

**SELECTED CANDIDATE GENES AND OBESITY
AMONG GHANAIAN ADULTS: A CASE CONTROL
STUDY AT THE KORLE-BU TEACHING
HOSPITAL (DIETHERAPY UNIT) ACCRA**

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JULY, 2014

DECLARATION

I, hereby declare that this is the result of my own research work under the supervision of Dr. Charles A. Brown and Dr. Samuel Antwi-Baffour. There has not been any previous submission for a Master of Science degree here or elsewhere. Also, references to works of other authors cited have been duly acknowledged.

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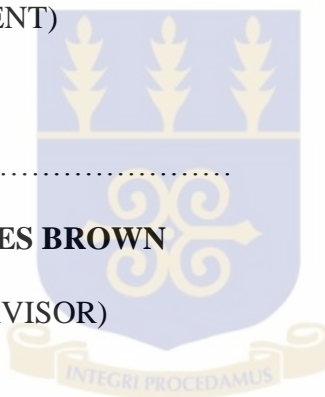
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ABSTRACT

Background: Obesity is a complex, heterogeneous group of disorders that is determined by genes, environmental factors and interaction between genes and environment. Several studies have all established associations of certain candidate genes to obesity and their variations with respect to race, geographical location/country of origin. A study of this nature to investigate the relationship between some of these selected genes and obesity has not yet been conducted in Ghana.

Aim: The aim of this study was to investigate the relationship between single nucleotide polymorphisms (SNPS) in selected candidate genes (β 3-adrenergic receptors (ADRB3), leptin receptor (LEPR), and peroxisome proliferator activator γ receptor (PPAR γ)) and obesity among Ghanaian adults.

Methods: This was a case-control study. The cases were newly diagnosed obese (BMI \geq 30 kg/m²) male and female Ghanaian adults (18 and 60 years) who visited the Dietherapy Unit (KBTH) for dietary counselling. Controls were students of the School of Allied Health Sciences and various staff members of Korle bu Teaching Hospital. A validated questionnaire was used to collect data on demographic characteristics, feeding habits, dietary history and anthropometric measures. Dietary intake was assessed using a food frequency questionnaire. DNA was extracted from mouth rinse water samples and SNPS in the ADRB3, LEPR and PPAR γ genes were determined by polymerase chain reaction restriction fragment length polymorphism (PCR –RFLP).

Results: A total of 56 subjects (71.4% females), consisting of 24 cases (41.63 ± 11.50 years) and 32 controls (26.42 ± 5.48 years), were recruited for the study. All the subjects were non-smokers, with 80.3% not consuming alcohol. There were statistically significant differences in BMI, waist to hip ration (WHR) and mid-upper arm circumference (MUAC) measurements between cases and controls (all $ps < 0.0001$).

There was no statistically significant difference ($p = 0.3032$) between the mean energy intakes of the cases and controls. DNA fragments for the PPAR γ gene was amplified in 55 out of the 56 respondents. Only the presence of CC (Pro12Pro) genotype was detected after the RFLP. No relationship was observed between PPAR γ gene polymorphism and BMI, MUAC and WHR among the participants. For LEPR, amplification was successful in 15 cases and 14 controls. However, only amplicons of six cases, all in the OBClass 1 to OBClass 3 range, after RFLP indicated the presence of the Gln223Arg genotype. A significant difference (all $ps < 0.05$) emerged for the LEPR gene Gln223Arg genotype polymorphism frequencies between the cases and the controls for BMI, MUAC and WHR. For ADRB3, amplification was successful in 19 cases and 24 controls. RFLP indicated the presence of the Trp64Trp and the Trp64Arg genotypes. No significant difference (all $ps > 0.05$) emerged for the ADRB3 gene Trp64Arg genotype polymorphism frequencies between the cases and the controls for BMI, MUAC and WHR.

Conclusions: No relationships were observed between ADRB3 and PPAR γ genotype polymorphisms and BMI, MUAC and WHR. However, LEPR gene Gln223Arg genotype polymorphism was statistically associated with BMI, MUAC and WHR. A larger sample size would have to be considered in order to establish any potential associations between LEPR and obesity in the Ghanaian population.

DEDICATION

This dissertation is dedicated to THE LORD GOD ALMIGHTY for bringing me this far.

It is also to Yauniuck my “friend” and Patrick my “brother” for their encouragement and assistance in diverse ways in the completion of this work.



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ABBREVIATIONS

ADRB3	Adrenergic Beta 3 Receptor
BMI	Body Mass Index
BP	Blood Pressure
CDC	Centre for Disease Control
CPHA	Canadian Public Health Association
DHS	Demographic Health Survey
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
FAO	Food and Agriculture Organisation
GHS	Ghana Health Service –
GSS	Ghana Statistical Service
GWAS	Genome Wide Association Studies
KBTH	Korle Bu Teaching Hospital
LEPR	Leptin Receptor
MESA	Multi-Ethnic protocol for the Study of Atherosclerosis
mRNA	messenger RNA
MUAC	Mid Upper Arm Circumference
NHLBI	National Heart Lung and Blood Institute
NIH	National Institute of Health
PCR	Polymerase Chain Reaction
PPARG	Peroxisome Proliferator Activated Receptor Gamma
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
SNPs	Single Nucleotide Polymorphisms
TNES	A buffer comprising; Tris, NaOH, EDTA, and SDS
UNICEF	United Nations International Children’s Emergency Fund
WHO	World Health Organization
WHR	Waist to Hip Ratio

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Obesity is an accumulation of excess body fat, to such an extent that health might be impaired (WHO, 2000; 2013a). Obesity is a complex condition involving environmental, behavioural and genetic factors as well as interactions among them. Despite its advancement in pandemic proportions, associated morbidity and mortality, and the financial burden for governments and society, it remains a major unsolved medical problem (Kopelman. 2005; Arora & Anubhuti 2006). Obesity increases the risk for developing chronic diseases, including type 2 diabetes (T2M), coronary heart diseases (CHDs), hypertension, osteoarthritis, and certain cancers (endometrial, breast, and colon), besides the social and psychological consequences (Neary *et al.*, 2003; WHO, 2013a).

Overweight and obesity are the fifth leading risk for global deaths. At least 2.8 million adults die each year as a result of being overweight or obese. In addition, 44% of the diabetes burden, 23% of the ischaemic heart disease burden and between 7% and 41% of certain cancer burdens are attributable to overweight and obesity (WHO 2013a).

Obesity is the most common nutritional disorder in the world. Globally, there are more than 1.4 billion overweight adults, of these over 500 million are obese (WHO, 2013a). About 65% of the world's population live in countries with high mortality rates from overweight and obesity compared to underweight (WHO, 2013a). The United States has the highest prevalence of obesity among the developed nations (CDC, 2010; WHO,

2013a). However, increases in the prevalence of overweight and obesity have been observed throughout the world.

An estimated 68.8% of U.S. adults are overweight, and 35.7% are obese (CDC, 2012). In Canada 36% of adults are overweight and another 23% are obese (Statistics Canada, 2010). In Europe, between 30% and 80% of the adult population are overweight. About 31.9% to 79.3% of the men, and 27.8% to 77.8% of the women are overweight while 5.4% to 22.8% adult males and 7.1% to 35.6% adult females are obese (WHO, 2007; WHO, 2013b). In the United States, prevalence of obesity is higher in black and Hispanic populations, especially among Mexican American women (CDC, 2012).

The prevalence of obesity in West Africa rapidly increased during the last two decades of the 20th century and continues to increase in the 21st century. A recent review of obesity prevalence in West Africa indicates that between 2000 and 2004, 10% of West African adults were obese. According to the same study, half of the urban population in general and 60% of urban West African women were either overweight or obese (Abubakari *et al.*, 2008). In Ghana, the Demographic and Health Survey (DHS) in 2008 indicated that the percentage of women aged 15-49 overweight or obese grew from 25% to 30% between 2003 and 2008 with the highest values among urban women (GSS, GHS, and ICF Macro, 2009). Wave 1 of the Women's Health Study of Accra (WHS-1) found 62.2% of 1,237 non-pregnant women living in Accra to be overweight or obese (Duda *et al.*, 2007).

Genetic factors may influence the development of obesity in a number of ways (Thomas & Bishop 2007). The number of fat cells, regional distribution of body fat, and resting metabolic rate (RMR) are also influenced by genes. Studies of twins confirm that genes

determine 50% to 70% of the predisposition to obesity (Prentice *et al.*, 2005). However in 2007, genome-wide association studies to identify obesity-related genes discovered the first obesity-related gene variants; the fat mass and obesity-associated (FTO) gene on chromosome 16 (Dina *et al.*, 2007; Frayling *et al.*, 2007). These gene variants are fairly common, and those carrying them do have a 20 – 30% higher risk of obesity than people without it (Loos *et al.*, 2008; Qi, 2008).

There are numerous genes involved in genetic predisposition to obesity. Some have received much attention, the *ob* gene (produces leptin), the adiponectin (ADIPOQ) gene, the B3-adrenoreceptor gene located primarily in the adipose tissue, which is thought to regulate RMR and fat oxidation in humans, and the FTO gene (predisposes to diabetes by its effect on body mass) (Frayling *et al.*, 2007). Mutations in the *ob* gene, leptin receptor (LEPR), or ADIPOQ genes can result in obesity or metabolic syndrome (MetS), especially if diet provides too much saturated fat (Ferguson *et al.*, 2010). A much greater proportion of the population is also likely to exhibit polymorphisms in other genes which have a significant but less dramatic effect on body weight (e.g. mutations in uncoupling proteins, the B3-adrenergic receptor (ADRB3) or peroxisome proliferator activator receptor γ (PPAR γ). These genes may influence a wide range of metabolic and behavioural characteristics that together determine an individual's susceptibility to obesity (Thomas & Bishop 2007).

A candidate gene is defined as that part of the DNA molecule that directs the synthesis of a specific polypeptide chain closely associated with a particular disease. Candidate genes for obesity can be chosen for their possible effects on body fat composition, anatomical distribution of fat, food intake and energy expenditure (Kopelman, 2000). Despite the evidence that genetic factors play a role in determining changes in adiposity, very few

studies have investigated the role of obesity candidate genes. Results from other studies have shown that the B-2/B-3 adrenergic receptor (ADRB2/ADRB3), LEP/LEPR and PPAR γ genes are associated with adiposity changes over time (van Rossum *et al.*, 2002).

1.2 PROBLEM STATEMENT

In Ghana, obesity was formally regarded as a disease of the ‘western world’ and of the affluent. Current statistical data, however, indicates this trend has changed (Sobal and Stunkard, 1989; Duda *et al.*, 2007) and obesity now runs across the entire socio-economic divide of the population.

Several associations have been linked with obesity such as environmental and behavioural. Lately, genetic linkage is showing dominance in most recent scientific researches on obesity. The observation in this trend is not so in Ghana. Several studies (Bouchard *et al.*, 1988; Malczewska-Malec *et al.*, 2004; Podolsky *et al.*, 2007) have all established associations of certain candidate genes to obesity and their variations with respect to race, geographical location/country of origin. While little or no scientifically researched information in this area of obesity research exist in Ghana, the evidences from other published works call for more studies by Ghanaian medical researchers to investigate variations if any that exist within the Ghanaian population.

1.3 SIGNIFICANCE OF THE STUDY

Investigation into these genes will help clarify the aetiology of obesity, its metabolic consequences and complications. In addition, it will also identify at-risk individuals or groups in terms of their genetic profile with the goal of developing personalized prevention and treatment strategies to enhance the quality of life. It will provide insight during dietetic counselling for obese individuals.

This project will also provide baseline information for future research in this field, since there is lack of scientific information on the genetic bases of obesity in Ghana.

1.4 HYPOTHESIS

There is no relationship between the selected candidate genes and obesity.

1.5 AIM

The aim of this study was to investigate the relationship between single nucleotide polymorphisms (SNPS) in selected candidate genes (β 3-adrenergic receptors (ADRB3), leptin receptor (LEPR), and peroxisome proliferator activator γ receptor (PPAR γ) and obesity among Ghanaian adults.

1.6 SPECIFIC OBJECTIVES

- a) To detect single nucleotide polymorphisms (SNPS) in the receptors of the ADRB3, LEPR and PPAR γ in the Ghanaian population.
- b) To determine whether there is a relation between the SNPS in the receptors of the selected candidate genes and obesity in the Ghanaian population.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 OBESITY

2.1.1 Definition

WHO (2000; 2013a) defined obesity as an accumulation of excess body fat, to such an extent that health might be impaired. Kumar and Clark (2009), also explain that obesity is an excess storage of fat, which can easily be detected by looking at the undressed patient. They further explained that, overweight, a precursor to obesity, occurs as a result of an imbalance between food consumed and physical activity. If energy expenditure (mechanical work and heat) is smaller than the intake, energy will be stored. As emphasized by O’Rahilly (2009), obesity results from a chronic surplus of energy intake compared to energy expenditure, which leads to storage of excessive amounts of triglycerides in adipose tissue. The World Health Organization uses body mass index (BMI) as a surrogate measure of overall obesity, and thus classifies a person with a $BMI \geq 30 \text{ kg/m}^2$ as obese and a $BMI \geq 40 \text{ kg/m}^2$ as extremely obese (WHO, 1998; 2010).

2.1.2 Description

The case of obesity is a complex one, involving environmental, behavioural and genetic factors as well as interactions among them (Kumar and Clark, 2009). The multifactorial facets of obesity: genetics, behavioural (diet and physical activity patterns), environmental, psychosocial and sociocultural factors with emphasis on genetics influencing the development of obesity in an individual have been established by several authors (Stunkard *et al.*, 1990; Maes *et al.*, 1997; Farooqi *et al.*, 1999; Farooqi and O’Rahilly, 2005; Ruiz *et al.*, 2010).

The causes of this imbalance can be traced to a number of factors including specific genetic traits which may convey a metabolic predisposition, behavioural factors and environmental circumstances (Jebb, 1997). These factors may act alone or in combination to modulate intake and/or expenditure and hence determine the likelihood of an individual becoming obese (Thomas & Bishop 2007).

Over the years, many hypotheses have evolved to explain why some people become fat and others remain lean, and why it is so difficult for reduced-obese persons to maintain weight loss (Mahan *et al.*, 2012). No single theory can completely explain all manifestations of obesity or apply consistently to all persons.

Moreover, it is a reputable fact that rapid globalization of the westernized lifestyle is enhancing this emerging obesity epidemic. Nonetheless, not everyone in this contemporary obesogenic environment develops obesity, highlighting the multifactorial nature of the condition. Undeniably, obesity arises through the combined actions of multiple genetic and environmental factors, i.e. the obesogenic environment increases the risk of obesity, particular in those who are already genetically susceptible (Loos, 2009). Obesity causes or exacerbates many health problems, both independently and in association with other diseases. In particular, it is associated with the development of type 2 diabetes mellitus, coronary heart disease (CHD), hypertension, an increased incidence of certain forms of cancer, respiratory complications (obstructive sleep apnoea), osteoarthritis of large and small joints (Kopelman, 2000), besides the social and psychological consequences (Neary *et al.*, 2003). Despite its advancement in pandemic proportions, associated morbidity and mortality, and the financial burden for government and society, it also remains a major unsolved medical problem (Kopelman, 2005; Arora & Anubhuti, 2006; Shamseddeen *et al.*, 2011).

