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ADRENOCEPTOR BETA 3 (ADRB3) GENE VARIANTS AND CHILDHOOD OBESITY

BY

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DECLARATION

This is to certify that this thesis is as a result of an independent research undertaken by Elizabeth Oduro under the supervision of Dr Charles Brown and Dr Matilda Asante towards the award of Master of Science Degree in Dietetics at the Department of Nutrition and Dietetics, School of Biomedical and Allied Health Sciences, College of Health Sciences, University of Ghana.

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ABSTRACT

Background: Childhood obesity is becoming very common worldwide and similar trends are being seen in Ghana. Various researches have shown that several genes are linked to obesity. Several genes including adrenoceptor beta 3 (ADRB3) gene have been implicated. ADRB3, is mainly expressed in adipose tissue, and contributes to variations in energy expenditure and body fat distribution. Polymorphisms of the ADRB3 have been suggested to participate in the pathogenesis of obesity. There is no information on the association between ADRB3 genotype polymorphisms and obesity in children in Ghana.

Aim: This study was aimed at investigating the role of ADRB3 gene variants in obesity in children in Ghana.

Methods: Sixty eight boys and girls selected from two primary schools, St Mark Basic School (Atomic) and GAEC Basic School (Atomic), were used in the study. A food frequency questionnaire was used to assess dietary patterns. Buccal rinse samples were collected from the children after assessment of waist-to-hip ratio (WHR), weight, height and body mass index (BMI) calculated. DNA was extracted from the buccal rinse samples and ADRB3 gene variants analyzed by polymerase chain reaction and restriction fragment length polymorphism. Correlations between ADRB3 gene variants and obesity in the children were determined.

Results: A total of 68 subjects, 39 cases (obese) and 29 controls (non-obese), were recruited for the study. There were more females (55.9%) compared to males (44.1%). The average age for the cases was 11.92 ±1.69 years and that of the controls was 13.17 ± 1.77 years. BMI values of the children were statistically significant different (all *ps*< 0.0001) between cases and the controls for both males and females. WHR measurements between cases and controls for female children were also statistically significant (*p* =

0.0039). Amplification was successful in 58 children (35 cases and 23 controls). A significant difference emerged for the ADRB3 gene Try64Arg genotype polymorphism frequencies between the cases and the controls (p = 0.0377, OR = 0.1471, 95% CI = 0.02666 to 0.8113). No significant differences (all ps > 0.05) emerged between the ADRB3 gene Try64Arg genotype polymorphism frequencies and BMI and WHR. More males consume fruits and vegetables daily compared to females. Females compared to their male counterparts frequently consume sweet drinks, fatty meat and fast foods.

Conclusions: No relationships were observed between ADRB3 genotype polymorphisms and obesity among the school children.

DEDICATION

I thank God for the grace and favour that kept me to the end. This dissertation is dedicated to Dr Charles Brown.

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I am most grateful to my supervisors Dr Charles Brown and Dr Matilda Asante for the guidance, patience and dedication to ensure this project is a success. God richly bless you.

I would like to appreciate Mr Prince Oduro, my parents and siblings for all you did for me. Prince Pappoe, Mr & Mrs Sowah and Mr & Mrs Opoku, you are all part of the success story. I appreciate you all and I love you loads.

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LIST OF ABBREVIATIONS

ADRB3	Adrenergic Beta 3 Receptor
BMI	Body Mass Index
cAMP	Cyclic Adenosine Monophosphate
CDC	Centre for Disease Control
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
GSS	Ghana School Survey
GWAS	Genome Wide Association Studies
IOTF	International Obesity Task Force
LEPR	Leptin Receptor
mRNA	messenger RNA
NCMP	National Child Measurement Programme
NHANES	National Health and Nutrition Examination Survey
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, NaCl, EDTA, and SDS
USDHHS	U.S. Department of Health and Human Services
WHO	World Health Organization
WHR	Waist to Hip Ratio

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Overweight and obesity are defined as abnormal or excessive fat accumulation that may impair health. Worldwide obesity has more than doubled since 1980 (WHO, 2015). According to the WHO, overweight and obesity are considered the fifth leading risk for global deaths (WHO, 2015). Overweight and obesity are independent risk factors for increased morbidity and mortality throughout the lifecycle. For example, overweight and obesity in women are predictors of gestational diabetes during pregnancy and new-borns with excessive birth weight (Institute of Medicine, National Academy of Sciences, 1990). High birth weight is a predictor of overweight and obesity in adulthood and in cofactors associated with insulin resistance (Curhan *et al*., 1996).

