistance

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RESEARCH PAPER SUBMITTED FOR PUBLICATION

The following is the first draft of a research paper prepared for submission and publication in the following journal: Medical Science Monitoring (http://www.medscimonit.com).

No association between adrenergic receptor gene polymorphisms and some characteristics of the metabolic syndrome in African women

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ABSTRACT

Introduction

Worldwide the incidence of the metabolic syndrome increases annually at an alarming rate. Two conditions associated with this are obesity and hypertension (high blood pressure). Both have negative health and lifestyle consequences. Numerous studies on adrenergic receptor (AR) gene polymorphisms in population groups in other countries have proved, but not exclusively, that these polymorphisms may be positively associated with susceptibility to and progression of obesity and hypertension. The AR encoding genes are attractive targets for such studies because the ARs, as part of the sympathetic nervous system, perform important functions like vasoconstriction, vasodilation, lipolysis and influence the heart's contraction. These functions accentuate the possible role of AR gene polymorphisms in the onset or progression of obesity and hypertension.

Objectives

The objectives of this study were to determine the incidence of the β_1-AR Ser49Gly and β_2-AR Arg16Gly polymorphisms in 102 black South African female volunteers and that of the β_1-AR Ser49Gly, β_2-AR Arg16Gly, Gln27Glu and β_3-AR Trp64Arg in 115 white South African female volunteers and calculate the minor allele frequency in both groups; identify possible diplotypes and haplotypes in the study groups; take relevant physiological parameters (measured in the POWIRS studies) into account in the search for possible associations of these polymorphisms, diplotypes and haplotypes with obesity and high blood pressure as characteristics of the metabolic syndrome; compare the black and the white study groups with regards to the above mentioned objectives.

Methods

DNA was isolated from blood spots on Guthrie cards and the respective AR gene fragments amplified by polymerase chain reaction (PCR). After restriction enzyme digestion, the DNA fragments were separated by agarose gel electrophoresis. Genotypic findings were examined along with measured physiological parameters (measured during the POWIRS studies) and

statistically processed. AUC analysis was performed on parameters measured during the oral glucose tolerance test. Diplotype and haplotype analyses were also performed on both subject groups.

Results

The minor allele frequencies for both groups were calculated and compared to that reported in other published studies for other ethnicities. For the black group, the minor allele frequencies were: 84% (β_1-AR Ser49Gly), 16% (β_2-AR Gln27Glu), 49% (β_2-AR Arg16Gly) and 28% (β_3-AR Trp64Arg) and for the white group: 94%, 46%, 50% and 7%. When the measured physiological parameters were compared between the genotypes, diplotypes and haplotypes in each group of women, only a few significant differences were observed. Comparison of the two groups of test subjects showed only minimal significant differences.

Conclusions

Although no associations were found between the different investigated AR gene polymorphisms, or diplotypes and haplotypes and obesity and hypertension or high blood pressure, it may be that they rather act as contributors to risk factors for the onset and progression of these characteristics of the metabolic syndrome.

Keywords

Adrenergic receptor genes, polymorphism, metabolic syndrome, obesity, hypertension.

INTRODUCTION

The 2003/2004 South African Health Review stated that chronic non-infectious diseases that are usually associated with lifestyle (cardiovascular disease, chronic obstructive pulmonary disease and diabetes) result in 37% of deaths in this country (Health24, 2005; News24, 2005). Some of the risk factors for these conditions include obesity, sedentary lifestyle, poor diet and hypertension. These are also risk factors for development of the metabolic syndrome, although it is in some cases unclear whether these factors give rise to the onset of this syndrome, or develop as a result of it (Johnson & Terra, 2002; Van der Linde, 2004; Health24, 2005; News24, 2005).

Worldwide the incidence of the metabolic syndrome increases annually at an alarming rate. The literature becoming available on the prevalence of the metabolic syndrome in South Africa is rather scanty but progressing. Results show that the situation in South Africa parallels that of the USA as being close to 25% of the population, including children and adolescents (American Association for Clinical Chemistry, 2004; Van der Linde, 2004; Health24, 2005; News24, 2005).

The metabolic syndrome has rapidly become one of the most important public-health concerns and challenges worldwide (Mehta & Reilly, 2004). Obesity and hypertension are two conditions associated with this syndrome, both having negative health and lifestyle consequences. Both are caused by (and contributed to) by environmental and genetic factors possibly including inter-individual genetic diversity (Siani & Strazzullo, 2006). The metabolic syndrome is the collective name given to a set of risk factors/characteristic components including central adiposity, dyslipidemia, hypertension and insulin resistance with or without glucose intolerance (Grundy, 2003; Girod & Brotman, 2003; American Association for Clinical Chemistry, 2004; Mehta & Reilly, 2004). One of the risk factors for the development of the metabolic syndrome that have been getting increasingly more attention is genetic predisposition, the susceptibility to certain diseases and/or physiological states as a result of the genetic makeup of man Grundy, 2003; American Association for Clinical Chemistry, 2004; Hughes & Aitman, 2004; Safar *et al.*, 2006). The AR (adrenergic receptor) genes have been implicated as playing a role as possible polygenic predisposing genes.