Most patients suffer from simple obesity but in certain conditions such as Prader-Willi syndrome, Laurence-moon-biedl syndrome, hypothyroidism/Cushing's syndrome, Stein-leventhal syndrome and hypothalamic damage due to trauma/tumours, obesity is an associated feature (Kumar and Clark, 2009). In these and other cases of obesity, excess weight arises as a consequence of a long term excess of energy consumed relative to an individual's energy requirement.

The Build and Blood Pressure Study has shown that the adverse effects of excess weight tend to be delayed, sometimes for ten years or longer (Society of Actuaries, 1979).

Life-insurance data and epidemiological studies confirm that increasing degrees of overweight and obesity are important predictors of decreased longevity (Lew, 1985).

In the Framingham Heart Study, the risk of death within 26 years increased by 1% for each extra pound (0.45 kg) increase in weight between the ages of 30 years and 42 years, and by 2% between the ages of 50 years and 62 years (Hubert, 1986). Despite this evidence, many clinicians consider obesity to be a self-inflicted condition of little medical significance (Kopelman, 2000).

2.1.3 Prevalence

2.1.3.1 Global

Obesity is the most common nutritional disorder in the world, globally; there are more than 1.4 billion overweight adults, of whom more than 500 million are obese (WHO, 2013a). The prevalence is generally higher in developed than developing countries, but the rate of increase in some areas, especially in countries undergoing rapid economic transition, is marked.

There are marked trends in obesity with age. Among 16-24 year olds, only 10% are obese, rising to over 30% in 55-64 year olds, after which there is a modest decline (Thomas & Bishop 2007).

The United States has the highest prevalence of obesity among the developed nations (Figs. 1 and 2) (CDC, 2010; WHO, 2013a). However, increases in the prevalence of overweight and obesity have been observed throughout the world. The international trend is often called "globesity." The estimates of overweight and obesity among children and adults are based on measured weights and heights from the National Health and Nutrition Examination Survey (NHANES), conducted by the National Center for Health Statistics (CDC, 2007). An estimated 66% of U.S. adults are overweight, and 32% are obese. In Canada 36% of adults are overweight and another 23% are obese (Statistics Canada, 2010). In Europe between 15% and 25% of adults are obese. In the United States, prevalence of obesity is higher in Non-Hispanic black women and Hispanic populations, with about 41.9% and 30.7% respectively (CDC, 2010).

Unfortunately, children are not immune to the epidemic. Obesity is now the most common paediatric nutritional problem in the United States. Nearly a third of children and adolescents between ages 2 and 19 years are overweight (Mahan *et al.*, 2012).

Obesity is almost invariable in developed countries and almost all people accumulate some fat as they get older. The WHO acknowledges that obesity (body mass index $> 30 \text{ kg/m}^2$) is a worldwide problem which also affects many developing countries (Kumar and Clark, 2009). Obesity is a worldwide communal health problem. The problem does not only affect developed countries, as there is now a significant increase in overweight and obesity throughout the developing world (CPHA. 2006).

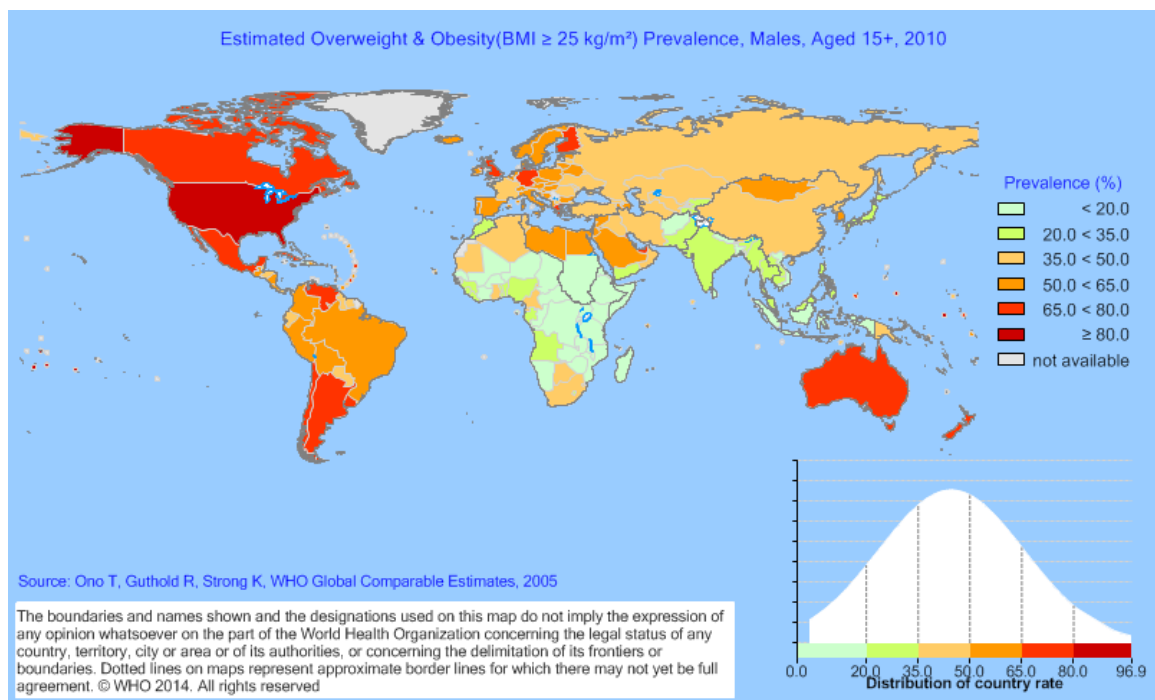


Fig. 1: WHO estimated global overweight and obesity prevalence for males, Aged 15+, 2010

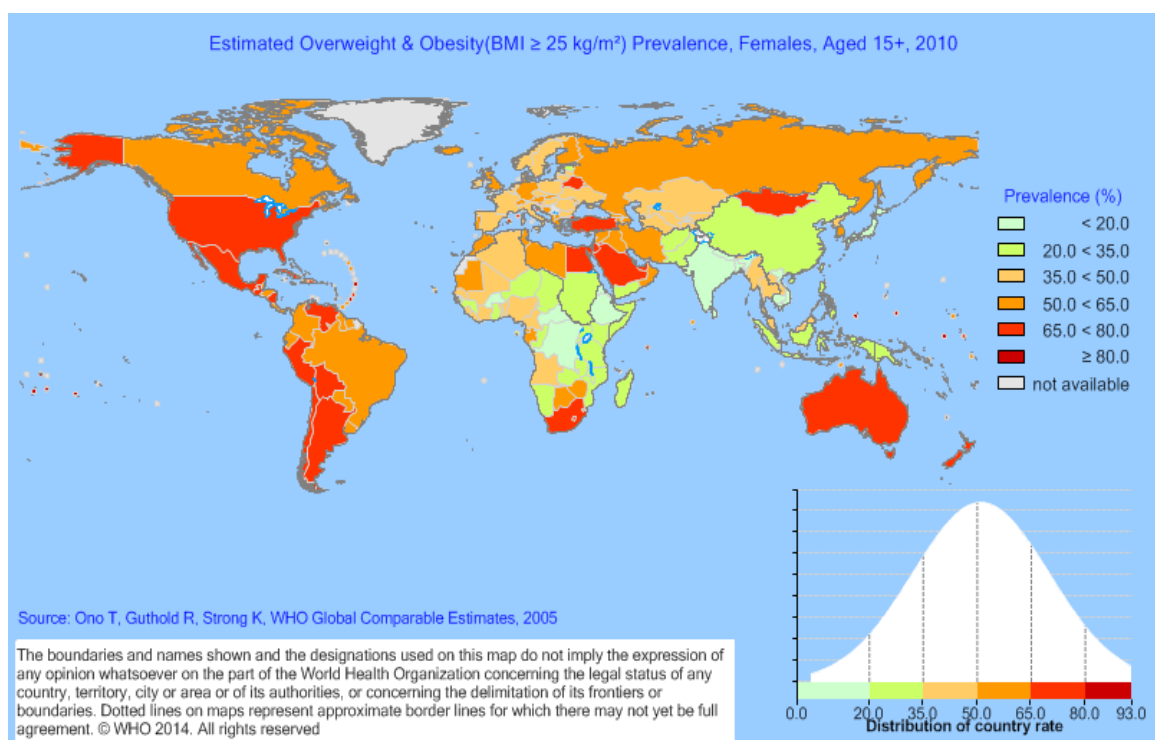


Fig. 2: WHO estimated global overweight and obesity prevalence for females, Aged 15+, 2010.

2.3.1.2 Africa

It is projected by the WHO that about three quarters of the obese population worldwide will be in non-industrialised countries by 2025 (WHO, 2005). There is a rapid ascendency in the prevalence of obesity in sub-Saharan Africa, especially among women (Figs. 3 and 4). The prevalence of obesity in West Africa rapidly increased during the last two decades of the 20th century and continues to increase in the 21st century. A review of obesity prevalence in West Africa indicated that between 2000 and 2004, 10% of West African adults were obese. According to the same study, half of the urban population in general and 60% of urban, West African women were either overweight or obese (Abubakari *et al.*, 2008).

A Demographic and Health Survey data review from seven African countries by Ziraba, *et al.* (2009) showed that the prevalence of urban overweight and obesity increased by nearly 35% during the period, with an estimated annual increase of about 5%. They also found that overweight/obesity did not differ significantly between the poor and the rich, but was significantly higher among non-educated women than educated women. The prevalence of overweight and obesity in urban sub-Saharan Africa was found to range between 23% in Malawi to 35% in Niger and Ghana, and 38% in Kenya.

Another study conducted in two North African countries, Morocco and Tunisia, also gave similar findings. Half of the women were overweight or obese (BMI > 25) with 51.3% in Morocco and 50.9% in Tunisia (Mokhtar *et al.*, 2001). The study also showed significantly higher rates of obesity among women than men in both countries (18% versus 5.7% in Morocco and 22.7% versus 6.7% in Tunisia).

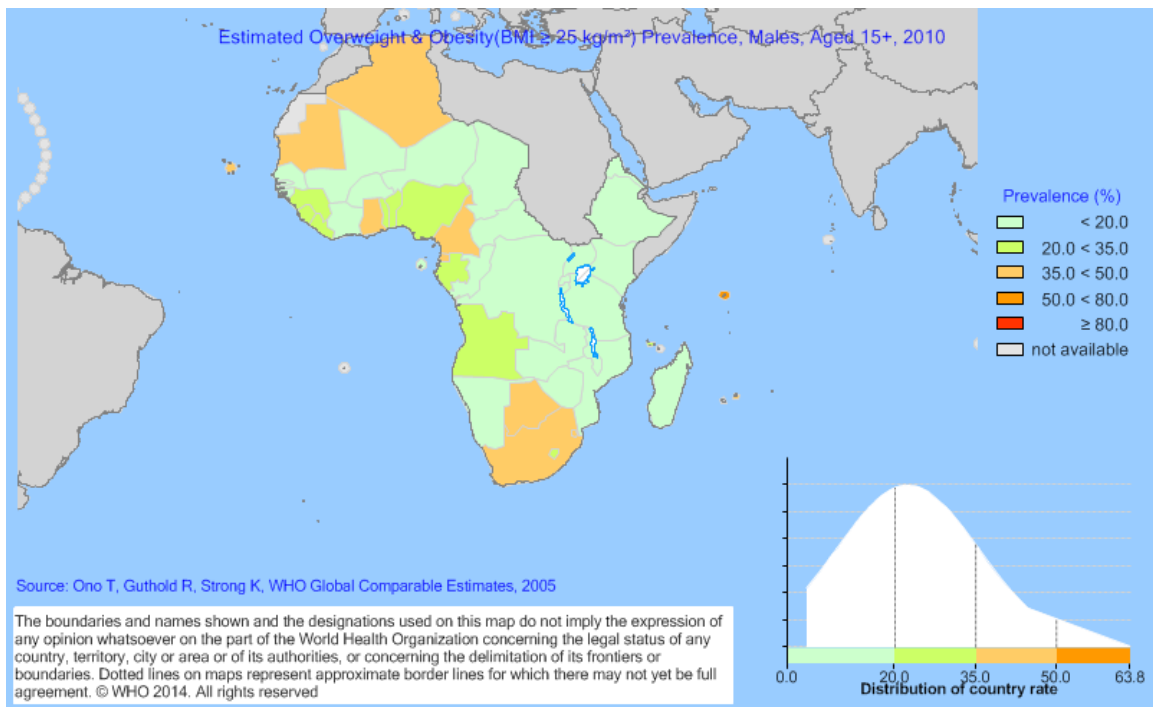


Fig. 3: WHO estimated African overweight and obesity prevalence for males, Aged 15+, 2010

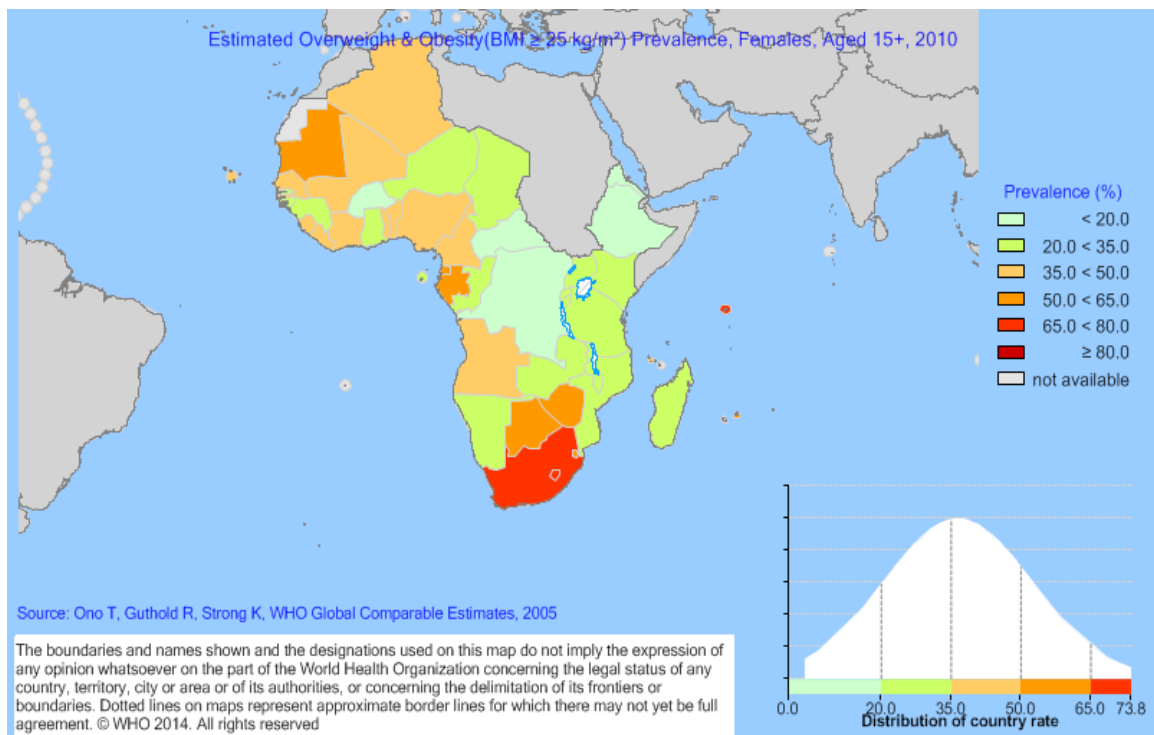


Fig. 4: WHO estimated African overweight and obesity prevalence for females, Aged 15+, 2010

Overweight and obesity was found to be prevalent among women in urban areas and lower education levels (Puoane *et al.*, 2002; Ziraba *et al.*, 2009). Puoane *et al.*, (2002) conducted studies in South Africa which divulged the prevalence of overweight and obesity to be higher among women (56.5%) than in men (29.2%). They also found that the risk of obesity increased with age.