In parallel with the worldwide increases in obesity prevalence, overweight and obesity in children are rising (WHO, 2015). In 2013, 42 million children under the age of 5 years were overweight or obese (WHO, 2015). Childhood obesity has reached epidemic proportions in the United States, with a prevalence of 14–18.4% between 2000 and 2004 (U.S. Department of Health and Human Services [USDHHS], 2006).

Once considered a high-income country problem, overweight and obesity are now on the rise in low- and middle-income countries, particularly in urban settings. In developing countries with emerging economies (classified by the World Bank as lower- and middle-income countries) the rate of increase of childhood overweight and obesity has been more than 30% higher than that of developed countries (WHO, 2015). In sub-Saharan lower income countries, reliable and nationally representative prevalence of childhood

obesity is rare. A prevalence of 2.5% was captured for obesity in school-aged children between 5-17 years (Muthuri *et al*., 2014). Kramoh *et al*. (2012) and Musa *et al*., (2012) both stated childhood obesity prevalence of 1.8% and 5% in Ivory Coast, Abidjan and Nigeria, Benue State, respectively. In Ghana, prevalence of childhood obesity is sharply increasing more in the urban areas and also among the high socio-economic class. A recent publication by Mohammed and Vuvor (2012), states a 10.6% prevalence of childhood obesity in University Primary School, Legon in Accra.

Obesity is a complex condition caused by many factors including environmental and genetic factors. Obesity in children is a body mass index (BMI) at or above the 95th percentile for children of the same age and sex (CDC, 2006). It is the result of an imbalance between food intake and energy expenditure (Seth & Sharma, 2013). The role of genetic factors in the aetiology of obesity has been recognized for a long time, but the identification of genes and mutations contributing to body weight gain over time has been sluggish. The development of microarray-based high-throughput single-nucleotide polymorphism (SNP) genotyping technology has made genome-wide association studies (GWAS) feasible (Rankinen *et al.,* 2010).

Several genes influence the development of obesity in varying ways (Thomas *et al*., 2007). The adrenoceptor beta 3 gene (β3-adrenergic receptor), also known as ADRB3, is mainly expressed in adipose tissue, and contributes to variations in energy expenditure and body fat distribution (Lowell & Bachman, 2003; Clément *et al.,* 1995). Polymorphisms of the ADRB3 have been suggested to participate in the pathogenesis of obesity (Clément *et al.,* 1995; Arner, 1995). In particular, a single nucleotide polymorphism in the ADRB3 gene (substitution of tryptophan 64 with arginine

(Trp64Arg)) has been associated with obesity, insulin resistance, abnormal lipid profile and arterial hypertension (Hallman *et al.*, 2004; Hao *et al.*, 2004).

1.2 PROBLEM STATEMENT

Childhood obesity predisposes individuals to obesity in adulthood (Freedman *et al.*, 2005). Obesity is a very important risk factor for several non-communicable diseases such as diabetes, cardiovascular diseases, hypertension, cancer (Deckelbaum & Williams, 2001), psychological problems and sleep apnea (Barlow & the Expert Committee, 2007). These diseases have adverse consequences which can be life threatening and warrant early prevention strategies.

Single nucleotide polymorphisms of the ADRB3 gene are strongly correlated positively with BMI and obesity. However, since the prevalence of especially the Trp64Arg mutation differs among ethnic groups (Shuldiner & Sabra, 2001), other studies have failed to show any relationship between this polymorphism and obesity (Frederiksen *et al.*, 2003; Kurokawa *et al.*, 2001). Furthermore, it has been suggested that the association of this ADRB3 polymorphism with body weight and obesity related phenotypes may be dependent upon the presence of other susceptibility genes and/or exposure to other environmental factors (Shuldiner & Sabra, 2001).

However, the potential role of this polymorphism of the ADRB3 gene has not been explored in Ghanaians. In addition, most researches on obesity are focused on dietary intake and physical inactivity mostly in adults. Research in genetics of obesity is new and there exist insufficient data in this field in Ghana.

1.3 SIGNIFICANCE OF STUDY

Childhood obesity is becoming very common worldwide and similar trends are seen in Ghana. Children express the same comorbidities that are associated with being overweight and obese as adults (Dietz & Gortmaker, 2001; Williams, 2001). Thus, being overweight during childhood brings with it comorbidities that will increase the duration of comorbidities in an individual by one to two decades, a factor that can increase the impact of a number of risk factors on adult diseases.