The adrenergic receptors (ARs) are members of the superfamily of cell surface receptors expressed by virtually every cell type in the human body and are receptors for the neurohormones (catecholamines) epinephrine and norepinephrine within the sympathetic nervous system (Hein & Kobilka, 1997; Johnson & Terra, 2002; Small *et al.,* 2003). They carry out cell signalling functions via coupling to G-proteins (guanine nucleotide binding proteins) (Small *et al.,* 2003). The ARs form part of the autonomic nervous system which controls numerous physiological and metabolic

functions such as energy homeostasis, metabolism of carbohydrates and lipids, neuronal firing and blood pressure homeostasis (Buscher et al., 1998; Brodde & Michel, 1999; Mersmann, 2001; Port & Bristow, 2001; Small et al., 2003; Flordellis et al., 2004, Yasuda et al., 2006). Alterations in AR function may play a role in the pathophysiology of diseases and states such as obesity and related metabolic disorders. The genes encoding these proteins are therefore suggested to be "candidate genes" for the development of obesity and disorders in carbohydrate and lipid metabolism (Azuma et al., 1998; Fujisawa et al., 1998; Arner & Hoffstedt, 1999; Buscher et al., 1999; Mershmann, 2001; Malczewska-Malek et al., 2003; Small et al., 2003; Yasuda et al., 2006).

Significant inconsistencies exist in the findings of studies performed on AR gene polymorphisms with regards to possible associations with characteristics of the metabolic syndrome (Buscher et al., 1999; Johnson & Terra, 2002; Santos et al., 2002; Malczewska-Malec et al., 2003 and Small et al., 2003). The β_1-AR Ser49Gly polymorphism has been linked to obesity, but more strongly to hypertension and cardiovascular phenotypes (Small et al., 2003; Flordellis et al., 2004; Leineweber et al., 2004). Genetic polymorphisms of the β_2- and β_3-AR genes have been shown, although not exclusively, to have possible effects on an individual's susceptibility to some of the features of the metabolic syndrome, i.e. obesity and hypertension (Fujisawa et al., 1998; Arner & Hoffstedt, 1999; Buscher et al., 1999; Candy et al., 2000; Kato et al., 2000; Bengtsson et al., 2001; Strazzullo et al., 2001; Johnson & Terra, 2002; Malczewska-Malec et al., 2003; Small et al., 2003). Currently, it seems that the β-AR polymorphisms are more likely to be risk factors rather than disease-causing genes (Leineweber et al., 2004; Siani & Strazzullo, 2006).

Small differences in minor allele frequencies of AR gene polymorphisms have been reported by various studies conducted on the same population group (ethnic group). This might be attributed to the difference in the number of subjects used in the particular studies or homogeny of descent (Small et al., 2003; Leineweber et al., 2004). Other possible reasons for differences in results from the various studies include differences in the age, gender and ethnicity (region-specific polymorphisms as well as ancestral descent) of the persons in the study groups (Arner & Hoffstedt, 1999; Strazzullo et al., 2001; Santos et al., 2002; Malczewska-Malec et al., 2003; Flordellis et al., 2004), insufficient statistical power (study group too small) and a lack of standardized definition for the phenotype for hypertension (Johnson & Terra, 2002). Another possible reason for the observed inconsistency is the fact that a genetic variant may interact with others (polymorphisms) to influence body fat. It is said that genes do not speak in monologues, but sing in choirs, hence the idea of haplotypes progressed (Buscher et al., 1999; Malczewska-Malec et al., 2003; Mehrian-Shai et al., 2004; Reichardt, 2006).

The aim of this investigation was to look for a possible association between four adrenergic receptor gene polymorphisms, diplotypes and haplotypes with parameters of carbohydrate and lipid metabolism, index of insulin resistance, BMI and percentage body fat in two groups of South African women.

MATERIALS AND METHODS

Study design

During 2003 – 2004 the POWIRS (Profile of Obese Women with the Insulin Resistance Syndrome) a case-control study was conducted by the School of Physiology, Nutrition and Consumer Sciences of the Faculty of Health Sciences, North-West University. The study consisted of female volunteers from the North-West Province of South Africa (Schutte *et al.,* 2005). The aim of this investigation was to look for a possible association between the β_2-AR Gln27Glu and β_3-AR Trp64Arg gene polymorphisms with parameters of carbohydrate and lipid metabolism, index of insulin resistance, BMI and percentage body fat in African women and white women in South Africa.