2.1.3.3 Ghana

In Ghana, the Demographic and Health Surveys (DHS) indicate that the percentage of women aged 15-49 overweight or obese grew from 25% to 30% between 2003 and 2008 with the highest values among urban women (GSS, GHS, and ICF Macro; 2009). Wave 1 of the Women's Health Study of Accra (WHSA-1) found 62.2% of 1,237 non-pregnant women living in Accra were overweight or obese; the same study found no relationship between obesity prevalence and socio-economic status (Duda *et al.*, 2007). Amoah, (2003) conducted a similar study in Ghana, which buttressed the fact that overweight and obesity actually were higher among women (47.3%) than men (21.1%), with a ratio of more than 2:1 respectively in the capital, Accra. The Kumasi Metropolitan Health Profile Report (2008) provided a startling revelation in the prevalence of obesity among adult residents in Kumasi. In that report, about three times more women (26%) were obese compared to the (8%) men. The national prevalence of obesity was 5.5% as at 2005 (Biritwum *et al.*, 2005)

2.1.4 Risk Factors

2.1.4.1 Modifiable risk factors

2.1.4.1.1 Environmental factors

On the global level, the marked increase in obesity which accompanies urbanization and economic development provides strong evidence that environmental factors per se play a

strong part in the aetiology of obesity or unleash a latent genetic predisposition to obesity (Prentice *et al.*, 2005). The environment has changed dramatically during the last century and the increased availability of highly palatable energy dense foods as well as a lower physical activity levels are thought to play a major role in the development of obesity (Prentice & Jebb, 1995; 2003).

It is clear that the modern urban environment promotes consumption through the accessibility, affordability and advertising of food, while simultaneously decreasing energy needs through increasing reliance on vehicles, labour-saving devices in the home and garden and a decline in manual occupation (Thomas & Bishop 2007). Several factors such as finance and the availability of sweets and snacks, tend to affect food intake. Some people consume more during situations of intense exercise or pregnancy and are unable to return to previous eating patterns. It should be noted that the increase in obesity is usually related to the type of food consumed such as food containing sugar and fats. According to Kumar and Clark (2009), obese patients eat more than they admit, and over the years a very small daily excess of intake over expenditure can lead to a large accumulation of fat.

2.1.4.1.2 Behavioural factors

Our diet and physical activity patterns are the source of day to day variations in energy balance. The state of positive energy balance that started the obesity epidemic must have resulted from changes in behaviour. Experts debate about the extent to which changes in diet versus changes in physical activity led to the obesity epidemic (Hill, 2006). Overweight and obesity are well understood to be the result of an energy imbalance; consuming more calories than are equivalently expended in physical activity.

Two popularly cited theories for the West African change in energy balance include increases in urbanization and westernization. In theory, urbanization and westernization lead to decreased physical activity and increased food supply, including access to high caloric fast foods and sugar sweetened beverages (Duda *et al.*, 2007). Although there is some indication that energy intake has increased and physical activity has declined over the past few decades, it is surprisingly difficult to accurately quantify these changes (Heitmann *et al.*, 2000). Increased time spent in sedentary behaviours and decreased time spent in moderate-to-vigorous-intensity physical activity have been reported to be independently associated with the risk of metabolic syndrome and its components (Bertrais *et al.*, 2005; Ford *et al.*, 2005; Dunstan *et al.*, 2005, 2007; Healy *et al.*, 2006; Li *et al.*, 2007). Furthermore, it is difficult to separate cause from effect between energy intake and obesity. For example, energy intake in the NHANES surveys aligns well with body weight.

The jump in body weight and obesity seen from NHANES II to NHANES III was mirrored by a jump in average energy intake (Briefel *et al.*, 1995). Most experts speculate that technological changes have reduced lifestyle physical activity (Hill & Peters, 1998). One of the most controversial areas of obesity research is the role of diet composition on body weight. Flatt, (1988) provided a theoretical basis for how diet composition could impact body weight regulation. He argued that achieving energy balance is largely a matter of achieving substrate balance and particularly fat balance.

There is little functional capacity for storage of additional protein or carbohydrate in the body, but capacity for fat storage is essentially unlimited. It is important that protein and carbohydrate balance be regulated acutely, and the body developing effective means for oxidizing excesses of these nutrients. Fat balance can be regulated over the long term

because fat can be brought in and out of storage as needed (Hill, 2006). Thus, the way the body re-achieves energy balance after a perturbation (e.g. overfeeding) will involve restoration of protein and carbohydrate balance before fat balance. Nordmann *et al.*, (2006) conducted a meta-analysis of non-energy-restricted, low-carbohydrate diets and concluded that they were at least as effective as low-fat diets over a period of one year.

Diet composition can impact body weight in individuals who are in energy balance.

Astrup *et al.*, (2000) reviewed a large number of studies where diet composition was altered in individuals who were in energy balance and found that body weight is reduced slightly as dietary fat content of the diet is lowered. This is because carbohydrate produces more thermic effect than fat, reducing dietary fat and increasing dietary carbohydrate would also be expected to produce a slight increase in the thermic effect of food (Hill *et al.*, 1993).

Portion size is another factor that can influence total energy intake. Kral *et al.* (2004) demonstrated that energy intake increases as the portion size of the food offered increases. Other factors, such as increased variety of food, low cost, and accessibility also may increase energy intake (Woods *et al.*, 2000).

2.1.4.2 Non modifiable risk factors

2.1.4.2.1 Genetic factors

Body weight is established by a combination of genetic and environmental factors related to lifestyle (diet, physical activity or sedentary lifestyle), as well as by interactions between those factors. Therefore, obesity appears as a consequence of a positive energy balance involving any alteration in one or several of these factors (Razquin *et al.*, 2011; Ordovaz *et al.*, 2011).

There are a number of ways in which genetic factors may influence the development of obesity (Thomas *et al.*, 2007) and an individual's response to 'obesogenic' environments is largely determined by genetic susceptibility (Podolsky *et al.*, 2007).

Halaas *et al.* (1995) accentuated that hereditary component of bodyweight regulation was studied for the first time in the first decades of the twentieth century, but there were no objective and consistent data about specific genes involved in obesity development and onset. In 1994, the discovery of *ob* gene and leptin, contributed to promoting a momentous progress in the knowledge and understanding on the genetic component of body weight regulation.

Family and twin studies showed that genetic factors contribute 40–70% to the inter individual variation in common obesity (Maes *et al.*, 1997). Furthermore, Hinney *et al.*, (2010) suggested that about 50- 80 % of a population BMI variation could be due to genetic factors as revealed from their twin, families and adoption studies.

Many hormonal and neural factors involved in weight regulation are determined genetically. These include the short and long term signals that determine satiety and feeding activity. Small defects in their expression or interaction could contribute significantly to weight gain (Lenard *et al.*, 2009).

The number of fat cells, regional distribution of body fat, and resting metabolic rate (RMR) are also influenced by genes. Studies of twins confirm that genes determine 50% to 70% of the predisposition to obesity (Prentice *et al.*, 2005). Although numerous genes are involved, several have received much attention such as the *ob* gene which produces leptin, the adiponectin (ADIPOQ) gene, the FTO gene, and the B3-adrenoreceptor gene.

Mutations in the *ob* gene, leptin receptor (LEPR), or ADIPOQ genes can result in obesity or metabolic syndrome (MetS), especially if the diet provides too much saturated fat (Ferguson *et al.*, 2010). The B3-adrenoreceptor gene, located primarily in the adipose tissue, is thought to regulate resting metabolic rate (RMR) and fat oxidation in humans. The FTO gene predisposes to diabetes by its effect on body mass (Frayling *et al.*, 2007).

In laboratory animals, a number of monogenic disorders are associated with severe obesity. A number of human obesity syndromes characterized by similar gene defects, including defects in the *ob* gene, resulting in congenital leptin deficiency, mutations in the leptin (LEP/LEPR) and melanocortin (MC4) receptor genes, and defective pro-opiomelaninocortin (POMC) processing has been reported (Farooqi and O'Rahilly 2005). In humans, each of these syndromes is associated with marked hyperphagia and severe obesity, although in animal models there may be additional effects on energy expenditure.

A much greater proportion of the population is likely to exhibit polymorphisms in other genes which have a significant but less dramatic effect on body weight (e.g. mutations in uncoupling proteins, the B3-adrenergic receptor (ADBR) or peroxisome proliferator activated receptor gamma (PPAR γ). These genes may influence a wide range of metabolic and behavioural characteristics that together determine an individual's susceptibility to obesity (Thomas & Bishop 2007).

2.2 GENETIC AETIOLOGY OF OBESITY

Obesity as a result of genetic causes has been grouped into two major categories namely monogenic and polygenic obesity due to their varied causal association pathways (Farooqi and O’Rahilly, 2005; Hinney & Hebebrand, 2008). Recently, Herrera, & Lindgren, (2010) also indicate that there are three commonly classified subgroups depending on suspected aetiology: monogenic obesity (extremely severe obesity in the absence of developmental delays), syndromic obesity (clinically obese subjects additionally distinguished by mental retardation, dysmorphic features, and organ-specific developmental abnormalities), and polygenic or common obesity, which affects the general population (but may have associated health risks, such as increased risk of CVD).

2.2.1 Monogenic Obesity

Present data indicates that, monogenic obesity due to a single chromosome’ polymorphism is responsible for almost 5% of the entire obesity cases. It has been proven that some genetic variations in several genes that codify for proteins implicated in appetite regulation and satiety are responsible for pathologic changes leading to the phenotypic manifestations of obesity (Farooqi and O’Rahilly, 2005). Up to now, about 11 genes have been associated to this type of obesity (Fig. 5).

Among these genetic variants, the LEP, LEPR, POMC, MC4R and PCSK1 are especially important, since they account for the 5% of severe obesity cases of early development in children (Farooqi and O’Rahilly, 2005; Moleres *et al.*, 2013).

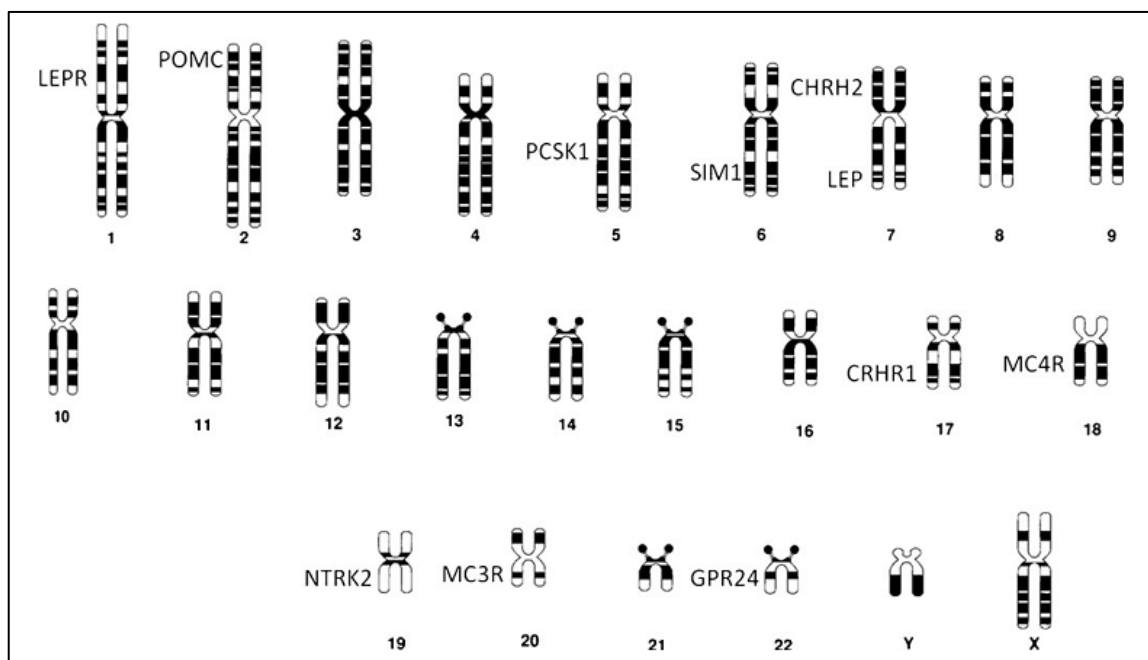


Fig. 5: Genetic map for monogenic obesity genes and their distribution range throughout chromosome (Moleres *et al.*, 2012).

2.2.2 Polygenic Obesity

Polygenic, or common, obesity arises when an individual's genetic makeup is susceptible to an environment that promotes energy consumption over energy expenditure. It is determined by the presence of genes of enough prevalence but with a relatively low effect (Perusse *et al.*, 2005).

Most Westernized societies have an environment that favours weight gain rather than loss because of food abundance and lack of physical activity, thus positioning common obesity as a major epidemic currently challenging these societies (Mutch and Cle'ment, 2006). The last 2005 update of the Human Obesity Gene Map (Rankinen *et al.*, 2006) describes more than a hundred obesity candidate genes associated with polygenic obesity (Moleres *et al.*, 2013).

In relation to polygenic obesity studies, two main approaches have been applied namely, candidate gene studies and genome-wide association studies (GWAS). Genome-wide association study is now the more frequent tool used for the discovery of new genetic variants associated to obesity. In addition to these two approaches, a new tool, copy number variations (CNVs), a structural alteration of DNA that results in an abnormal number of copies of one or more sections of the DNA as well as epigenetic processes and nutrigenetics have been developed as new strategies to search for obesity causes (Moleres *et al.*, 2013).