Genes contribute to pathogenesis of obesity. Probing and examining the ADRB3 gene polymorphism will elucidate the role of this gene in the aetiology, metabolic consequences and complications of obesity in children. The assessment of these ADRB3 polymorphisms will help dietitians and other health professionals to provide scientific evidence-based counselling to individuals or groups at risk. It will also aid in the development early prevention strategies and therapeutic options.

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1.4 HYPOTHESIS

There is no relationship between the ADRB3 gene variants and childhood obesity in Ghana.

1.5 AIM

This aim of this study was to investigate the relation between ADRB3 gene variants and obesity in school children Ghana.

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1.6 SPECIFIC OBJECTIVES

The specific objectives were to:

1. screen for ADRB3 gene variants among the school children.
2. observe the dietary pattern of the school children using food frequency questionnaire.
3. determine whether there is a relation between ADRB3 gene variants, dietary pattern and obesity among the school children.

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CHAPTER TWO

LITERATURE REVIEW

2.1 CHILDHOOD OBESITY

2.1.1 Definition

Childhood obesity is defined as body mass index (BMI) greater than two (2) standard deviations above the WHO growth standard median (WHO, 2006).

Body Mass Index (BMI) is a measure of the body fat or adipose tissue based on a person's weight and height and expressed as a person's weight in kilograms divided by height in meters squared (kg/m^2). Individuals with BMI of 30 kg/m^2 or more are classified as obese (Ogden and Carroll, 2010). The globally accepted indicator of obesity is the BMI but in children cut-offs of 30kg/m^2 for obesity is not applicable since they do not reach such levels until they are fully matured. Hence growth curves appropriate for age and sex are used to determine ranges of obesity children (Cameron *et al*, 2006).

The British 1990 growth reference (UK90) is the most commonly used reference within the UK, and the International Obesity Task Force (IOTF) thresholds, World Health Organization Growth Reference and the Centers for Disease Control (CDC) Growth Reference are more frequently used in other countries.

2.1.2 Description

Obesity is not associated with or caused by a single variable. Rather, it is multi-factorial in nature. Obesity is a complex issue related to lifestyle, the environment and genes. These factors compound with psychological, cultural and physiological influence (Mahan *et al*., 2012).

Obesity is not just a matter of size of the body but related to serious health, social and psychological implications. According to (Seth & Sharma 2013), it encompasses both short or immediate and long term harmful effects such as diabetes, cardiovascular diseases, cancer, sleep apnoea, depression and poor self-esteem. Furthermore, obesity is also very costly. Several thousands of dollars is spent as obesity attributable expenditure (Trogdon *et al.*, 2012). An amount of $127 million was estimated for hospital cost associated with childhood obesity during 1997-1999, up from $35 million in 1979-1981. In 2000 according to CDC (year), the United States estimated a total cost of $117 billion for obesity in children and adults. Child obesity often persists in adulthood.

There are consistent increasing body of data that early life environment is an important determinant of risk of obesity in later life. It is also widely acknowledged that early prevention is of utmost significance. Childhood obesity is of serious concern in developing countries experiencing a nutrition transition: the displacement of traditional diets with foods high in saturated fats, sodium, and cholesterol and an increase in sedentary lifestyles (Duncan *et al.*, 2011).

2.1.3 Prevalence

Globally**,** though absolute numbers of prevalence of childhood obesity vary from different geographical locations (Fig. 1) and even among studies in the same year and location, the trend remains the same; a dramatic upward rise (Bagchi, 2011). The international trend of increase in the prevalence of overweight and obesity observed throughout the world is often called "globesity."