Study subjects

All the subjects involved in this study were female employees of a governmental institution in Potchefstroom, North-West Province of South Africa. The POWIRS I study consisted of 102 urban African female volunteers (heterogenous group consisting of 58% Setswana, 17% Xhosa and 25% Zulu mother tongue speakers) . Exclusion criteria included pregnancy, lactation and temperatures above 37°C. For the POWIRS II study, 115 white South African female volunteers (homogenous group) were used with inclusion criteria being apparently healthy white women, between 19 and 55 years of age and the same exclusion criteria applying (Schutte *et al,* 2005). The POWIRS studies were approved by the Ethics Committee of the North-West University (Potchefstroom Campus) and the study protocols conformed to ethical guidelines prescribed by the Helsinki Declaration of 1975 (ethics approval number 03M03). The subjects were all fully informed about the study objectives and procedures prior to their inclusion and with assistance the information could be provided to them in their own home language when required. An informed consent form was signed by all the volunteers

Physiological parameters measured

The cut-off point of BMI was set at 25 in dividing the subjects into a overweight and non-overweight group for comparison. Parameters of carbohydrate and lipid metabolism, HOMA-IR (fasting glucose x fasting insulin/22.5), BMI and percentage body fat were measured. Weight, height and waist

circumference were measured with calibrated instruments and standard methods (Schutte *et al*, 2005). The percentage body fat and HOMA-IR were calculated. A stethoscope and mercury sphygmomanometer were used to monitor blood pressure. Fasting blood samples were taken, after which a two-hour oral glucose tolerance test was performed. Blood samples were collected every 30 minutes after glucose ingestion. Plasma glucose, insulin and various lipid levels were determined using standard methods (Schutte *et al*, 2005).

Genotypic analysis

DNA was isolated from blood spots on Guthrie cards collected from the volunteers during the POWIRS I and II studies by the method described by Gregersen *et al* (1991). The published oligonucleotide sequences used in this study were obtained from Maqbool *et al*, 1999 (β_1-Ser49Gly), Santos *et al*, 2002 (β_2-Gln27Glu), Kim *et al*, 2002 (β_2-Arg16Gly) and Yuan *et al*, 1997 (β_3-Trp64Arg). PCR was performed on a Hybaid Thermocycler to amplify the relevant AR gene fragments. The resulting PCR products were digested with the appropriate restriction enzyme and the fragments separated on 3% agarose gels (Roche, 1 816 586). The ethidium bromide stained gels were visualized on a GelDoc system from Syngene and the genotypes noted.

Statistical analysis

Statistical analysis was performed using the Statistica 7 software package (Statsoft, Inc., 2000), Microsoft Excel 2002 and GraphPad Prizm 4 (GraphPad software, Inc., 2003). The t-test, independent by group analysis was performed in order to compare differences in measured parameters. The level of statistical significance was set at P ≤ 0.05. Hardy-Weinberg equilibrium was tested with the Chi-square test and the minor allele frequency determined. Area under the curve (AUC) analysis was performed on the parameters measured during the oral glucose tolerance test (OGTT, FFA and INS) with significant difference set at > 5%. These analyses were performed on both groups of women for each of the investigated AR gene polymorphisms, as well as for the diplotypes and the haplotypes.

RESULTS

In table 1 the descriptive statistics of both the black and the white groups of women are given for the measured physiological parameters. The genotype distributions for each of the investigated AR gene polymorphisms were consistent with a population in Hardy-Weinberg equilibrium. Normal distributions of data were observed in both the study groups. These results (table 1) show that both the SBP and DBP were significantly higher in the black group relative to the white group but that the Tc, LDH and Trig were higher in the white women. Only three of the parameters measured

during the oral glucose tolerance test (OGTT30, OGTT60 and FFA120) were found to be significantly higher in the white women, however, these parameters were still within the normal ranges.

The β_1-AR Ser49Gly, β_2-AR Gln27Glu, β_2-AR Arg16Gly and β_3-AR Trp64Arg minor allele frequencies observed for the black women were 84%, 16%, 49% and 28%, respectively, and for the white women these frequencies were, in the same order, 94%, 46%, 50% and 7% (figure 1). The minor allele frequency for the β_1-AR Ser49Gly polymorphism was the highest in both study groups. For the β_1-AR Ser49Gly polymorphism the minor allele frequencies observed for the two groups differed by only 10 % and for β_2-AR Arg16Gly the allele frequencies were virtually the same. In contrast the β_2-AR Gln27Glu and β_3-AR Trp64Arg polymorphism minor allele frequencies differed to a great extent between the groups. The β_2-AR Gln27Glu polymorphism minor allele frequency of the black women exceeded that of the white women by 30 %. For the β_3-AR Trp64Arg polymorphism the minor allele frequency observed in the group of white women was 21% higher than that of the black women. Of the four polymorphisms investigated, this is the only case where the minor allele in the black women was more common than in the white women.