2.3 CANDIDATE GENES STUDIES

Candidate obesity gene approach analyses genes involved in key metabolic pathways or those that have been shown to be important for obesity development in animal studies (Hinney *et al.*, 2010). A candidate gene is defined as that part of the deoxyribonucleic acid (DNA) molecule that directs the synthesis of a specific polypeptide chain closely associated with a particular disease (Kopelman, 2000).

The search for obesity genes requires a multifaceted approach that involves studies of potential candidate genes derived from animal models, human obesity syndromes and a genome-wide search using microsatellites covering the human genome. Candidate genes for obesity can be chosen for their possible effects on body fat composition, anatomical distribution of fat, food intake and energy expenditure (Kopelman, 2000).

Loos (2009) also outlined a detailed description of candidate genes studies as a hypothesis-driven study which relied on the current understanding of the biology and pathophysiology that underlied the susceptibility to obesity. Genes, for which there is

evidence for a role in regulation of the energy balance in animal models or in extreme/monogenic forms of obesity, are tested for association with obesity-related traits at the population level. In the mid-1990s, when genotyping was still an expensive and tedious task, candidate gene studies typically examined only one or a few variants per gene, with a focus on non-synonymous variants because of their potential functional implications. Recent advancements and publicly available datasets, such as dbSNP and the International HapMap, have provided deeper insight into genetic variation in genes. This has also led to more comprehensive studies that systematically examine the association of all common variation in a gene of interest by means of carefully selected tagSNPs and their haplotypes under the supposition that a causal variant would be in high linkage disequilibrium with one of the tagSNPs or at least captured by the haplotypes.

Nonetheless, a significant limitation of this study approach is the reduced sample size ($n < 1000$) that prevents it from having the necessary power to identify modest genetic effects on obesity. To overcome this limitation, in the last years, studies with a high number of participants and meta-analyses that gather the published information have been carried out and from them, strong associations between obesity and several genetic variants have been found (Vimaleswaran & Loos, 2010).

Since the first candidate gene studies for obesity related traits, more than fifteen years ago, the number of proposed obesity susceptibility genes has grown steadily (Table 1). The latest update of the Human Obesity Gene Map reported one-hundred and twenty seven candidate genes for which at least one study reported a positive association with obesity-related traits (Rankinen *et al.*, 2006).

Table 1: Some genes showing polymorphisms involved in obesity development according to candidate genes studies along with their location and their main function within metabolism

FUNCTION	GENE	LOCATION
Involved in dietary intake^a	INS	11p15.5
	LEP	7q31.3
	MC4R	18q22
	NPY	7p15.3
	POMC	2p23.3
	AGRP	16q22
	CARTPT	5q13.2
	FTO	16q12.2
	LEPR	1p31
Involved in energy expenditure^b	ADBR2	5q31-q32
	ADRB3	8p12
	UCP1	4q28-q31
	UCP2	11q13
	UCP3	11q13.4
	CLOCK	4q12
Adipose tissue growth and development^c	PPARG2	3p25
	CEBPA	19q13.11
	IL6	7p21-p15
	FAB94	8q21.13
	PNPLA3	22q13.31
	PLPIN5	19p13.3

^aLarsen *et al.*, 2012; Molerres *et al.*, 2012

^bOchoa *et al.*, 2006; Galbete *et al.*, 2012

^cRazquin *et al.*, 2009; Corpeleijn *et al.*, 2010).

The rapid expansion reflects the availability of efficient genotyping technologies to a wider scientific community. Nonetheless, replication in subsequent studies for many of the proposed candidate genes has been incoherent, making the overall conclusion on association indistinct. Meta-analyses and the use of large sample sizes ($n > 5000$) has proven robust associations for non-synonymous variants in the melanocortin 4 receptor (MC4R), pro-hormone convertase 1/3 (PCSK1), brain-derived neurotrophic factor (BDNF), and β -adrenergic receptor 3 (ADRB3) genes (Loos, 2009).

2.4 GENE IDENTIFICATION BEFORE THE GENOME-WIDE ASSOCIATION ERA

Although recent success of GWAS has drawn a lot of attention, gene identification in recent years has been based on two broad genetic epidemiological approaches, i.e. candidate gene and genome-wide linkage studies. For over a decade, the scientific community interested in obesity genetics was privileged to have a Human Obesity Gene Map that catalogued all genetic variants and chromosomal loci ever associated with or linked to obesity-related traits (Rankinen *et al.*, 2006). Its first edition was published in 1996, followed by ten yearly updates, the latest in 2006 covering the literature available as of the end of 2005. The map continues to be a useful resource, in print and web-based versions (<http://obesitygene.pbrc.edu>), providing a comprehensive and easily accessible overview of the literature on candidate gene and genome-wide linkage studies published before 2006 (Loos, 2009).

2.5 GENOME-WIDE LINKAGE STUDIES

Genome wide linkage scans exploit familial relationship and rely on use of highly polymorphic markers that spread across the whole genome to pinpoint the location of

genes, followed by calculating the degree of linkage of the marker to a disease trait (Bell *et al.*, 2005).

Genome-wide linkage studies rely on the relatedness of study participants and test whether certain chromosomal regions co-segregate with a disease or trait across generations. A whole-genome linkage survey requires 400–600 highly polymorphic markers, genotyped at 10-cM intervals. Genome-wide linkage studies have a rather coarse resolution and typically identify broad intervals that require follow-up genotyping to pinpoint the genes that underlie the linkage signal. Since the first genome-wide linkage study was published in 1997, the number of chromosomal loci linked to obesity-related traits has grown exponentially. The latest Human Obesity Gene Map update reported 253 loci from 61 genome-wide linkage scans, of which 15 loci have been replicated in at least three studies. Yet none of these replicated loci could be narrowed down sufficiently to pinpoint the genes or variants that underlie the linkage signal (Rankinen *et al* 2006).

Despite substantial power, a meta-analysis of 37 genome-wide linkage studies with data on more than 31,000 individuals from 10,000 families of European origin could not locate a single obesity or BMI locus with convincing evidence (Saunders *et al.*, 2007). This meta-analysis indicates that genome-wide linkage might not be an effective approach for identifying genetic variants for common obesity.

2.6 GENOME WIDE ASSOCIATION STUDIES

Genome Wide Association Studies (GWAS) is one of the most recent, common and useful tool in discovering new genetic variants associated with obesity and its related co-morbidities. In this study method, the genome of a group of people with the trait under study (cases) is compared with another group of people without it (controls) in order to

detect genetic variants associated with this trait. In relation to candidate gene studies, there are major advantages when GWAS is used. These include having a much higher resolution, being more profitable, and allowing for the analyses of larger sample sizes hence providing solid findings (Frayling, 2007).

The fat mass and obesity associated gene (FTO) was the first gene from GWAS to be indisputably related to obesity after being replicated in different populations (Dina *et al.*, 2007; Frayling *et al.*, 2007; Scuteri *et al.*, 2007). Afterwards, more waves of GWAS for body mass index (BMI), with a sample size of about 123,865 individuals, have been performed identifying 32 loci extensively linked with this trait (Willer *et al.*, 2009; Speliotes *et al.*, 2010; Loos, 2012).

The discovery of these new loci is of great importance since it has allowed for new biological pathways involved in the development and onset of obesity to be studied in depth. In the midst of the waves of GWAS for BMI, six loci have been confirmed to have a strong association with this trait. These loci are located in FTO gene, near MC4R, near KCTD15, near NEGR1, near TMEM18 and in SH2B1 gene (Loos, 2012). The identified loci associated with BMI after GWAS are shown in the Table 2.

Despite the evidence that genetic factors play a role in determining changes in adiposity, very few studies have investigated the role of obesity candidate genes. Results from other studies have shown that the ADRB2/ADRB3, LEP/LEPR, NOS3 and PPAR γ genes are associated with adiposity changes over time (van Rossum *et al.*, 2002).

Table 2: Nearest genes to loci discovered throughout Genome Wide Association Studies (GWAS), and BMI increase (kg/m²) per risk allele in the genotype (Thorleifsson *et al.*, 2009; Willer *et al.*, 2009; Loos, 2012)

Loci	Genome Wide Association Studies (GWAS)	BMI increase (kg/m²) per risk allele
FTO	[19–21]	0.39
Near TMEM18	[19–21]	0.31
NearMC4R	[19–21]	0.23
SH2B1	[19–21]	0.15
Near NEGR1	[19–21]	0.13
Near KCTD15	[19–21]	0.06
Near SEC16B	[19–21]	0.22
BDNF	[19–21]	0.19
Near GNPDA2	[19–21]	0.18
NearETV5	[19–21]	0.14
Near FAIM2	[19–21]	0.12
MTCH2	[19–21]	0.06
SLC39A8	19	0.19
Near GPRC5B	19	0.17
Near PRKD1	19	0.17
QCPTL	19	0.15
Near RBJ	19	0.14
TFAP2B	19	0.13
MAP2K5	19	0.13
NRXN3	19	0.13
Near FLJ35779	19	0.10
Near FANCL	19	0.10
CADM2	19	0.10
Near TMEM160	19	0.09
Near LRP1B	19	0.09
MTIF3	19	0.09
TNN13K	19	0.07
Near ZNF608	19	0.07
Near PTBP2	19	0.06
Near RPL27A	19	0.06
NUDT3	19	0.06

Although much effort has been and continues to be invested in finding genetic polymorphisms associated with obesity itself, much less attention has been paid to finding genetic polymorphisms associated with the development of obesity (Rankinen *et al.*, 2006).

2.7 PEROXISOME PROLIFERATOR ACTIVATED- RECEPTOR GENE AND OBESITY

Obesity is a complex metabolic disorder with strong genetic components (Bouchard *et al.*, 1988). Candidate genes are numerous and may involve both structural and regulatory proteins from various tissues. The development of obesity requires the continuous differentiation of new adipocytes throughout life (Flier, 1995). This process of adipocyte differentiation from pre-adipocytes has been shown to be controlled by members of the peroxisome proliferator activated receptor gamma (PPAR γ), adipocyte determination and differentiation-dependent factor-1 (ADD1)/SREBP1, and CCAAT/enhancer-binding protein (C/EBP) nuclear receptor families (Lin & Lane, 1994; Tontonoz *et al.*, 1994a; Tontonoz *et al.*, 1994b; MacDougald and Lane, 1995; Kim and Spiegelman, 1996).

The PPAR γ gene is located on chromosome 3 (Beamer *et al.*, 1997). There are three types of PPAR due to the alternative splicing of its mRNA into these isoforms. Alternative promoter regions within the PPAR γ gene allow for the formation of three PPAR γ isoforms: PPAR γ 1, γ 2 and γ 3 (Vidal-Puig *et al.*, 1997). Among these isoforms, PPAR γ has been shown to regulate adipocyte differentiation and lipid metabolism through the activation of adipocyte- specific genes (Spiegelman & Flier, 1996). It is also secreted in adipose tissue and controls adipocyte differentiation (Tontonoz *et al.*, 1994; Forman *et al.*, 1995; Kliewer *et al.*, 1995) and regulates lipid and glucose homeostasis (Auwerx *et al.*, 1996; Spiegelman & Flier, 1996).

PPAR γ is an important regulator of adipocyte differentiation and a modulator of intracellular insulin-signalling events (Spiegelman, 1998). It also acts through the transactivation of adipose specific genes, including those that encode for proteins involved in lipid storage and metabolism to result in fat cell differentiation (Tontonoz *et al.*, 1994; Tontonoz *et al.*, 1995; Sears *et al.*, 1996).

Forced expression of PPAR γ 2 and C/EBP-A in fibroblasts (Tontonoz *et al.*, 1994a) and myoblasts (Hu *et al.*, 1995) is capable of differentiating these cells into adipocytes. PPAR γ has been shown to have the highest adipogenic activity among the members of the PPAR family (Brun *et al.*, 1996). PPAR γ also controls the transcription of target genes by the formation of a heterodimeric complex with retinoid x receptors, and this complex binds to specific PPAR γ response elements in the promoter regions of these genes (Tontonoz *et al.*, 1994a). While PPAR γ 1 mRNA is found in a wide range of tissues (including heart, liver, skeletal muscle and adipose tissue), PPAR γ 2 is most abundant in adipose tissue). PPAR γ 3 is expressed in macrophages, colon epithelium and adipose tissue (Fajas *et al.*, 1998). PPAR γ 2 contains 30 additional amino acids at its N-terminus, which may confer a greater ligand-independent activation function (augmented by insulin) upon this isoform (Werman *et al.*, 1997). PPAR γ 2 expression is increased in adipose tissue taken from obese subjects compared with lean subjects, and is reduced during weight loss (Vidal-Puig *et al.*, 1997). Results from animal models experimentation and *in vitro* cell culture systems indicate that PPAR γ enhances expression of genes such as lipoprotein lipase (Schoonjans *et al.*, 1996), aP2 (Tontonoz *et al.*, 1994), phosphoenolpyruvate carboxykinase (Tontonoz *et al.*, 1995), and acylCoA synthase (Schoonjans *et al.*, 1995), while it down-regulates expression of the leptin gene (Kallen & Lazar, 1996; De Vos *et al.*, 1996; Zhang *et al.*, 1996; Hollenberg *et al.*, 1997).

Dominant negative PPAR γ mutations are associated with severe insulin resistance, hypertension and alterations in lipid profile (low high-density lipoprotein (LDL), and high triglycerides (Barosso *et al.*, 1999). The Pro12Ala polymorphism of the PPAR γ 2 isoform has been shown to decrease PPAR γ activity (Hara *et al.*, 2000; Masugi *et al.*, 2000) and to be associated with higher BMI (Deeb *et al.*, 1998). In addition, prospective and intervention studies have shown that the Pro12Ala polymorphism could be associated with weight regain after a diet-induced weight loss (Ek *et al.* 1999; Nicklas *et al.*, 2001). Pro115Gln PPAR γ mutation is associated with excessive adipose tissue accumulation through an intrinsic increase in adipogenic activity (Ristow *et al.*, 1998).

2.8 BETA-2/3 ADRENERGIC RECEPTOR GENE AND OBESITY

Adipose tissue forms an integral part of the regulation, storage and mobilization of energy, and has been the focus of the efforts to identify candidate genes for obesity. One such gene is the B3-adrenergic receptor (Emorine *et al.*,1989; Nahmias *et al.*, 1991) which is the main receptor involved in the regulation of thermogenesis and lipolysis in brown and white adipose tissue in rodents (Arch & Kaumann, 1993). In humans, the B3-adrenergic receptor (Emorine *et al.*, 1989) is expressed predominantly in fat and adipocytes lining the gastrointestinal tract (Krief *et al.*, 1993). The receptor's primary role is thought to be the regulation of the resting metabolic rate and lipolysis. (Emorine *et al.*, 1994). The suggested role of the B3-adrenergic receptor in fat metabolism, its functional deficiency in genetically obese mice (Collins *et al.*, 1994; Arbeeny *et al.*, 1994) and the results of studies in which the gene for the receptor has been disrupted in mice (Susulic *et al.*, 1994) has impelled numerous investigations into the function of this gene and its mutated variants such as (Trp64Arg) in patients with morbid obesity.