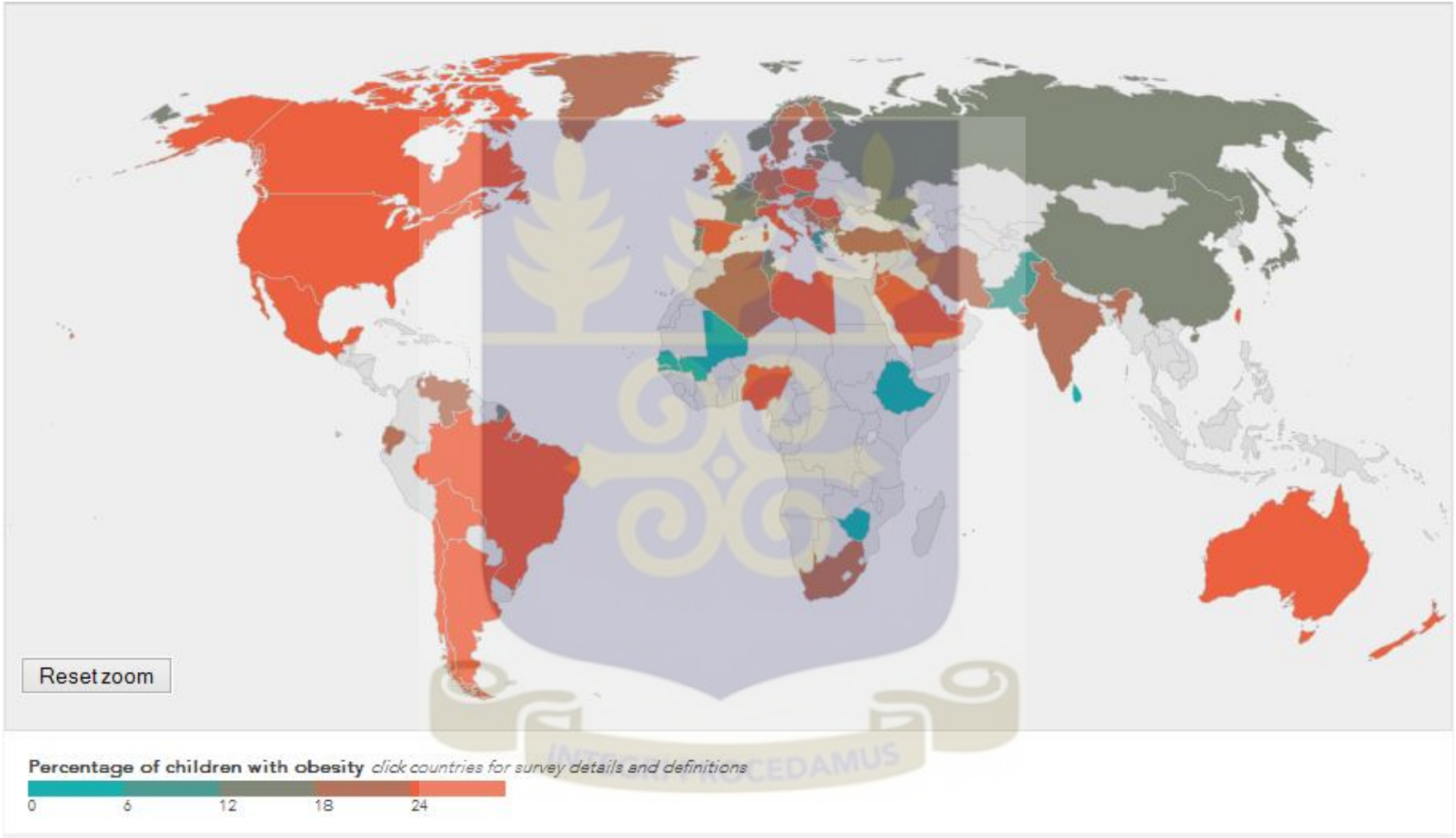


Fig. 1. Overweight children around the world (Source: http://www.worldobesity.org)

2.1.3.1 United Kingdom

Children's BMI is classified as overweight (including obese) where it is greater or equal to the 85th of the UK90 growth reference for population monitoring purposes. The National Child Measurement Programme (NCMP) measures the height and weight of around one million school children in England every year, providing a detailed picture of the prevalence of child obesity (NCMP, 2014).

The latest figures, for 2013/14 (Table 1), show that 19.1% of children in Year 6 (aged 10-11) were obese and a further 14.4% were overweight. Of children aged 4-5years, 9.5% were obese and another 13.1% were overweight. This means a third of 10-11 year olds and over a fifth of 4-5 year olds were overweight or obese. Wales and Scotland appear to have a higher prevalence of childhood obesity than England.

Table 1: Percentage of children obese and overweight (including obese) in the UK and Ireland **(**Data from Public Health England, 2015).

Country	Year	Age (years)	Obese			Overweight (including obese)			Data source
			ALL	Boys	Girls	ALL	Boys	Girls	
England	2013	2-15	15%	16%	15%	29%	30%	29%	Health Survey for England
Scotland	2013	2-15	16%	17%	15%	29%	31%	27%	Scottish Health Survey
Wales	2012	2-15	19%	20%	19%	34%	35%	33%	Welsh Health Survey

All estimates in the table are based on measured height and weight

2.1.3.2 United States

Childhood obesity prevalence remains high. Overall, obesity among young people, aged 2 to 19 years, has not changed meaningfully since 2003-2004 and remains at about 17%. However, among 2-5 years old, obesity has dropped based on CDC's National Health and Nutrition Examination Survey (NHANES) data (USDHHS 2013). Approximately 17% (or 12.7 million) of children and adolescents aged 2—19 years had obesity. The prevalence of obesity among children aged 2 to 5 years decreased significantly from 13.9% in 2003-2004 to 8.4% in 2011-2012 (USDHHS 2013).