The physiological parameters measured and the genotypes determined in the POWIRS I and II studies along with the results obtained from this study, are provided in tables 2 to 5. The only physiological parameter found to differ significantly between the Hm (homozygote for the polymorphism) and the Ht (heterozygote) genotypes of the β_1-AR Ser49Gly polymorphism in the black women was OGTT30 (Table 2). Significant differences were observed between four of the physiological parameters (INS0, INS30, INS120, HOMA-IR) measured between the Hm and Ht genotypes for the white women (table 2), three of which (INS) are related to the oral glucose tolerance test.

For the β_2-AR Gln27Glu polymorphism, several parameters measured during the oral glucose tolerance test differed significantly (table 3) for the black women, but in the case of the white women no significant differences were found between the three genotypes at this locus (table 3).

The differences that were observed for the FFA parameters measured during the oral glucose tolerance test for the black women were between the Hm and Ht, and the Hm and N genotypes of the β_2-AR Arg16Gly polymorphism (table 4). All the FFA parameters differed significantly between the Hm and N genotypes except for the fasting value. For the white women, however, no significant differences were found between the Hm and N genotypes (table 4).

For the β_3-AR Trp64Arg polymorphism, there were only two significant differences observed in the entire group of black women (table 5). Both were measured during the oral glucose tolerance test. Table 5 shows that only one significant difference was found for the white women between the Ht and N genotypes (OGTT30).

The Gln27/Arg16 diplotype was observed in 46% of the black women - group 2 with 47 individuals (table 6). Only four of the possible nine combined genotypes were present in less than 5 individuals and there were no combinations that were not represented in this cohort. A comparison of the prevalence of the diplotype combinations between the two groups showed that for the white women, the diplotype Gln/Glu/Arg/Gly was present in the biggest group (table 6). This diplotype was represented by 31 of the 115 white women (27%). There were only 2 of the possible 9 diplotypes not present in more than 5 individuals in the group of white women and each of the possible diplotypes were represented (no zeros).

The measured physiological parameters were compared between the different diplotypes. For the black women, the SBP was found to be the only parameter differing significantly and found to be elevated (between group 3 and 7, table 6). In the case of diplotypes 2 and 5 in the white women (table 6), the HOMA-IR was found to differ significantly and diplotype 2 exceeded the cut-off point. All the other parameters showing significant differences between the diplotypes for both the black and the white women were still within the normal ranges.

It is apparent from table 7 that for the group of black women the majority of possible haplotypes was not present in any of the subjects. Clustering of individuals was only observed in five of the possible haplotypes. The Gly49/Gln27/Arg16Gly/Trp64Arg haplotype had the greatest representation in the group: 17 individuals (17% of the total group). For the white female group the largest number of subjects representing a single haplotype is 24 and thus 21 % of the total group. The white group of women showed a more homogenous distribution of individuals across the possible haplotypes (table 8). The measured physiological parameters were compared with the different haplotypes for both groups of women, but those observed which differ significantly were found to be still within the normal ranges (Schutte *et al.*, 2005).

When AUC analyses were performed, most of the physiological parameters measured during the oral glucose tolerance test differed significantly between the genotypes of the investigated AR gene polymorphisms. However, all these parameters were found to still be within the normal ranges. This

analysis was also performed for the diplotypes and haplotypes investigated in the two groups of women and the same results were obtained.

DISCUSSION

Since the two subject groups are almost equal in numbers (102 black and 115 white women respectively) it is not expected that the numbers will play a role in causing the differences that may be observed in the allele frequencies between the two groups. The differences observed in minor allele frequencies may be ascribed to the test group being selected volunteers or can be attributed to the fact that the two groups are of different ethnicities as results from studies around the world support this observation (Leineweber *et al.*, 2004). Differences in ancestral descent have also been introduced as a contributing factor (Small *et al.*, 2003; Leineweber *et al.*, 2004). The fact that the study groups were selected volunteers may have a possible impact upon the validity of the calculated minor allele frequencies as being representative of that in the rest of these respective populations. This can be said because some genotypes were not present in the study groups which could affect the calculation of the minor allele frequencies. The observed allele frequency might be a true reflection of the prevalence of the polymorphic alleles in these population groups, or not, as a result of the study groups consisting of the so-called volunteers.

Comparing the minor allele frequencies calculated for the subjects studied in this investigation with those from published studies, significant differences, but also resemblances, became apparent. The minor allele frequencies of both the groups are much higher than those in the published studies when considering the β_1-AR Ser49Gly polymorphism. For the remaining three investigated polymorphisms, the minor allele frequencies observed are fairly similar to those in other ethnicities and population groups (Buscher *et al.*, 1999; Johnson & Terra, 2002; Santos *et al.*, 2002; Malczewska-Malec *et al.*, 2003 and Small *et al.*, 2003). Noteworthy is the fact that the white female group had the lowest minor allele frequency for the β_3-AR Trp64Arg polymorphism (7%).