The B3-adrenergic receptor is expressed in visceral fat in humans (Krief *et al.*, 1993) and is considered responsible for increases in lipolysis and the delivery of free fatty acid into the portal vein (Lonnqvist *et al.*, 1995). An increase in visceral fat mass, in turn, correlates with resistance to insulin in skeletal muscle (Colberg *et al.*, 1995). An abnormality in the B3-adrenergic receptor could therefore explain the link between abdominal obesity and insulin resistance.

2.9 LEPTIN/LEPTIN RECEPTOR GENE AND OBESITY

The ground-breaking discovery of leptin by the year 1994 ignited the field of obesity research in providing the first direct evidence for a hormonal system primarily involved in body weight regulation (Oswal & Yeo, 2010). According to Zhang *et al.* (1994), leptin, is the circulating product of the ob gene, which is secreted by adipose tissue, and the expression of this hormone rises with increasing fat stores in animals and humans (Maffei *et al.*, 1995; Considine *et al.*, 1996).

Considine *et al.* (1996) asserted that the hormone acts as a sensor of fat mass through a negative feedback loop to maintain body fat stores at required levels. Klok *et al.* (2007) did confirm the fact that, the concentration of leptin in circulation is parallel to body fat stores, to such an extent that, a rise in adiposity increases leptin production, hence restraining food intake and vice versa. Accordingly, humans and mice with inherited loss of function mutations of the genes encoding either leptin or its receptor display severe early onset obesity (Pellemounter *et al.*, 1995; White & Tartaglia, 1996; Montague *et al.*, 1997; Clément *et al.*, 1998; Farooqi *et al.*, 2007). Studies have shown that leptin deficiency is usually common among people with monogenic obesity syndromes who do respond well to treatment with recombinant leptin (Farooqi *et al.*, 1999; Licinio *et al.*, 2004; Farooqi and O'Rahilly, 2005).

There are several systemic effects that are attributed to leptin secretion such as body mass control, lipid and glucose metabolism, thermogenesis, angiogenesis, immunity, reproductive, endocrine and cardiovascular functions, among others (Korner *et al.*, 2009). Mutations in the *ob* gene lead to defective/elevated leptin production and obesity in the *ob/ob* mouse (Zhang *et al.*, 1994), but no such mutations had yet been identified in humans (Considine *et al.*, 1996b; Niki *et al.*, 1996; Maffei *et al.*, 1996). However, Maffei *et al.* (1995) and Considine *et al.* (1996) both asserted that most obese humans also have elevated blood leptin levels, possibly implying that defects in the leptin receptor (OB-R) gene contribute to obesity in humans.

The leptin receptor is a single membrane spanning protein with structural similarity to the class 1 cytokine receptor family (Tartaglia *et al.*, 1995; Lee *et al.*, 1996). Though the array of clinical phenotypes of congenital leptin and leptin receptor deficiency is related, the leptin receptor deficiency in humans leads to a less severe phenotype (Farooqi *et al.*, 2007). It is also considered to be less prevailing than leptin deficiency, accounting for up to 3% of all cases of extreme early onset obesity.

The hypothalamic arcuate nucleus (ARC) is the main locus for leptin signalling and leptin resistance (Chen *et al.*, 1996; Mercer *et al.*, 1996; Thornton *et al.*, 1997). Within it are two distinct classes of neurons; one class expressing the POMC (proopiomelanocortin) and cocaine and amphetamine-related transcript peptides which reduce food intake, while the other expresses the NPY (neuropeptide Y) and AgRP (agouti-related protein) peptides which stimulate feeding behaviour (Coll *et al.*, 2007; Coll *et al.*, 2008). Leptin receptors which are highly expressed on the membranes of

these neurons allow leptin to reciprocally regulate their populations (Oswal and Yeo, 2010).

Several varied alternatively spliced isoforms of the receptor (ObR) exist (Lee *et al.*, 1996), with each having a characteristic intracellular domain. The isoforms are either classified as short or long depending upon the length of the intracellular domain. The signaling capacity of the short isoforms (ObRa, ObRb, ObRc, ObRd, ObRe, and ObRf) are limited whereas the long isoform ObRb is believed to be the primary signalling form of the receptor (Lee *et al.*, 1996; Bjorbaek *et al.*, 1997; Fei *et al.*, 1997). The cerebral micro vessels constituting the blood–brain barrier expresses the ObRa and ObRc isoforms at high levels and play key roles in the transport of leptin into the central nervous system (Hileman *et al.*, 2002).

Both diet-induced obese mice and mice lacking the ObR have distinctly impaired leptin transport (Hileman *et al.*, 2002). It is generally assumed that this impairment results from a saturation of the leptin transporter due to the high endogenous leptin levels of these mice. To buttress this argument, the ObR deficient Koletsky rat has shown normalized leptin transport when a perfusion method that negates the effect of serum leptin levels was used. It appears however, that the activity of a separate transporter for leptin is actually modulated by the short isoforms of the receptor, whose identity remains unknown (Banks *et al.*, 2002). The long form of the receptor, ObRb is markedly expressed in the hypothalamus where it regulates the effects of leptin on energy homeostasis (Fei *et al.*, 1997; Elmquist *et al.*, 1998).

The OB-R gene–related protein which controls endocytic internalization, in part regulates the membrane expression of the ObRb (Couturier *et al.*, 2007).

A lack of functional ObRb is responsible for the obesity and metabolic syndrome observed in the db/db mouse model (White & Tartaglia, 1996).

In mice, only the Ob-Rb isoform with a long cytoplasmic tail appears fully functional and is thought to mediate the hypothalamic actions of leptin (Lee *et al.*, 1996). A mutation in the OB-R gene of db/db mice leads to abnormal mRNA splicing that replaces the Ob-Rb with the short Ob-Ra isoform (Lee *et al.*, 1996, Chen *et al.*, 1996), giving rise to abnormal signal transduction (Ghilardi *et al.*, 1996), complete leptin resistance and obesity (Halass *et al.*, 1995; Stephen *et al.*, 1995).

CHAPTER THREE

3.0 METHODOLOGY

3.1 STUDY DESIGN

The research design was a case-control study using a convenient sampling method to select Ghanaian adults at the Dietherapy Unit of the Korle-Bu Teaching Hospital, Accra.

3.2 STUDY SITE

The study was conducted at the Dietherapy Unit, Korle-Bu Teaching Hospital (KBTH), Accra. Korle Bu Teaching Hospital was established on October 9, 1923, as a general hospital to address the health needs of the indigenous people of Accra. Today, it is the third largest hospital in Africa and the leading national referral centre in Ghana and has grown from an initial 200 bed capacity to 2,000, with 17 clinical and diagnostic Departments/Units and a daily average attendance of 1,500 patients (KBTH, 2014).

The Dietherapy Department of KBTH provides essential services to individuals and groups as part of the hospital's multidisciplinary approach to health care delivery. Dietetic consultations start from 8 a.m. to 4 p.m., Mondays to Fridays. A patient can walk into the department for dietetic counselling on weight management, or requires a referral from a doctor for dietetic consultation on all other medical issues. Generally, expert consultations are provided by the department in several areas including nutritional screening, assessment and reassessment, nutritional education and counselling, community nutrition outreach programs and activities etc. Evidence-based, patient-centred dietetic counselling services are provided to individuals and groups visiting the department either on in-patient or out-patient basis.

3.3 PARTICIPANTS

Male and female Ghanaian adults who visited the Dietherapy Unit (KBTH) for dietary counselling were recruited as cases. The age range for inclusion was between 18 and 60 years. They were also newly diagnosed subjects, not on any drugs influencing lipid/glucose metabolism and on a normal/usual diet. Selected subjects had refrained from alcohol, and caffeine consumption for a month or more, prior to commencement of the study, and were non-smokers with $BMI \geq 30 \text{ kg/m}^2$, according to WHO classification for overweight and obesity (WHO, 2010).

Controls were mainly recruited from students of the School of Allied Health Sciences, staff of the Dietherapy Unit and hospital management staff of KBTH.

3.4 SAMPLE SIZE CALCULATION

A sample size of 80 was determined based on an absolute precision of 5% and a confidence interval of 95% (Daniel, 1999).

:

$$n = \frac{[z]^2 P[1-P]}{E^2}$$
$$n = \frac{(1.96)^2 \times 0.055 (1-0.055)}{(0.05)^2}$$
$$= 79.9, \text{ approximately } 80 \text{ participants}$$

Where:

n is estimated minimum sample size

E is the allowable margin of error.

z is the critical z score based on the desired level of significance.

P is estimated prevalence of 5.5% for obesity (Biritwum *et al.*, 2005)

However, due to time constraints, finances and logistics, a fraction 2/3 (50-56) of the sample size was used for the research.

3.5 ETHICAL CONSIDERATION

Permission was sought from the Ethics and Protocol Review Committee of the School of Allied Health Sciences (SAHS), College of Health Sciences, University of Ghana before commencement of this study. Authorization to conduct the study was also obtained from the management of Korle-Bu Teaching Hospital (Acting Chief Administrator, Director of Medical Affairs, Head' Public/Occupational Health Unit, and Head' Department of Dietetics) to use their facility as a study site, before data collection was done. Voluntary written informed consent (Appendix II) was also obtained after a detailed explanation of the study to the subjects before recruitment. Patient confidentiality was ensured at all times and participation in this study was absolutely voluntary.

3.6 DATA COLLECTION

3.6.1 Questionnaire Administration

A structured questionnaire (Appendix I) to identify newly diagnosed obese ($BMI \geq 30\text{kg/m}^2$) subjects was administered. The first part of the questionnaire had a detailed consent form which was signed by both the participant and researcher. The second part comprised a checklist for baseline assessment, with four sub-divisions: demography, anthropometry, lifestyle information and diet history.

The lifestyle information dealt with frequency of alcohol consumption, smoking and caffeine use. A diet history of subject's meal pattern, encompassing meal timings, types and quantities was also recorded.

3.6.2 Anthropometric Measurements

Height was measured with a stadiometer (SECA 213, Humberg, Germany) to the nearest millimeter (mm). Weight was measured to the nearest kilogram (kg) at one decimal place with a standardised weighing scale (TANITA BC-533, Tokyo, Japan), blood pressure (mmHg), was taken with Omron MX2 Basic (HEM-742-E2, Kyoto, Japan) sphygmomanometer for three consecutive times while patient was seated and the mean calculated. Body mass index ($\text{weight}/\text{height}^2$) in kg/m^2 was then calculated. Mid upper arm circumference (MUAC) was measured, preferably on the right arm at the midpoint between the tips of the shoulder (acromion process) and elbow (olecranon process), in centimetres using S0145630 MUAC tape (Ramsey, 1991; Taylor *et al.*, 1998; CDC, 1998; Bild *et al.*, 2002; CDC, 2007; UNICEF, 2009).

3.6.3 Waist to Hip Ratio (WHR) Determination

According to the WHO STEPS protocol for measuring waist circumference, the measurement should be made at the approximate midpoint between the lower margin of the last palpable rib and the top of the iliac crest. Taylor *et al.*, (1998) and quite a few protocols from the United States also indicated that waist girth should be measured at the minimum circumference top of or between the iliac crest and the rib cage. However, the recommendation by the United States National Institute of Health (NIH) Multi-Ethnic protocol for the Study of Atherosclerosis (MESA) which stated that, the measurement of waist circumference should be made at the level of the umbilicus, or navel was used. Waist and hip girths in (cm) were measured with an anthropometric tape over light

clothing. The measurement was taken at the end of a normal expiration, when the lungs were at their functional residual capacity. The subjects were made to relax, and took a few deep, natural breaths before the actual measurement was made. This was to minimize the inward pull of the abdominal contents during the waist measurement.

The hip circumference measurement was taken around the widest portion of the buttocks, at the maximum width over the greater trochanters with the use of a stretch-resistant tape that was snugged around the body, but not pulled so tightly, preventing constriction. The tape provided a constant tension throughout its use, reducing differences in tightness and ensuring parallelism to the floor at the level at which the measurement was made.

The subject stood with body erect, arms at the sides, feet positioned close together, and weight evenly distributed across the feet. The waist-to-hip ratio (WHR) was then calculated (CDC, 1998; Taylor *et al.*, 1998; NHLBI, 2000; Bild *et al.*, 2002; CDC, 2007; WHO, 2008).

3.7 MOLECULAR LABORATORY METHODS

3.7.1 DNA Extraction

Participants were provided with 500 ml sachet water and asked to rinse mouth three times; discarding after each rinse to get rid of any food particles. They were then required to fill mouth with water upon the fourth rinse, rubbing the tongue against inner cheeks, palate, and inner lips against teeth, while swigging for about 30 seconds to one minute. Mouth content was spat into a 50ml conical tube, ensuring the 50 ml mark was reached. Samples were placed in a pre-packed ice container and transported to the Department of Clinical Pathology, Noguchi Memorial Institute for Medical Research (NMIMR).

Samples were spun down at 2000 rpm for 20 minutes using table top centrifuge (Tomy LC-200, Digital Biology Co., Tokyo, Japan). The supernatants were carefully poured off and the pellets were then re-suspended in 600 μ l TNES digestion buffer (10 mM Tris-HCl (pH 7.5), 400 mM NaCl, 100 mM EDTA, 0.60% SDS) by pipetting up and down. The re-suspended pellets were separately transferred into a 1.7 ml Eppendorf tubes, the 35 μ l of proteinase K were added to each tube and incubated overnight in a water bath (Memmert WNB 10, Memmert GmbH+ Co. KG, Schwabach, Germany) at 55°C. Samples were retrieved, 200 μ l 5M NaCl was added to each tube and vortexed briefly. The contents were then spun down at 14000rpm for 30 minutes in a microcentrifuge (Hettich Mikro 200, Andreas Hettich GmbH & Co. KG Hettich, Tuttlingen, Germany). The supernatants were transferred into 2ml Eppendorf tubes, and 800ul 100% ethanol added to each tube and rocked gently back and forth.

Deoxyribonucleic acid (DNA) precipitation was visible at this point. Samples were then stored in a -80°C freezer for two hours. They were retrieved, allowed to thaw, and spun down at 14000rpm for 30 minutes. The absolute ethanol was carefully poured off pellet, 500ul 70% ethanol added and spun down again at 14000rpm for five minutes. The 70% ethanol was also poured off after spinning; the tubes were blotted, and air-dried. The formed pellets were finally re-suspended in 500ul TE and stored at -20°C

3.7.2 PCR Amplification

Primers (Table 3) and PCR methods described by Liao *et al.* (2006) for PPAR γ (Tables 4a and 4b), Widén *et al.* (1995) for ADRB3 (Tables 5a and 5b), Gotoda *et al.* (1997) for LEPR (Tables 6a and 6b) were used. The PCR assays and reaction conditions were slightly modified.