There are significant racial and age disparities in obesity prevalence among children and adolescents. In 2011-2012, obesity prevalence was higher among Hispanics (22.4%) and non-Hispanic black youth (20.2%) than non-Hispanic white youth (14.1%) (USDHHS 2013). The prevalence of obesity was lower in non-Hispanic Asian youth (8.6%) than in youth who were non-Hispanic white, non-Hispanic black or Hispanic. In 2011-2012, 8.4% of 2- to 5-year-olds had obesity compared with 17.7% of 6- to 11-year-olds and 20.5% of 12- to 19-year-olds (USDHHS 2013).

2.1.3.3 Africa

Trends in Africa are assuming a similar picture. Childhood obesity is a serious concern in developing countries experiencing a nutrition transition. Data on childhood overweight/obesity and thinness in Sub-Saharan Africa including Ghana is limited and probably scarce. The issue of childhood obesity is less documented in developing countries, making less information available (McDonald *et al*., 2009).

According to (Mogre *et al*., 2013), childhood overweight/obesity prevalence of 17.4%, was found in their study which involved 218 school-aged children (5-14 years) in

Tamale, Ghana. This is reported to be one of the highest in Sub-Saharan Africa. A study among children aged 2 to 18 years in Nigeria reported a childhood overweight/obesity prevalence of 14.2% (Ene-Obong *et al.*, 2012). The Nigerian study defined overweight/obesity using the International Obesity Task Force (IOTF) cut-off points. The IOTF makes use of BMI-for-age percentile curves instead of the WHO BMI-for-age Z scores. A study by Peltzer and Pengpid (2011) among a sample of children from Ghana and Uganda found a prevalence of overweight or obesity of 10.4% among girls and 3.2% among boys, and 0.9% and 0.5% obesity only among girls and boys, respectively. The Ugandan and Ghanaian study measured the heights and weights of the studied participants based on self-report. The use of self-reported weight and heights may lead to underestimation of overweight and obesity (Elgar et al., 2005). The differences could be due to the different methods used in the classification of weight status of the children. Ghana School Survey overall prevalence of overweight and obesity among children is 15% (Report of Ghana School Survey Dissemination Workshop, 2013).

2.1.4 Risk Factors

Risk factors of childhood obesity are variables possessing the potential to increase the likelihood of developing obesity in early life. It is important to comprehend that while a particular risk factor or set of risk factors increases the likelihood of becoming overweight or obese, this does not mean that there is a certainty of suffering from either condition (Black, 1992). Determinants of childhood obesity can be classified into two categories: modifiable and non-modifiable risk factors.

Obesity in childhood results from the combination of both modifiable and non-modifiable risk factors. The modifiable risk factors include dietary factors and eating habits, sedentary lifestyle, psychological factors, socioeconomic factors, parental obesity,

environment, and certain socio-demographics such as income level (Pleuss and Matfin, 2009). Non-modifiable risk factors for childhood obesity are permanent and cannot be changed and these include genetics, race, and family history of obesity. Understanding the risk factors or causes of childhood obesity can provide the opportunity to focus resources, interventions and research in directions that would be profitable in solving the problem.

2.1.4.1 Modifiable risk factors

These determinants of childhood obesity include physical activity level, dietary habits especially during screen times, among others (Seth & Sharma 2013). Although the mechanism of obesity development is not fully understood, it is confirmed that obesity occurs when energy intake exceeds energy expenditure. There are multiple aetiologies for this imbalance, hence, the rising prevalence of obesity cannot be addressed by a single aetiology. In a lesser number cases, childhood obesity is due to genes such as leptin deficiency or medical causes such as hypothyroidism and growth hormone deficiency or side effects due to drugs like steroids (Link *et al*., 2004). Most of the time, however, personal lifestyle choices and cultural environment significantly influence obesity.

2.1.4.1.1 Dietary behaviour

Excessive caloric intake leads to the development of obesity. However high fat diets promote fat accumulation significantly more than high carbohydrate diets since high fat diets have high energy density, metabolic efficiency, palatability, poor regulation and weak satiating effect (Prentice & Poppitt, 1996). A high dietary fat intake may in part cause weight gain since the ability to oxidize fat is not increased. Some cross-sectional studies have found a positive relationship between fat intake and adiposity in children

even after controlling for confounding factors (Tucker, 1997; Heaney, 2002). The main objection to the notion that dietary fat is responsible for the fast-tracked paediatric obesity epidemic is the fact that at the same time the prevalence of childhood obesity was increasing, the consumption of dietary fat in different populations was decreasing. Although fat eaten in excess leads to obesity, there is not strong enough evidence that fat intake is the main reason for the ascending trend of childhood obesity.