In order to prove association with either obesity or elevated blood pressure, a sufficient number of the measured parameters must differ and be above the cut-off point (outside the normal range).

In the group of black women only the OGTT30 was found to be significantly different between the Hm and Ht genotypes for the β_1-AR Ser49Gly polymorphism (table 2). The only significantly differing parameter is insufficient to support the notion of possible associations with obesity or high blood pressure. The insulin parameters of the white women of the Ht genotype were significantly higher than those of the Hm (table 5), but the HOMA-IR was still below the cut-off point of 2.5 for

insulin resistance (Keskin *et al.,* 2006). Thus, no associations were found in the case of the white women.

Although the body fat % of the Ht and N genotypes showed a significant difference in the black women for the β_2-AR Gln27Glu polymorphism, the values were still within the normal ranges (table 2). This was the case with the SBP as well. For the white women there were no significant differences found between the genotypes (table 6). In both groups, no association was found between this AR gene polymorphism and obesity or high blood pressure.

For the β_2-AR Arg16Gly polymorphism in the black women, it is clear from table 3 that all the FFA parameters were found to differ significantly between the Hm and other two genotypes except the fasting value. This might be explained by the fact that differences only come to light when the system is challenged – in this case with the oral glucose tolerance test. The difference seen might be a result of over- or hyperactivity of the adrenergic receptors as a result of the polymorphism. The other parameters found to be significantly different between the genotypes were all within the normal ranges, thus no associations were found for this AR gene polymorphism in the black women with either obesity or high blood pressure. The BMI of the Ht genotype was significantly different to that of the N genotype for this AR gene polymorphism in the group of white women (table 7), but the BMI of all three genotypes still fell into the overweight category (BMI between 25 and 30). The WC of the Ht genotype was higher than that of N and above the cut-off value of 88cm but those of the Hm and N genotypes was still within the normal range. No association with obesity or high blood pressure was therefore found in the group of white women.

The three parameters found to differ significantly (tables 4 and 8) for the β_3-AR Trp64Arg polymorphism are insufficient to prove possible associations between this AR gene polymorphism and obesity or high blood pressure in either of the subject groups.

Studies have shown that the Arg16 (N) genotype rarely occurs with Glu27 (Hm). The haplotype Arg16Glu27 occurs in less than 1% of the population in a study by Bruck *et al.* (2005). In our study this combination was present in 1% of the black women and 4% of the white women.

In some population groups, associations of certain AR gene polymorphisms with obesity and hypertension (genotype-phenotype correlations) were both proved and disproved, for example the Japanese study groups (Yuan *et al.,* 1997; Arner & Hoffstedt, 1999; Breitwieser, 2002; Masuo *et al.,* 2005)The investigated AR gene polymorphisms were compared within each of the subject groups and compared between the two groups of women. The findings of this study supports the notion of

no association existing between characteristics of the metabolic syndrome and adrenergic receptor polymorphisms, but suggest that they may act as contributing factors to risk factors themselves.

When the diplotypes observed in the black women were compared, only the SBP was found to differ significantly between groups 3 and 7 and both were somewhat elevated. Although some of the parameters measured during the oral glucose tolerance test showed significant differences between some of the diplotypes, all the values were still within the normal ranges. No association was therefore demonstrated between the observed β_2-AR diplotypes for the black women. In all except one comparison parameters were found to be significant different still within the normal ranges and therefore no possible associations found. The HOMA-IR of diplotype 2 and 5 was compared and found to differ significantly. For diplotype 2 the HOMA-IR value was found to be 3.43 and over the cut-off point, while that of diplotype 5 was normal. The INS parameters measured for diplotype 2 was also significantly higher than that of diplotype 5. This could possibly link this diplotype to the onset and progression of insulin resistance along with the effects thereof such as hyperinsulinemia, (further) weight gain and increases in blood cholesterol.

Clustering of individuals into certain haplotypes for the white women was observed more so than in the black women (tables 11 and 12). This may be explained by the fact that the group of black women was not of homogenous descent (not all Zulu or Xhosa for instance). Statistically significant differences observed between different haplotypes for the black women are not sufficient to prove possible associations of any of these haplotypes with obesity or high blood pressure. Although the HOMA-IR differed significantly between haplotypes 1, 2 and 3 of the white women and the mean of this parameter exceeded the cut-off point, the fasting plasma glucose as well as the other parameters measured during the oral glucose tolerance test were not significantly different between these 3 haplotypes. No other parameters found to be significantly different were outside normal ranges, thus showing no possible associations between haplotypes observed for the white women with obesity or high blood pressure.