Table 3: Sequence details of primers used for the genotyping of the selected candidate genes

Gene and Primer	Primer sequence (5' - 3')	SNP
PPAR-γ		
PPARG2f	GCCAATTCAAGCCCAGTC	Pro12Ala
PPARG2r	GATATGTTTGCAGACAGTGTATCAGTGAAGGAATCGCTTTCCG	
Leptin		
LEPRf	AAACTCAACGACACTCTCCTT	Gln223Arg
LEPRr	TGAACTGACATTAGAGGTGAC	
ADRB3		
BSTNUP	CGCCAATACCGCCAACAC	Trp64Arg
BSTNDOWN	CCACCAGGAGTCCCATCACC	

Table 4a: PPAR- γ PCR reaction mixture

Reagents	Volume per sample (μ l)	Final concentration
Sterile distilled water	9.075	
10X PCR buffer	3.0	1X
10Mm DNTP mix	2	0.2mM each
10 μ M PPARG2f	0.4	0.2 μ M
10 μ M PPARG2r	0.4	0.2 μ M
<i>Taq</i> polymerase (5U/ μ l)	0.125	0.025U
DNA template	5	
Total volume	20	

Table 4b: PPAR- γ PCR PCR cycling condition

Cycle	Temperature	Time	No. of cycles
Initial Denaturation	94°C	5 minutes	1
Denaturation	94°C	30 seconds	
Annealing	51°C	40 seconds	35
Extension	72°C	40 seconds	
Final extension	72°C	7 minutes	1

PCR product size = 270bp

Table 5a: ADRB3 PCR reaction mixture

Reagents	Volume per sample (μl)	Final concentration
Sterile distilled water	9.075	
10X PCR buffer	3.0	1X
10Mm DNTP mix	2	0.2mM each
10 μ M BSTN UP	0.4	0.2 μ M
10 μ M BSTN DOWN	0.4	0.2 μ M
<i>Taq</i> polymerase (5U/ μ l)	0.125	0.025U
DNA template	5	
Total volume	20	

Table 5b: ADRB3 PCR cycling conditions

Cycle	Temperature	Time	No. of cycles
Initial Denaturation	94°C	5 minutes	1
Denaturation	94°C	30 seconds	
Annealing	61°C	30 seconds	35
Extension	72°C	30 seconds	
Final extension	70°C	7 minutes	1

PCR product size = 210bp

Table 6a: Leptin PCR reaction mixture

Reagents	Volume per sample (μ l)	Final concentration
Sterile distilled water	9.075	
10X PCR buffer	3.0	1X
10Mm DNTP mix	2	0.2mM each
10 μ M LEPRf	0.4	0.2 μ M
10 μ M LEPRr	0.4	0.2 μ M
<i>Taq</i> polymerase (5U/ μ l)	0.125	0.025U
DNA template	5	
Total volume	20	

Table 6b: Leptin PCR cycling conditions

Cycle	Temperature	Time	No. of cycles
Initial Denaturation	94°C	5 minutes	1
Denaturation	94°C	30 seconds	
Annealing	50°C	45 seconds	35
Extension	72°C	60 seconds	
Final extension	70°C	7 minutes	1

PCR product size = 80bp

3.7.3 Restriction Fragment Length Polymorphism (RFLP) Analysis

After PCR amplification, the amplified products were subjected to restriction enzyme digestion. Appropriate restriction enzymes were added to the amplified PCR products and the digestions were carried out as recommended by the manufacturers (New England Biolabs, USA). The final reaction volume of 20 μ l contained 5-10 μ l of the amplified products.

For ADRB3, the amplified fragments of 210 bp were digested with *Mva* I (*Bst*NI). The fragment size was judged as the Arg64Arg type if 161 base pairs; the Trp64Trp type if 99 and 62 base pairs; the Trp64Arg type if 161, 99, and 62 base pairs.

For PPAR γ , the amplified fragments of 270 bp were digested with *Bst*UI. A single 270 bp fragment indicates the presence of CC (Pro12Pro) genotype, two fragments of 227 and 43 bp indicate the presence of GG (Ala12Ala) genotype and three fragments of 270, 227 and 43 bp indicate the presence of GC (Pro12Ala) genotype.

For LEPR, the amplified fragments of 80 bp were digested with *Msp*I. A single 80 bp fragment indicates the presence of Gln223Gln genotype, two fragments of 80 and 58 bp indicate the presence of Gln223Arg genotype and a single fragment of 58 bp indicate the presence of Arg223Arg genotype

3.7.4 Agarose Gel Electrophoresis

Following the PCR, 10 μ l of each PCR product was added to 2 μ l of 6 x bromophenol blue loading dye and electrophoresed in 2% agarose gel stained with 0.5 μ g/ml ethidium bromide. The gels were prepared and run in 1x TAE buffer at 100V for 30 -45 mins and

were observed and photographed over a UV transilluminator at short wavelength using a Kodak EDAS 290 gel documentation system. The sizes of the PCR products were estimated by comparing with the mobility of a standard 100bp DNA ladder.

Restriction digests were run in 3% agarose gels, following the same procedure.

3.8 STATISTICAL ANALYSIS

Results are presented as mean \pm standard deviation (SD), and categorical variables are expressed as numbers counted. The exact test was used to verify Hardy Weinberg equilibrium of genotype frequencies. Differences in demographic characteristics between cases and controls were tested by chi-square test for categorical data and Student's t-test for numerical data. Odds ratio (OR) and 95% confidence interval (CI) for the association between genotype and BMI, MUAC and WHR was computed. The level of statistical significance was set at $P < .05$. All analyses were performed using IBM SPSS 21.

CHAPTER FOUR

4.0 RESULTS

4.1 SOCIO-DEMOGRAPHIC CHARACTERISTICS OF RESPONDENTS

A total of 56 subjects, 24 cases and 32 controls, were recruited for the study. The average age for the cases was 41.63 ± 11.50 years and that of the controls was 26.42 ± 5.48 years (Table 7). There were more females (71.4%) compared to males (28.5%). There were also more married people among the cases (30.4%) compared to singles (51.8%) within the controls. All the respondents were non-smokers, with the majority (80.3%) not consuming alcohol.

Table 7: Socio-demographic characteristics of respondents

Variable	Cases (%)	Controls (%)	Total
N	24 (42.9)	32 (57.1)	56
Age (years)	41.63±11.50	26.42±5.48	
Sex			
Male	4 (7.1)	12 (21.4)	16
Female	20 (35.7)	20 (35.7)	40
Marital status			
Yes	17 (30.4)	3 (5.4)	20
No	7 (12.5)	29 (51.8)	36
Alcohol consumption			
Yes	5 (8.9)	6 (10.7)	11
No	19 (33.9)	26 (46.4)	45
Smoking			
Yes	0	0	0
No	24 (42.9)	32 (57.1)	56

4.2 ANTHROPOMETRIC INDICES

4.2.1 BMI

There was a statistically significant difference in BMI between cases and the controls ($p < 0.0001$) [Figure 6]. All the cases were in the WHO classification of overweight to obese class 3: eight were within the obese class III range (Table 8). The majority of the controls fell within the normal range: there were five overweight respondents.

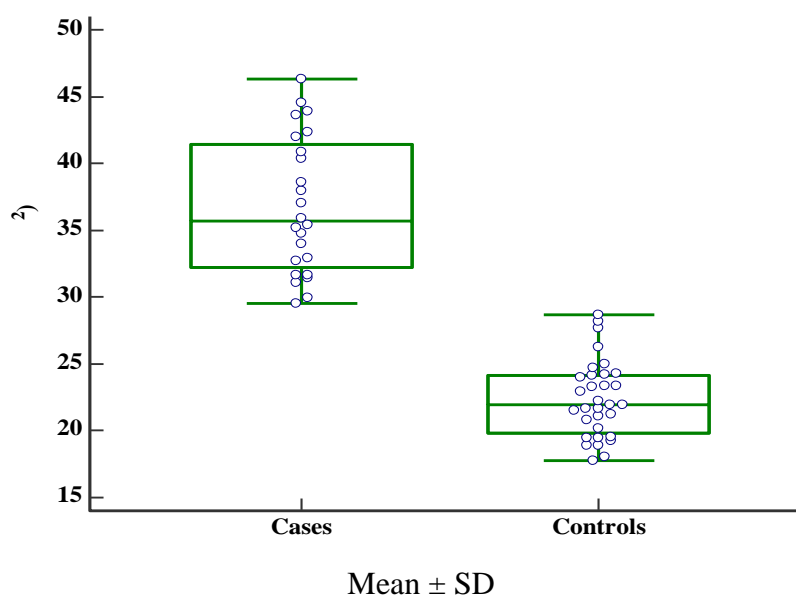


Figure 6. Mean body mass index (BMI) of respondents.

Table 8. Weight classification of respondents

Weight classification*	BMI (kg/m ²)	Subjects	
		Cases (%)	Control (%) s
Underweight	<18.5	0	2 (3.6)
Normal	18.5 - 24.9	0	25 (44.6)
Overweight	25 - 29.9	2 (3.6)	5 (8.9)
Obese Class 1	30 - 34.9	7 (12.5)	0
Obese Class 2	35 - 39.9	7 (12.5)	0
Obese Class 3	≥ 40	8 (14.3)	0
Total		24	32

*WHO 2010.

4.2.2 MUAC

Mid-upper arm circumference (MUAC) measurements (Figure 7) between cases and controls were statistically significant ($p < 0.00001$). All the cases had MUAC measurements and corresponding BMI values which indicated that they were likely to be obese (Table 9). Two controls had MUAC measures < 23.5 cm and corresponding BMI < 20 kg/m².

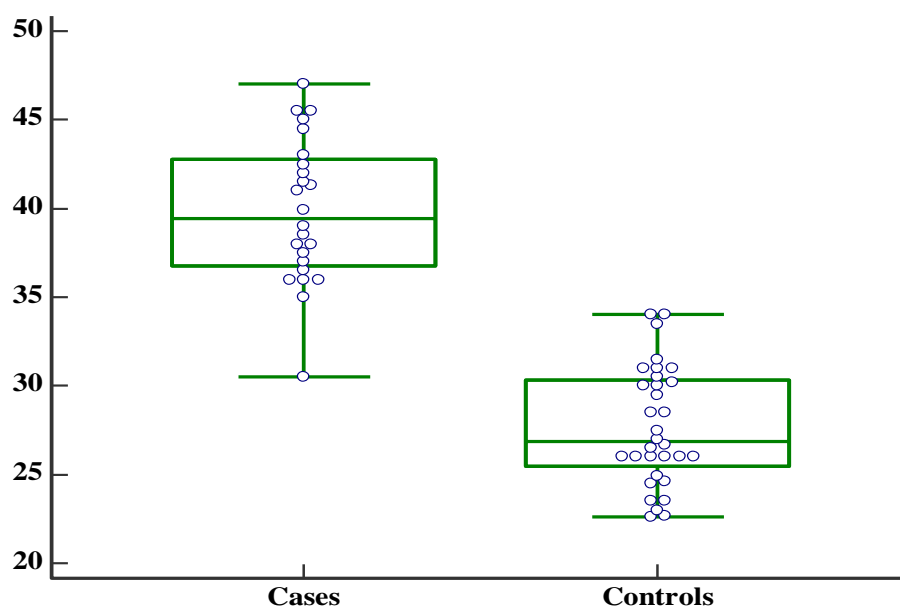


Figure 7: Mean mid-upper arm circumference (MUAC) of respondents.

Table 9. Respondent's MUAC measurements

MUAC	Subjects	
	Cases (%)	Controls (%)
Yes*	23 (95.8)	0 (0.0)
No	1 (4.2)	32 (100.0)
Total	24	32

*MUAC Cut-off: Yes = MUAC is >32.0 cm and BMI is >30 kg/m² i.e. subject is likely to be obese (The Malnutrition Action Group, *British Association of Parenteral and Enteral Nutrition* (BAPEN). 1 (2003); UNICEF/WHO (2009).

4.2.3 WHR

Majority (75.0%) of the cases as compared to the controls (3.1%) were obese based on WHR classifications (Table 10). WHR measurements between cases and controls were also statistically significant ($p < 0.00001$).

Table 10. Waist-to-hip measurements of respondents

Waist to hip ratio	Subjects		Total
	Cases (%)	Control (%) s	
Yes*	18 (75.0)	1 (3.1)	19
No	6 (25.0)	31 (96.9)	37
Total	24	32	56

*Yes: Men (M) = more than 0.90, Women (F) = more 0.85, WHO (1999).

4.3 ENERGY INTAKE

Though, the minimum daily energy for the cases (1956.80 kcal) and controls (1938.40 kcal) was about the same, the maximum intakes (7214.90 kcal for controls and 11207 kcal for cases), differed. However, there was no statistically significant difference ($p = 0.3032$) between the mean energy intakes of the controls and the cases (Figure 8).