With simultaneous rise in childhood obesity prevalence in the USA, the National Health and Nutrition Examination Survey (NHANES) noted only subtle change in calorie intake among US children from the 1970s to 1988–1994 (Troiano *et al*., 2000). For this period, NHANES III found an upsurge in calorie intake only among white and black adolescent females. The same trend was observed by the NHANES (1999–2000) (Troiano *et al*., 2000). The Bogalusa study which has been following the health and nutrition of children since 1973 in Bogalusa (Louisiana), reported that total calorie intake of 10-year old children remained unchanged during 1973–1988 and a slight but significant decrease was observed when energy intake was expressed per kilogram body weight (Nicklas, 1995). The result of a survey carried out during the past few decades in the UK suggested that average energy intakes, for all age groups, are lower than they used to be (Prentice & Jebb, 1995). Some small studies also found similar energy intake among obese children and their lean counterparts (Maffeis *et al*., 1996). McDonald *et al*. (2000) found a significant association between snacking in between meals and overweight and obesity.

There is a growing body of evidence suggesting that increasing dairy intake by about two servings per day could reduce the risk of overweight by up to 70% (Heaney, 2002). Higher calcium intake and more dairy servings per day were associated with lessened

adiposity in children studied longitudinally (Skinner *et. al*., 2003; Carruth & Skinner, 2001).

2.4.1.1.2 Physical inactivity

It has been proposed that a steady drop in physical activity among all age groups has profoundly contributed to growing rates of obesity everywhere in the world. Physical activity is important in prevention and management of overweight and obesity because it has an impact on energy expenditure, body composition, substrate oxidation and metabolism (Donnelly *et al.*, 2004). Again those who exercise regularly are associated with improved self-efficacy, increased adherence to dietary intervention for weight loss and weight control (Donnelly *et al*, 2004).

In South Africa, according to the THUSA study (1996-1998) which recruited 1000 persons, physical inactivity was a major determinant of obesity in some women. Another survey, also in South Africa, emphasized that a self-reported inactivity was a major risk factor for overweight and obesity (Senekal *et al*, 2003). The protective effect of physical activity for obesity is not limited to adults but also in children (McVeigh *et al*., 2004).

African-American parents of the children 9-13 years report more barriers to the children's physical activity than white parents. Barriers such as transportation, safety and the expense and availability of local opportunities have been mentioned. Of African-American parents, 30.6% cite lack of opportunity as a barrier to physical activity compared with only 13.4% (Duke *et al*., 2002).

Parents report that they prefer having their children watch television at home rather than play outside unattended because parents are then able to complete their chores while

keeping an eye on their children (Gordon-Larsen, 2004). In addition, bigger proportions of children who are being driven to school and hardly involved in sports and physical education, chiefly among adolescent girls (Swinburn, 2002) are also associated with bloated obesity prevalence. Since both parental and children's choices fashion these behaviours, it is not shocking that overweight children tend to have overweight parents and are themselves more likely to grow into overweight adults than normal weight children (Carriere, 2003).

Several other studies have reported that childhood overweight and obesity was negatively associated with physical activity and positively associated with sedentary activities (Berkey *et al*., 2000).

2.4.1.1.3 Stress

According to Overgaard *et al*. (2004), psychological workload was associated with higher weight gain when 6704 female Danish nurses were studied between 1993 and 1999. More recently, Dallman *et al*. (2003), proposed that stressed or depressed people have a decreased cerebrospinal corticotrophic-releasing factor, catecholamine concentrations, and hypothalamic-pituitary-adrenal activity and may over eat to reduce the activity in the chronic stress response network. Goodman and Whitaker (2002) found in a longitudinal study that depressed adolescents are at increased risk for the development and persistence of obesity during adolescence. The importance of linking psychosocial stressors and childhood obesity has been stressed (Gundersen *et al*., 2011).

2.4.1.1.4 Screen times

The presence and influence of media pose a significant challenge to healthy eating and regular physical activity among many children. Television and other screen viewing may

confer risk of obesity through a reduction on energy expenditure or increased food intake (CDC, 2010; Narayan *et al*., 2003). Obesity in children is strongly associated with television viewing several hours a day (Delva *et al*., 2007; Berkey *et al*., 2000).