When AUC analysis was performed, most of the genotypes, diplotypes and haplotypes differed with regards to the parameters measured during the oral glucose tolerance test (OGTT, FFA and INS). The parameters found to be significantly different were still within the normal ranges. This could suggest that the adrenergic response differs between the genotypes of a specific AR gene, but not necessarily to an abnormal extent (under- or over-activity). The initial (fasting) concentration of the measured parameters (at time 0 minutes) in most cases did not differ significantly, thus the base value of operation for the ARs can be said to be more or less the same for all four polymorphic ARs. It is only when the system is put under stress / challenged with the loading test, that the differences

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in response (under- of over-activity) become noticeable. This could be possible by the result of altered ARs by these polymorphisms.

Insel *et al.* (2006) stated that most associations found between AR gene polymorphisms and disease states (such as the metabolic syndrome) were too weak to allow meaningful prediction of onset, progression or drug response in disease. The findings of our study therefore support the notion that there are no association between these AR genes and factors namely obesity and high blood pressure (hypertension) of the metabolic syndrome, but at the same time indications arise that these four investigated polymorphisms, diplotypes and haplotypes may in some cases in the two study groups, act not as risk factors themselves, but as contributors to risk factors for onset and progression of obesity, high blood pressure and the metabolic syndrome.

In conclusion, the number of measured physiological parameters found to be significantly different in the comparisons was insufficient to indicate possible association between the investigated AR gene polymorphisms, diplotypes or haplotypes and the two characteristics of the metabolic syndrome namely obesity and hypertension (high blood pressure). There were, however, indications that these AR gene polymorphisms, diplotypes and haplotypes investigated may act as contributors to the risk factors for development and progression of the metabolic syndrome.

In this study the four investigated AR gene polymorphisms were compared within each of the subject groups. Thereafter comparisons were made between the two subject groups. The results obtained can not, however, be extrapolated to the entire black and white populations in South Africa.

Gaps still exist in research on this topic however. Future approaches may include studying the functional role and interaction of the polymorphic ARs by shifting the focus from a single SNPs of individual AR genes as has been mostly the case up till now to haplotypes (combinations of SNPs within one gene) and "functional haplotypes" (combinations of SNPs within and between genes). A notion supported in the literature (Mehrian-Shai & Reichardt, 2004; Reichardt, 2006). Furthermore, population studies with the maximum amount of subjects should be undertaken to obtain statistically significant results with less room for doubt (increased statistical power).

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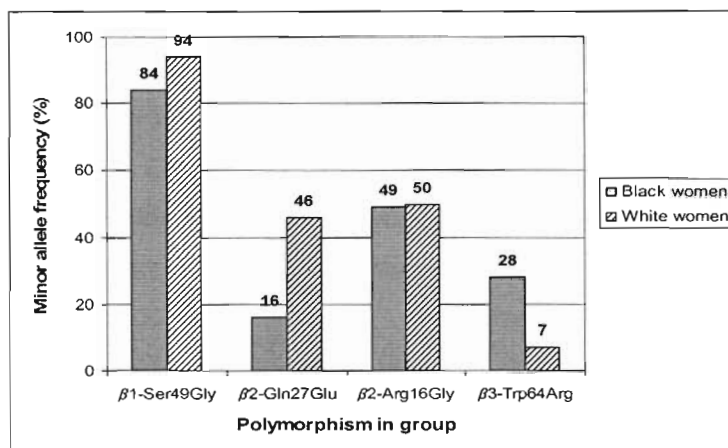
Figure 1: Minor allele frequencies observed for the two groups

Table 1: Statistical description of the study groups

Parameter	Black women (n=102) Mean	±SD	White women (n=115) Mean	±SD	P-values
Age (years)	31.25	8.64	31.34	9.18	0.945
BMI (kg/m^2)	27.98	6.33	28.48	7.15	0.588
WC (cm)	87.18	14.77	86.00	14.94	0.558
SBP (mmHg)	129.82	19.60	125.41	11.69	**0.043**
DBP (mmHg)	77.68	10.69	72.47	9.01	**0.000**
Tc (mmol/l)	4.25	0.93	4.94	1.03	**0.000**
HDL (mmol/l)	1.25	0.33	1.21	0.32	0.287
LDL (mmol/l)	2.85	0.87	3.15	0.93	**0.017**
Trig (mmol/l)	0.71	0.42	1.28	0.70	**0.000**
OGTT0 (mmol/l)	5.19	1.19	5.04	0.41	0.190
OGTT30	7.19	1.42	7.74	1.33	**0.004**
OGTT60	6.99	2.23	7.65	1.89	**0.021**
OGTT90	6.76	2.18	7.01	1.90	0.381
OGTT120	6.59	1.99	6.60	1.66	0.990
FFA0 (mmol/l)	0.54	0.27	0.56	0.19	0.621
FFA30	0.30	0.21	0.34	0.21	0.167
FFA60	0.09	0.09	0.08	0.08	0.858
FFA90	0.04	0.04	0.05	0.06	0.328
FFA120	0.03	0.02	0.04	0.07	**0.026**
INS0 (pmol/l)	92.94	41.47	92.66	33.47	0.956
INS30	659.05	444.11	603.47	375.75	0.326
INS60	597.84	400.91	576.99	361.49	0.692
INS90	477.61	373.92	479.23	330.75	0.973
INS120	428.58	325.75	385.33	277.42	0.300
HOMA-IR	3.09	1.55	3.04	1.29	0.791