4.4 CANDIDATE GENES

4.4.1 PPAR γ gene polymorphism among respondents

The expected 270 bp DNA fragment for the PPAR γ gene (Fig. 9) was amplified in 55 out of the 56 respondents. None of the 55 amplicons was digested with *Bst*UI which indicated the presence of CC (Pro12Pro) genotype

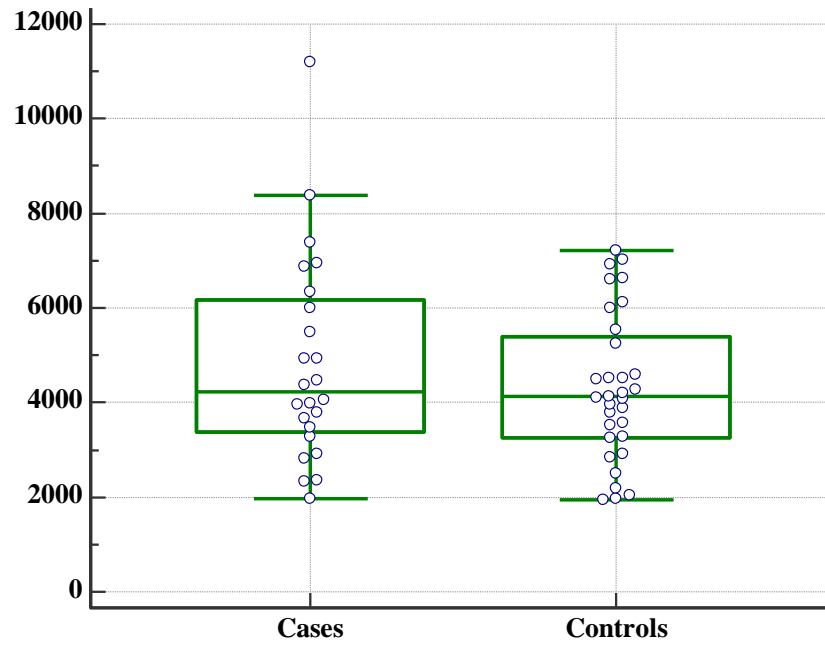


Figure 8: Mean energy intakes of the respondents

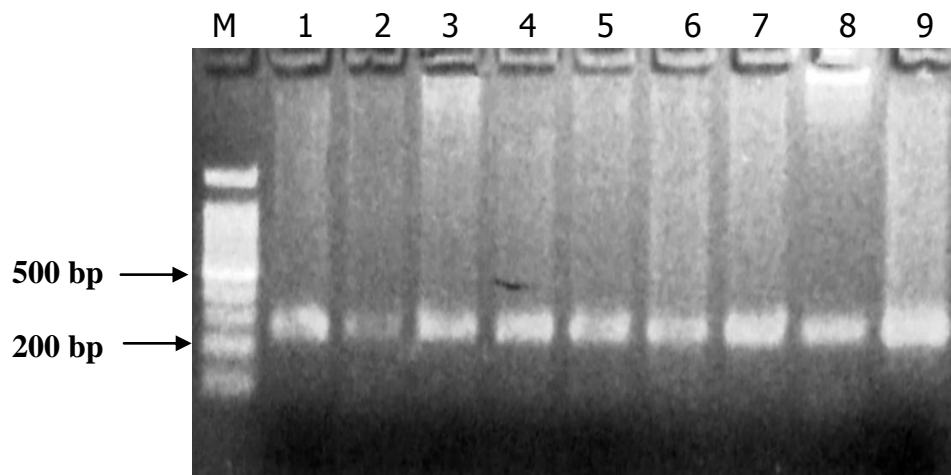


Figure 9. A representative agarose gel electrophoregram of PCR products (band size 270 bp) of PPAR- γ gene. Lane M = 100bp marker; Lanes 1-9 = PCR positives.

4.4.1.1 Relationship between PPAR γ gene polymorphism and BMI

No relationship was observed between PPAR γ gene polymorphism and BMI of the respondents. Only the Pro12Pro genotype, was observed among both the cases and controls.

4.4.1.2 Relationship between PPAR γ gene polymorphism and MUAC

No relationship was observed between PPAR γ gene polymorphism and MUAC of the respondents. Only the Pro12Pro genotype, was observed among both the cases and controls.

4.4.1.3 Relationship between PPAR γ gene polymorphism and WHR

No relationship was observed between PPAR γ gene polymorphism and WHR of the respondents. Only the Pro12Pro genotype, was observed among both the cases and controls.

4.4.2 Leptin receptor gene (LEPR) polymorphism among respondents

Amplification was successful in 29 respondents (15 cases and 14 controls). For each of these, the expected 80 bp fragment of the LEPR gene was seen (Fig. 10). However, only the amplicons of six of the cases out of the 29 respondents were digested with *MspI* (Table 11) which indicated the presence of the Gln223Arg genotype. The genotype frequencies for LEPR Gln223Arg SNP were distributed in compliance with Hardy Weinberg equilibrium ($p > 0.05$).

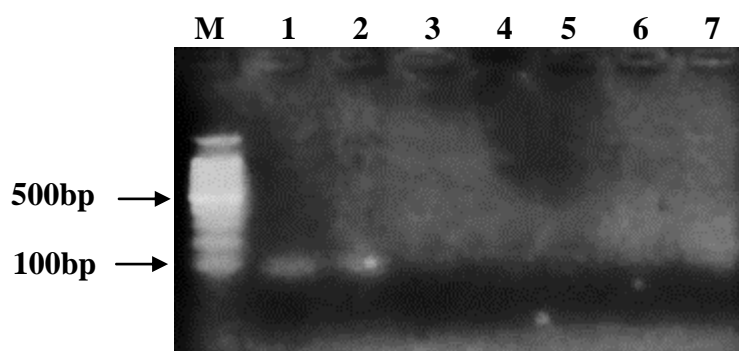


Figure 10. A representative agarose gel electrophoregram of PCR products (band size 80 bp) of LEPR gene. Lane M = 100bp marker; Lanes 1 and 2 = PCR positives; Lanes 3- 7 = PCR negatives.

Table 11: LEPR polymorphism among respondents

LEPR RFLP	Subjects	
	Cases	Controls
Yes (Gln223Arg)	6	0
No (Gln223Gln)	9	14
Total	15	14

4.4.2.1 Relationship between LEPR polymorphism and BMI

The six cases with LEPR SNPs were in the OBClass 1 to OBClass 3 range (Table 12). A significant difference emerged for the LEPR gene Gln223Arg genotype polymorphism frequencies between the cases and the controls ($p = 0.0169$, OR = 19.842, 95%CI = 0.9966 to 395.07).

Table 12: Relationship between LEPR polymorphism and BMI of respondents

LEPR RFLP			Subjects		Total	
			Cases	Controls		
Yes	Body Mass Index (kg/m ²)	OBClass1	2	0	2	
		OBClass2	1	0	1	
		OBClass3	3	0	3	
			Underweight	0	2	2
			Normal	0	10	10
No	Body Mass Index (kg/m ²)	Overweight	1	2	3	
		OBClass1	3	0	3	
		OBClass2	3	0	3	
		OBClass3	2	0	2	
Total			15	14	29	

4.4.2.2 Relationship between LEPR polymorphism and MUAC

Six cases had LEPR SNPs and all six had MUAC measurements >32 cm (Table 13). A significant difference emerged for the LEPR gene Gln223Arg genotype polymorphism frequencies between the cases and the controls ($p = 0.0169$, OR = 19.842, 95%CI = 0.9966 to 395.07).

4.4.2.3 Relationship between LEPR polymorphism and WHR

Six cases had LEPR SNPs and all six had WHR measurements >0.85 cm (Table 14). A significant difference emerged for the LEPR gene Gln223Arg genotype polymorphism frequencies between the cases and the controls ($p = 0.0169$, OR = 19.842, 95%CI = 0.9966 to 395.07).

Table 13: Relationship between LEPR polymorphism and MUAC of respondents

LEPR RFLP			Subjects		Total
			Cases	Controls	
Yes	Mid-upper arm	Yes*	6	0	6
	circumference (cm)	No	0	0	0
No	Mid-upper arm	Yes*	9	0	9
	circumference (cm)	No	0	14	14
Total			15	14	29

*MUAC Cut-offs: Yes = MUAC is >32.0 cm and BMI is >30 kg/m²

Table 14: Relationship between LEPR polymorphism and WHR of respondents

LEPR RFLP			Subjects		Total
			Cases	Controls	
Yes	WHR	Yes*	6	0	10
		No	0	0	5
No	WHR	Yes*	9	0	6
		No	0	14	8
Total			15	14	29

*Yes: Men (M) = more than 0.90, Women (F) = more 0.85, WHO (1999).

4.4.3 ADRB3 genotype polymorphism among respondents

Amplification was successful in 43 respondents (19 cases and 24 controls). In each of these, the expected 210 bp fragment of the ADRB3 gene was seen (Fig. 11). Restriction enzyme digestion of the amplicons with *MvaI* indicated the presence of the Trp64Trp and the Trp64Arg genotypes (Table 15). The genotype frequencies were distributed in compliance with Hardy Weinberg equilibrium ($p > 0.05$).

4.4.3.1 Relationship between ADRB3 genotype polymorphism and BMI

Details of the ADRB3 genotype polymorphism and BMI is shown in table 16. No significant difference emerged for the ADRB3 gene Trp64Arg genotype polymorphism frequencies between the cases and the controls ($p = 0.2088$, OR = 0.3846, 95% CI = 0.1068 to 1.385).

4.4.3.2 Relationship between ADRB3 genotype polymorphism and MUAC

Details of the ADRB3 genotype polymorphism and MUAC is shown in table 17. No significant difference emerged for the ADRB3 gene Trp64Arg genotype polymorphism frequencies between the cases and the controls ($p = 0.2088$, OR = 0.3846, 95% CI = 0.1068 to 1.385).

4.4.3.3 Relationship between ADRB3 genotype polymorphism and WHR

Details of the ADRB3 genotype polymorphism and WHR is shown in table 16. No significant difference emerged for the ADRB3 gene Trp64Arg genotype polymorphism frequencies between the cases and the controls ($p = 0.2088$, OR = 0.3846, 95% CI = 0.1068 to 1.385).

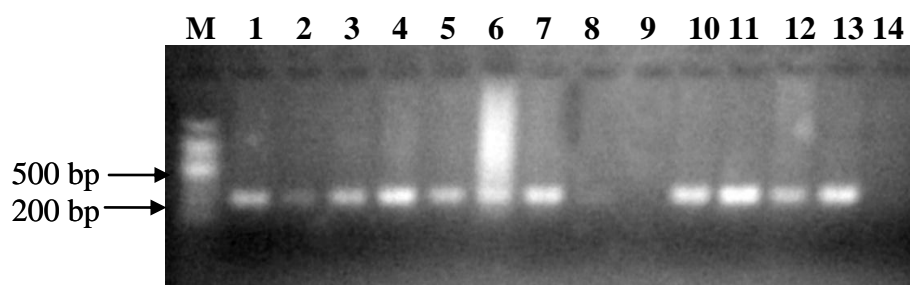


Figure 11. A representative agarose gel electrophoregram of PCR products (band size 210 bp) of the ADRB3 gene. Lane M = 100bp marker; Lanes 1-7, 10-13 = PCR positives; Lanes 8, 9 and 14 = PCR negatives

Table 15: ADRB3 polymorphism among respondents

ADRB3 RFLP		Subjects		Total
		Cases	Controls	
Yes	Trp64Arg	6	12	18
No	Trp64Trp	13	10	23
Total		19	22	41

Table 16: Relationship between ADRB3 polymorphism and BMI of respondents

ADRB3 RFLP			Subjects		Total
			Cases	Controls	
Yes	Body Mass Index (kg/m ²)	Underweight	0	1	1
		Normal	0	9	9
		Overweight	0	2	2
		OBClass1	2	0	2
		OBClass2	3	0	3
		OBClass3	1	0	1
No	Body Mass Index (kg/m ²)	Underweight	0	1	1
		Normal	0	7	7
		Overweight	1	2	3
		OBClass1	6	0	6
		OBClass2	1	0	1
		OBClass3	5	0	5
Total			19	22	41

Table 17. Relationship between ADRB3 polymorphism and MUAC of respondents

ADRB3 RFLP			Subjects		Total
			Cases	Controls	
Yes	Mid-upper arm	Yes*	6	0	6
	circumference (cm)	No	0	12	0
No	Mid-upper arm	Yes*	13	0	13
	circumference (cm)	No	0	10	14
Total			19	22	29

*MUAC Cut-offs: Yes = MUAC is >32.0 cm and BMI is >30 kg/m²

Table 18: Relationship between ADRB3 polymorphism and WHR of respondents

ADRB3 RFLP			Subjects		Total
			Cases	Controls	
Yes	WHR	Yes*	6	0	6
		No	0	12	12
No	WHR	Yes*	13	0	13
		No	0	10	8
Total			19	22	41

*Yes: Men (M) = more than 0.90, Women (F) = more 0.85, WHO (1999).

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSION

This study looked at selected candidate genes (PPAR γ , ADRB3 and LEPR) of obesity, in 24 newly diagnosed obese patients at the Dietherapy Unit (KBTH) and 32 controls.

All the cases studied had BMIs equal to or greater than 30. However, only five out of the controls were overweight but not obese. In comparison with the WHO obesity classification, 25 controls were within the normal limits, while two were underweight. There was a statistically significant difference ($p < 0.0001$) between the BMIs of the cases studied and the controls. This implies a positive association between higher BMIs and obesity. According to Hiza *et al.*, (2000), BMI is one of the most frequently used methods of evaluating body fat percentage. It is also used in an individual's nutritional and health status appraisal. Thus far, BMI is the best available anthropometric estimate of body fatness for public health purposes (Hall & Cole, 2008). Higher BMIs generally mean higher body fat and as BMI increases, particularly from values equal to or greater than 30, health risks increase (Hiza *et al.*, 2000). BMI measures excess body weight for a particular height and has been shown to correlate with body fat although it is not a direct measure of body fat (Nihiser, 2007).

BMI cut points recommended in 1998 by the WHO were the first such cut-offs accepted at the international level (Weisell, 2002). As argued by Ashwell (2011) and Kesavachandran (2012) who provided results from a cross-sectional and prospective epidemiological survey, it was evident that the cut-offs of BMI provided by the World Health Organisation (WHO, 1995), do not effectively reflect the overweight or obesity

status of all populations. A case in point is a higher body fat percentage correlating with lower BMIs among Asians while among Pacific Islanders, higher BMIs tend to be associated with more muscle mass and less body fat (Weisell, 2002). Excluding ethnicity, the universal applicability of BMI has also been raised concerning age, physical activity and most importantly, as a less reliable predictor of cardiovascular risk (Muralidhara, 2008).

Debates regarding the usefulness of BMI in predicting body fat may significantly affect several specialised areas such as risks of coronary heart diseases, diabetes, weight loss programs, population health surveys, epidemiological studies of diet and health, sports and fitness training among others. Despite its drawbacks, BMI remains one of the most widely used tools to screen obesity risk in several target populations as it is simple, inexpensive and non-invasive (Bhurosy & Jeewon, 2013).

Twenty-two (41%) of the cases had MUAC measures greater than 32cm and BMI also greater than 30kgm^{-2} . This was in congruence with the MUAC and BMI cut-off set by The Malnutrition Action Group, from the British Association of Parenteral and Enteral Nutrition, for assessing malnutrition in individuals (The Malnutrition Action Group, 2003).

The use of MUAC is beneficial over BMI and skin-fold measures because it is non-invasive compared to other anthropometric measures. It also requires inexpensive equipment, which does not need to be calibrated or maintained. Moreover, in low-income settings, MUAC proves to be very useful in the absence of inappropriate weighing scales and stadiometer (Haboubi *et al.*, 2010). As proposed by Mazicioglu *et al.* (2010) MUAC closely reflects body fat tissue, it can therefore be used along with BMI to assess obesity

risk. Despite being a relatively easy method, small measurement errors such as incorrect choice of arm, inappropriate positioning of the tape measure at the midpoint between the acromion and the olecranon processes, incorrect horizontal positioning and insufficient compressive force can lead to invalid interpretations (Almeida *et al.*, 2003).

Majority of the cases (75%) had WHRs that were higher than the normal ranges for both men and women. This implies that, they were obese when WHR was used as the index for assessing obesity. According to Dekkers *et al.* (2008), waist circumference (WC) and WHR are measures of abdominal or central obesity. Assessment of central obesity is crucial since it is associated with high risks of cardiovascular and metabolic diseases (Mushtaq *et al.*, 2011).