Research shows that the number of hours spent watching TV is positively associated with increased caloric intake, overweight and obesity. A study of 6th-, 7th- and 8th- graders in Boston found that an extra hour of television is associated with the consumption of additional 167 calories daily (Delva *et al*., 2007; Wiecha *et al*., 2006).

African-American adolescents are exposed to more food advertisements than white adolescents. A study found that African-American aged 12 – 17 years viewed 14% more food product advertisements (Powell *et al*., 2007). The associated snacking on high sugar and high fat foods as a result of prompting from commercials and idleness is also a major factor in the development of childhood obesity. If long hours of TV viewing and other screen times such as video and computer games, have such negative consequences, it is just prudent to limit children's screen time.

In a study by Mogre *et al*. (2013) on overweight, obesity and thinness and associated factors among school aged children in Tamale, Ghana, significantly, 34.2% overweight and obese children and 13.9% of children who were thin watched TV during leisure times.

2.4.1.1.5 Sleep duration

Duration of night sleep, may alter later the risk of obesity through growth hormone secretion or because sleep reduces the child's exposure to factors in the environment that promote obesity. Research among school aged children have consistently revealed that

later bedtimes were associated with increased obesity risk (Snell *et al*., 2007; Sekine *et al*., 2002). Later bedtimes are simply a proxy for shorter sleep duration. However, a study conducted in Tamale, Ghana, by Mogre *et al*. (2013) reports that overweight and obese children went to bed an hour earlier than their non-obese counterpart.

2.1.4.2 Non modifiable risk factors

Predetermined, uncontrollable or unchangeable are terms used to describe non-modifiable risk. Race/ethnicity has an impact on people becoming overweight or obese.

2.1.4.2.1 Gender

While some studies have associated gender with childhood obesity, some other studies have found no association between childhood obesity and gender. A study by Kimani - Murage *et al*. (2011), in rural South Africa found combined overweight and obesity was higher in girls (15%) than boy (4%). A study in Ghana found a prevalence of overweight or obesity of 10.4% among girls and 3.2%among boys, and 0.9% and 0.5% obesity only among girls and boys, respectively (Peltzer & Pengpid, 2011).

However, in studies by McDonald *et al*. (2009) among Columbian children (aged 5 to 12 years), Schultz (2012) among Australian children (aged 5 to 15 years) and Mogre *et al*., (2013) among Ghanaian children, overweight/obesity was not associated to gender.

2.1.4.2.2 Genetics

Genetic factors influence the vulnerability of a given child to an obesity-conducive environment. However, environmental factors, lifestyle preferences, and cultural environment seem to play major roles in the rising prevalence of obesity worldwide (Hill 1998; Eckel & Krauss, 1998; Grundy, 1998). According to Bouchard *et al*. (1994), 75%

variation in percentage body fat and total fat mass is attributable to culture and lifestyle, whereas 25% can be attributable to genetic factors.

Correlational studies of BMI between family members, adoptees and their biological relatives, and between twins have emphasized evidence of genetic contribution to obesity (Bouchard *et al*., 1994). Genetics is implicated in the development of obesity. One way, genes, may contribute to obesity is their functional ability to regulate how humans capture, store, and release/burn energy from the food they eat.

2.2 GENETIC AETIOLOGY OF OBESITY

Polygenic obesity studies are presently based on the investigation of single nucleotide polymorphisms (SNPs) located within or near a candidate gene (Mutch &Clement, 2006).

2.2.1 Candidate Gene Studies

Candidate gene analysis, involves testing the association between obesity and a specific allele of a gene that seems to be influencing the regulation and metabolism of food (Clement & Ferre, 2003). According to Kopelman (2000), a candidate gene is defined as that part of the deoxyribonucleic acid (DNA) molecule that directs the synthesis of a specific polypeptide chain loosely associated with a particular disease. Identification of candidate genes of obesity involves animal studies, human obesity syndromes and a genome-wide search using microsatellites covering the human genome. These genes are chosen for their possible effects on body fat composition, anatomical distribution of fat, food intake and energy expenditure (Kopelman, 2000).

Candidate gene studies are hypothesis-driven (Loos, 2009). When there is evidence that a gene is involved in the regulation of energy balance in animal models or in extreme/monogenic forms of obesity, tests of association with obesity-related traits are conducted at population level (Loos, 2009). The Human Obesity Gene Map has reported one hundred and twenty seven candidate genes for which at least, a study reported a positive association with obesity-related traits (Rankinen *et al*., 2006).