Bold print indicates significant statistical difference (P ≤0.05)
BMI: body mass index; WC: waist circumference, SBP: Systolic blood pressure, DBP: diastolic blood pressure, Tc: total blood cholesterol, HDL: high density lipoproteins, LDL: low density lipoproteins, Trig: triglycerides, OGTT: oral glucose tolerance test, FFA: free fatty acids, INS: insulin, HOMA-IR: index of insulin resistance

Table 2: Comparison of measured parameters for the genotypes of the β_1-AR Ser49Gly polymorphism

| Parameter | Hm (n=71) | | Ht (n=30) | | P-values |
	Mean	±SD	Mean	±SD	Hm vs. Ht
Black women					
OGTT30 (mmol/l)	7.00	1.37	7.60	1.49	**0.054**
White women					
INS0 (pmol/l)	89.75	32.59	115.23	32.78	**0.009**
INS30	575.59	349.84	813.62	500.76	**0.031**
INS120	360.04	240.02	576.00	441.79	**0.008**
HOMA-IR	2.94	1.29	3.75	1.15	**0.034**

* indicates significant statistical difference (P ≤0.05)
SD: standard deviation; Hm: homozygotes for the polymorphic allele; Ht: heterozygotes; N: homozygotes for the wild type allele; OGTT: oral glucose tolerance test, FFA: free fatty acids, INS: insulin, HOMA-IR: index of insulin resistance

Table 3: Comparison of measured parameters for the genotypes of the β_2-AR Gln27Glu polymorphism

| Parameter | Hm (n=5) | | Ht (n=23) | | N (n=74) | | P-values | | |
	Mean	±SD	Mean	±SD	Mean	±SD	Hm vs. Ht	Hm vs. N	Ht Vs. N
Black women									
Body fat %	22.58	4.28	35.16	12.45	33.75	11.01	**0.037**	**0.027**	0.606
DBP (mmHg)	64.67	9.54	79.52	10.61	77.99	10.32	**0.008**	**0.006**	0.538
OGTT60 (mmol/l)	4.65	1.53	8.03	2.56	6.84	2.01	**0.009**	**0.020**	**0.025**
OGTT90	5.11	1.35	7.70	2.42	6.59	2.05	**0.031**	0.118	**0.036**
FFA30 (mmol/l)	0.08	0.07	0.35	0.21	0.30	0.20	**0.011**	**0.017**	0.333
INS60 (pmol/l)	222.00	132.04	749.00	458.89	580.10	377.50	**0.019**	**0.039**	0.089
INS90	179.40	52.06	636.19	553.43	452.41	301.54	0.082	**0.048**	**0.048**
White women									
None									

Bold print shows significant statistical difference (P ≤0.05)
Hm: homozygote for the polymorphism, Ht: heterozyogte, N: homozygote for the wild type allele
DBP: diastolic blood pressure, OGTT: oral glucose tolerance test, FFA: free fatty acids, INS: insulin
SD: standard deviation

Table 4: Comparison of measured parameters for the genotypes of the β_2-AR Arg16Gly polymorphism

	Hm (n=20)		Ht (n=61)		N (n=21)		P-values		
Parameter	**Mean**	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**	**Hm vs. Ht**	**Hm vs. N**	**Ht vs. N**
Black women									
FFA30 (mmol/l)	0.40	0.23	0.30	0.20	0.23	0.15	0.071	**0.008**	0.155
FFA60	0.13	0.09	0.08	0.10	0.05	0.03	0.066	**0.000**	0.147
FFA90	0.07	0.04	0.04	0.04	0.03	0.02	**0.008**	**0.004**	0.513
FFA120	0.04	0.03	0.03	0.01	0.02	0.01	**0.000**	**0.004**	0.628
White women									
BMI (kg/m^2)	27.01	6.00	30.12	7.69	26.22	6.13	0.062	0.634	**0.024**
WC (cm)	83.57	13.40	89.51	15.60	80.35	13.01	0.085	0.376	**0.010**
FFA30 (mmol/l)	0.28	0.16	0.38	0.21	0.32	0.23	**0.022**	0.400	0.254
INS60 (pmol/l)	465.32	316.18	636.64	356.87	563.68	400.84	**0.033**	0.323	0.413