No SNPs of the Pro12Ala allele for the PPAR γ gene was detected among all the participants involved in this research. According to Deeb *et al.* (1998), the Pro12Ala polymorphism of the PPAR γ 2 isoform has an association with higher BMI. In agreement with this, association studies performed by Beamer *et al.* (1998) in two independent Caucasian populations also linked the Pro12Ala PPAR γ 2 isoform with higher BMI which is suggestive of a susceptibility to obesity in humans. However, all 24 cases in this study did not show this SNP even though they were obese. This may confirm the fact that variations do exist with certain candidate genes of obesity with respect to race, geographical location/country of origin (Bouchard *et al.*, 1988; Malczewska-Malec *et al.* 2004; Podolsky *et al.*, 2007). Perhaps, a larger sample size would help to establish this fact among Ghanaian adults.

Six cases had the Gln223Arg polymorphism for LEPR. Although these cases were obese a strong association of this polymorphism with obesity could not be established as also

indicated by Gotoda *et al.* (1997). Leptin receptor gene, located on chromosome 1p31 (Thompson *et al.*, 1998) and leptin gene have several systemic effects attributed to them such as, body mass control, lipid and glucose metabolism, thermogenesis, angiogenesis, immunity, reproductive, endocrine and cardiovascular functions, among other (Korner *et al.*, 2009). Humans and mice with inherited loss of function mutations of LEP/LEPR display severe early onset obesity (Pellemounter *et al.*, 1995; White & Tartaglia, 1996; Montague *et al.*, 1997; Clément *et al.*, 1998; Farooqi *et al.*, 2007). Studies by Farooqi *et al.* (1999), Farooqi and O'Rahilly (2005), and Licinio *et al.* (2004) have shown that leptin deficiency is usually common among people with monogenic obesity syndromes and treatment with recombinant leptin yields positive outcome.

Adipose tissue forms an integral part of the regulation, storage and mobilization of energy, and has been the focus of the efforts to identify candidate genes for obesity. One such gene is the B3-adrenergic receptor (ADRB3) (Emorine *et al.*, 1989; Nahmias *et al.*, 1991) which is the main receptor involved in the regulation of thermogenesis and lipolysis in brown and white adipose tissue in rodents (Arch & Kaumann, 1993). In humans, ADRB3 receptor (Emorine *et al.*, 1989) is expressed predominantly in fat and adipocytes lining the gastrointestinal tract (Krief *et al.*, 1993). The receptor's primary role is thought to be the regulation of the resting metabolic rate and lipolysis (Emorine *et al.*, 1994).

SNPs were identified for the Trp64Arg allele for the ADRB3 gene. Six cases and 12 controls had the Trp64Arg polymorphism. No significant difference (all $ps > 0.05$) emerged for the ADRB3 gene Trp64Arg genotype polymorphism frequencies between the cases and the controls for BMI, MUAC and WHR and thus an association of this polymorphism with obesity could not be established.

The suggested role of the B3-adrenergic receptor in fat metabolism, its functional deficiency in genetically obese mice (Collins *et al.*, 1994; Arbeeny *et al.*, 1994) and the results of studies in which the gene for the receptor has been disrupted in mice (Susulic *et al.*, 1994) has impelled numerous investigations into the function of this gene and its mutated variants such as (Trp64Arg) in patients with morbid obesity. The B3-adrenergic receptor is expressed in visceral fat in humans (Krief *et al.*, 1993) and is considered responsible for increases in lipolysis and the delivery of free fatty acid into the portal vein (Lonnqvist *et al.*, 1995). An increase in visceral fat mass, in turn, correlates with resistance to insulin in skeletal muscle (Colberg *et al.*, 1995). The Trp64Arg allele of the B-3 Adrenergic receptor is associated with abdominal obesity and resistance to insulin and may contribute to the early onset of non-insulin dependent diabetes mellitus (Widén *et al.*, 1995).

Energy intakes for all the subjects were above the WHO/FAO (2002) recommendations. This alone may not be responsible for the weight gain in especially the cases. Long-term increases in energy intake without a corresponding increase in energy expenditure results in weight gain due to accumulation of excess body fat (Bray, 1987). It is possible that all the 56 subjects consumed significantly more carbohydrates than normal. There was no statistically significant difference in energy intake of both cases and control, though they all consumed a minimum of 2000 kcals, the cases had a higher upper limit above 8000 kcal, compared to 7000 kcal for the controls. Some studies also found no relationship between total dietary energy, dietary fat and overweight/obesity (Prentice & Jebb, 1995; Willet, 1998; Goran *et al.*, 1998; Treuth *et al.*, 1998). In contrast, other studies reported an association between dietary energy, dietary fat and weight gain (Bray & Popkin, 1998; Lanza *et al.*, 2001; Mokhtar *et al.*, 2001; Bes-Rastrollo *et al.*, 2008). The conflicting

reports could be linked to differences in research design and bias associated with self-reported food intake. High carbohydrate intake seems to affect the high prevalence of overweight/obesity among the subjects in this study. In a prospective longitudinal study, Bes-Rastrollo *et al.* (2008) found that dietary energy density was positively correlated with dietary carbohydrate. The authors also found a significantly greater weight gain among women who increased their dietary energy density during an 8-year time period than those who decreased their dietary energy density.

Food choices and preferences which also affect energy intakes in the long term are also influenced by an individual's sociocultural background and environmental factors. Preference for the traditional diets which are high in carbohydrates could explain the higher intake of carbohydrates, hence increased energy intake. The "westernized" dietary pattern which has become a common practice in urban Ghanaian cities could also account for the high energy intakes. A higher carbohydrate intake and more sedentary behaviour could explain the high rate of overweight/obesity among the cases.

The dietary pattern of the subjects in this study could reflect the impact of urbanisation, westernisation, increasing affluence and food market globalisation as has been observed in urban societies of some developing countries (Bourne *et al.*, 1993; Monteiro *et al.*, 2000; Popkin, 2001; Popkin & Gordon-Larsen, 2004; Prentice, 2006). Increasing affluence is usually followed by increased food accessibility and intake of larger food portions. Urbanisation, westernisation and food market globalisation has also led to the prevalence of fast food chains and energy dense exotic soft drinks on the Ghanaian market, especially in urban communities like Accra, Takoradi and Kumasi. The nutritional outcome is a shift from intake of traditional diets high in complex carbohydrates, pulses and low in fats toward an increased consumption of foods high in

fats, simple sugars and total calories. This situation is similar to that reported elsewhere by Bourne *et al.*, (1993) that urbanization led to imprudent dietary behaviour in South African populations. Thus, unhealthy dietary behaviour could impact on the high prevalence of overweight and obesity found among the cases in this study.

In the present study there were some limitations. The small sample size might limit the applications of the potential associations detected. The use of self-reported dietary intakes might have been subjected to under-reporting or over-reporting bias in subject's actual intakes. There were also no locally accepted handy measure estimates for the local soups, stews, and other uniquely Ghanaian dishes consumed. Estimates elsewhere were employed. This might have introduced bias in the caloric estimations, hence over or under –estimating energy intakes.

A conveniently randomized selection of newly diagnosed obese subjects within the stipulated age range was carried out, although a direct age matching for cases and controls, was not considered. This might have potentially influenced the weight distribution data for the various ages within the cases and controls.

5.2 CONCLUSION

There were statistically significant differences in BMI, WHR and MUAC measurements between cases and controls (all $p < 0.0001$). SNPS in the receptors of the ADRB3 and LEPR, but not PPAR γ genes, were detected in the subjects for the study. No relationships were observed between ADRB3 (Try64Arg) and PPAR γ (Pro12pro) genotype polymorphisms and BMI, MUAC and WHR of the subjects. However, significant differences emerged for the LEPR gene Gln223Arg genotype polymorphism frequencies between the cases and the controls ($p = 0.0169$, OR = 19.842, 95%CI = 0.9966 to 395.07)

and BMI, MUAC and WHR. Energy intakes for all the subjects were above the WHO/FAO recommendations. However, there was no statistically significant difference ($p = 0.3032$) between the mean energy intakes of the controls and the cases.

It is recommended that:

1. A larger sample size should be considered during further studies in this area in order to establish any potential associations between the selected candidate genes and obesity in the Ghanaian population.
2. Direct age matching for cases and controls, in addition to the other inclusion criteria used, should be considered for improved correlations during further studies.
3. Studies to establish accepted protocols for estimating dietary intakes other than self-reporting, and handy measures for local soups, stews, and other uniquely Ghanaian dishes consumed should be considered by the department of dietetics. This will help improve future localized researches requiring these information to avoid over or under –estimating subject’s energy intakes.

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APPENDIXES

APPENDIX I: INFORMED CONSENT FORM

Name of Researcher: AMOS AGYEI GYAMFI

Name of Institution: School of Allied Health Sciences
College of Health Sciences
University of Ghana.

Name of Supervisors: **DR. CHARLES A. BROWN**
(School of Allied Health Sciences, University of Ghana)

DR. SAMUEL ANTWI-BAFFOUR
(School of Allied Health Sciences, University of Ghana)

Project Title: **SELECTED CANDIDATE GENES AND OBESITY AMONG
GHANAIAN ADULTS: A CASE STUDY AT THE KORLE-
BU TEACHING HOSPITAL (DIETHERAPY UNIT) ACCRA**

Consent

You have been invited to take part in the above titled research. The purpose of which is to investigate the relationship between the selected candidate genes and their role in obesity among Ghanaian adults.

About 5 ml of your venous blood will be drawn for DNA analyses after your blood pressure, weight, height and waist-to-hip ratio have been measured.

Your confidentiality will be assured at all times. All information will be safeguarded. Your data will only be known to the researcher, it will also be kept under an encryption/password protection. Your privacy and anonymity will be ensured in the collection, storage and publication of the research material. Should the information be published in any scientific journal, you will not be identified by name. You have the right to refuse to participate at anytime you wish to.

I understand my participation is totally voluntary and free, and that I am not going to be subjected to any risk, danger or discomfort.

I have been informed that the confidentiality of the information will be safeguarded and that my privacy and anonymity will be ensured in the collection, storage and publication of the research material. I have the right to refuse to participate at anytime I wish to.

I have read the information provided. All questions have been answered to my satisfaction. I consent voluntarily to participate in this study.

Signature of participant:.....

Date:...../...../ 2013

Signature of researcher:.....

Date:...../...../ 2013

QUESTIONNAIRE FOR DATA COLLECTION**SELECTED CANDIDATE GENES AND OBESITY AMONG GHANAIAN ADULTS: A CASE CONTROL STUDY AT THE KORLE-BU TEACHING HOSPITAL (DIETHERAPY UNIT) ACCRA****Demography**

1. Age.....
2. Sex: M F
3. Religion: Christian Moslem Traditional Other
4. Marital Status: S M
5. Occupation.....
6. Location.....

Anthropometry

7. Height.....
8. Weight.....
9. BMI.....
10. BP.....
11. MUAC.....
12. Waist-to-hip ratio.....

Lifestyle Information

13. Smoking: Y N
14. Alcohol consumption Y N

If yes to 14, how often

15. Daily Weekly Monthly Other

16. Caffeine consumption Y N

If yes to 16, from which source

17. Tea bag Coffee Milo Energy drinks Other

How often to question 17,

18. Daily Weekly Monthly Other

19. DIET HISTORY

TIME	TYPE OF FOOD	PORTION SIZE (ESTIMATING FROM HANDY MEASURES)


20. SAMPLE COLLECTION (MOUTH RINSE (SALIVA)/BUCCAL SWAB/ 3ml VENOUS BLOOD)

Has venous blood/ buccal swab /mouth rinse been taken: Y N

APPENDIX II
ETHICAL IDENTIFICATION NUMBER

SCHOOL OF ALLIED HEALTH SCIENCES
COLLEGE OF HEALTH SCIENCES
UNIVERSITY OF GHANA
ACADEMIC AFFAIRS

Phone: +233-0302-687974/5
Fax: +233-0302-688291
My Ref. No. SAHS/ 10231377
Your Ref. No.



P. O .Box KB 143
Korle Bu
Accra
Ghana

20th March, 2013.

Mr. Amos Agyei Gyamfi,
Dept. of Dietetics,
SAHS,
Korle Bu.

Dear Mr. Gyamfi,

ETHICS CLEARANCE

Ethics Identification Number: SAHS – ET. /10231377/AA/1A/2012-2013.

Following a meeting of the Ethics and Protocol Review Committee of the School of Allied Health Sciences held on Friday 1st February, 2013, I write on behalf of the Committee to approve your research proposal as follows:

TITLE OF RESEARCH PROPOSAL: "Selected candidate genes of obesity among Ghanaian adults: A case study at the Dietherapy Unit of the Korle-Bu Teaching Hospital, Accra."

This approval requires that you submit six-monthly review reports of the protocol to the Committee and a final full review to the Committee on completion of the research. The Committee may observe the procedures and records of the research during and after implementation.

Please note that any significant modification of the research must be submitted to the Committee for review and approval before its implementation.

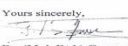
You are required to report all serious adverse events related to this research to the Committee within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this research. You will therefore, be required to furnish the Committee with any manuscript for publication.

Please always quote the ethical identification number in all future correspondence in relation to this protocol.

Thank you.

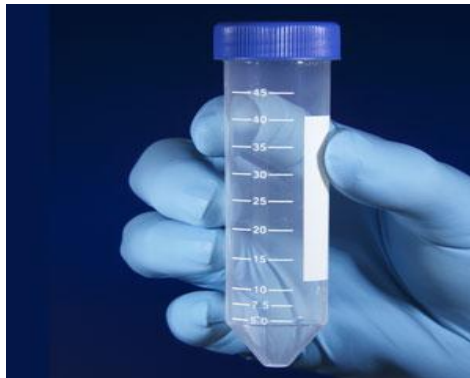
Yours sincerely,



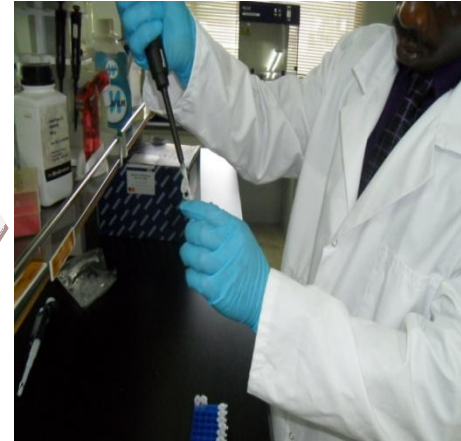
Dr. (Maj. Rtd.) George Asare
(Chairman, Ethics and Protocol Review Committee)

cc Dean
Co-ordinator, Dept. of Dietetics
Senior Assistant Registrar

APPENDIX III
SAMPLE COLLECTION



APPENDIX IV
SAMPLE PREPARATION (DNA EXTRACTION)



(Courtesy Prof. D. T. Burke. Department of Human Genetics, University of Michigan Medical School).