Bold print shows significant statistical difference (P ≤0.05)
Hm: homozygote for the polymorphism, Ht: heterozyogte, N: homozygote for the wild type allele
FFA: free fatty acids, BMI: body mass index, WC: waist circumference, INS: insulin
SD: standard deviation

Table 5: Comparison of the measured physiological parameters for the genotypes of the β_3-AR Trp64Arg polymorphism

	Hm (n=5)		Ht (n=48)		N (n=49)		P-values		
Parameter	**Mean**	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**	**Hm vs. Ht**	**Hm vs. N**	**Ht Vs. N**
Black women									
OGTT90 (mmol/l)	5.22	0.83	6.55	2.28	7.11	2.11	0.204	**0.054**	0.227
INS30 (pmol/l)	942.60	743.42	712.91	489.61	579.55	344.09	0.348	**0.052**	0.126
White women									
OGTT30	*	*	8.33	1.70	7.64	1.23	*	*	0.053

Bold print shows significant statistical difference (P ≤0.05)
*insufficient number of subjects with specific genotype
Hm: homozygote for the polymorphism, Ht: heterozyogte, N: homozygote for the wild type allele
OGTT: oral glucose tolerance test, INS: insulin
SD: standard deviation

Table 6: β_2-AR gene polymorphism diplotypes observed for the group of black women

β_2-AR Gln27Glu	β_2-AR Arg16Gly N	β_2-AR Arg16Gly Ht	β_2-AR Arg16Gly Hm
Black women			
Hm	1*	3	1
N	14 [1]	47 [2]	13 [3]
Ht	5	12 [4]	6 [5]
White women			
Hm	5	13 [3]	10 [5]
N	8 [7]	17 [2]	13 [4]
Ht	13 [6]	31 [1]	5

Number given to group for comparison indicated in brackets
Hm: homozygote for the polymorphism, Ht: heterozyogte, N: homozygote for the wild type allele

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Table 7: Haplotypes observed in the group of black women

B₁-AR Ser49Gly	β₂-AR Gln27Glu	β₂-AR Arg16Gly	β₃-AR Trp64Arg N	β₃-AR Trp64Arg Ht	β₃-AR Trp64Arg Hm
Hm	Hm	N	1*	0	0
Hm	Hm	Ht	2	1	0
Hm	Hm	Hm	0	1	0
Hm	N	N	5	2	0
Hm	N	Ht	13 [1]	17 [2]	3
Hm	N	Hm	6 [3]	6 [4]	0
Hm	Ht	N	0	2	0
Hm	Ht	Ht	4	4	0
Hm	Ht	Hm	3	1	0
Ht	Hm	N	0	0	0
Ht	Hm	Ht	0	0	0
Ht	Hm	Hm	0	0	0
Ht	N	N	3	4	0
Ht	N	Ht	6 [5]	6 [6]	2
Ht	N	Hm	0	1	0
Ht	Ht	N	2	1	0
Ht	Ht	Ht	2	2	0
Ht	Ht	Hm	1	0	0
N	Hm	N	0	0	0
N	Hm	Ht	0	0	0
N	Hm	Hm	0	0	0
N	N	N	0	0	0
N	N	Ht	0	0	0
N	N	Hm	0	0	0
N	Ht	N	0	0	0
N	Ht	Ht	0	0	0
N	Ht	Hm	1	0	0

*number of study subjects
Number given to group for comparison indicated in brackets
Hm: homozygote for the polymorphism, Ht: heterozyogte, N: homozygote for the wild type allele

Table 8: Haplotypes observed in the group of white women

B₁-AR Ser49Gly	β_2-AR Gln27Glu	β_2-AR Arg16Gly	β_3-AR Trp64Arg N	β_3-AR Trp64Arg Ht
Hm	Ht	Ht	24 [1]	4*
Hm	Ht	Hm	2	1
Hm	Ht	N	10 [2]	2
Hm	N	Ht	16 [3]	0
Hm	N	Hm	13 [4]	0
Hm	N	N	5	3
Hm	Hm	Ht	10 [5]	1
Hm	Hm	Hm	5	2
Hm	Hm	N	3	1
Ht	Ht	Ht	2	1
Ht	Ht	Hm	2	0
Ht	Ht	N	1	0
Ht	N	Ht	1	0
Ht	N	Hm	0	0
Ht	N	N	0	0
Ht	Hm	Ht	2	0
Ht	Hm	Hm	3	0
Ht	Hm	N	0	1

*number of study subjects
Number given to group for comparison indicated in brackets
Hm: homozygote for the polymorphism, Ht: heterozyogte, N: homozygote for the wild type allele

Vir my oupa Johannes Hercules de Beer wat my geleer het: "Al keer hulle jou in 'n hoek vas, jy gaan nie lê nie, jy baklei terug".