2.2.1.1 Genome-wide linkage studies

Genome wide linkage studies aim to detect chromosomal regions sheltering one or more genes relevant for a respective phenotype by making use of linkage data (Bell *et al*., 2005). Those regions underlying linkage peaks are narrowed down by fine mapping, so that candidate gene analyses can be pursued. Although more than 40 microsatellite-based genome wide linkage scans have been performed and single candidate genes have been detected, none of the results have been validated unequivocally (Saunders *et al*., 2007). This supports the contention that the effect sizes of genes influencing adiposity are small and/or that substantial heterogeneity exists

2.2.1.2 Genome wide association studies

Genome wide association studies (GWAS) makes it possible for the entire human genome to be studied. GWAS provide a better design to identify common variants with low to moderate penetrance which are relevant as risk factors for the trait of interest. Within a short time span, GWAS have proven to be very successful for the detection of polygenic variants (Frayling, 2007). The production of high density SNP-chips has made GWAS feasible, which only recently have led to the identification of a number of confirmed genes for different complex disorders, thereby revolutionizing the molecular genetic analyses of complex disorders (Liu *et al*., 2013). Stringent efforts to confirm (or

reject) an original finding are absolutely crucial; due to the low effect sizes and possible gene–environment interactions it is to be expected that not every study will be able to confirm a true positive finding; meta-analyses are required. GWAS performed for obesity or body mass index have already documented that this approach is extremely powerful to detect genetic variants relevant for the analyzed phenotype(s). .

2.3 THE ADRENERGIC SYSTEM

The adrenergic system is one of the body's major stress-response mechanisms. The classic example of adrenergic stimulation is the "fight-or-flight" response, in which situational factors stimulate massive release of adrenal catecholamines into the bloodstream (Dorn, 2010). The adrenergic system also plays a central role in the regulation of energy balance by stimulating both thermogenesis and lipolysis in brown and white adipose tissues in humans and various animal models (Lafontan & Berlan, 1993).

2.3.1 β-adrenergic receptors (β-ARs)

β-adrenergic receptors are members of the seven-transmembrane superfamily of G protein coupled receptors, which are very important in both cardiovascular and metabolic regulation. In the heart, for example, ADRB pathways are the primary means of increasing cardiac performance in response to acute or chronic stress (Bardou *et al.*, 2007).

β-adrenergic receptors have been divided into at least 4 sub- types; β_1-AR, β_2-AR, β_3-AR and β_4-AR. The β_1-AR and β_4-AR are mainly expressed in the cardiovascular system, β_2-AR in airway smooth muscle and β_3-AR in adipose tissue (Frielle *et al.*, 1988). The β-

ARs function carry out their functions by coupling to the stimulatory G protein, which induces the adenylyl cyclase, thus leading to an increase of intracellular cAMP level. cAMP acts as a second messenger in the cell through its activation of protein kinase A (PKA), which results in the phosphorylation of multiple targets (Liggett, 1997). The physiological responses of β-ARs to various endogenous and exogenous ligands dis- play substantial inter-individual and interethnic differences, which might he due to genetic variation in these receptors in humans (Mason *et al*., 1999; Green *et al*., 1994).

Available information has demonstrated the significant association between the impaired activity of β-adrenergic receptors (β-ARs) and obesity (Scherrer *et al*., 1994; Spraul *et al*., 1993). Therefore, β-ARs genes may be of particular importance for the development of human obesity and have become the focus of studies in recent years (Liu *et al*., 2007).

2.3.1.1 The β3-Adrenergic Receptor (β_3-AR)

The β_3-AR is located primarily in the small intestine, adipose tissue and vascular endothelium (Berkowitz *et al*., 1995) where it is are involved in lipolysis, glucose uptake, cardio-inhibition and relaxation of colon, oesophagus and bladder (Bond *et al*., 2015). The human β_3-AR gene has been localized to chromosome 8 (8p12-8p11.1) [Nahmias *et al*., 1991]. It is composed of 2 exons separated by a small intron. A 1.4 kb exon encodes 402 amino acid residues, while a small exon of 0.7 kb contains the sequence coding for the 6 C-terminal residues of the receptor and the entire mRNA 3'-untranslated region (Granneman *et al*., 1993).

The β_3-AR crosses the cell membrane seven times (Fig. 2), is coupled to guanine-nucleotide binding (G) proteins, and is localized in adipose tissue. Stimulation of the

receptor by β--adrenergic agonists activates adenylate cyclase, which increases intracellular concentrations of cyclic AMP (cAMP) and results in increased lipolysis and thermogenesis (van Spronsen *et al*., 1993; Krief *et al*., 1993; Emorine *et al*., 1989).

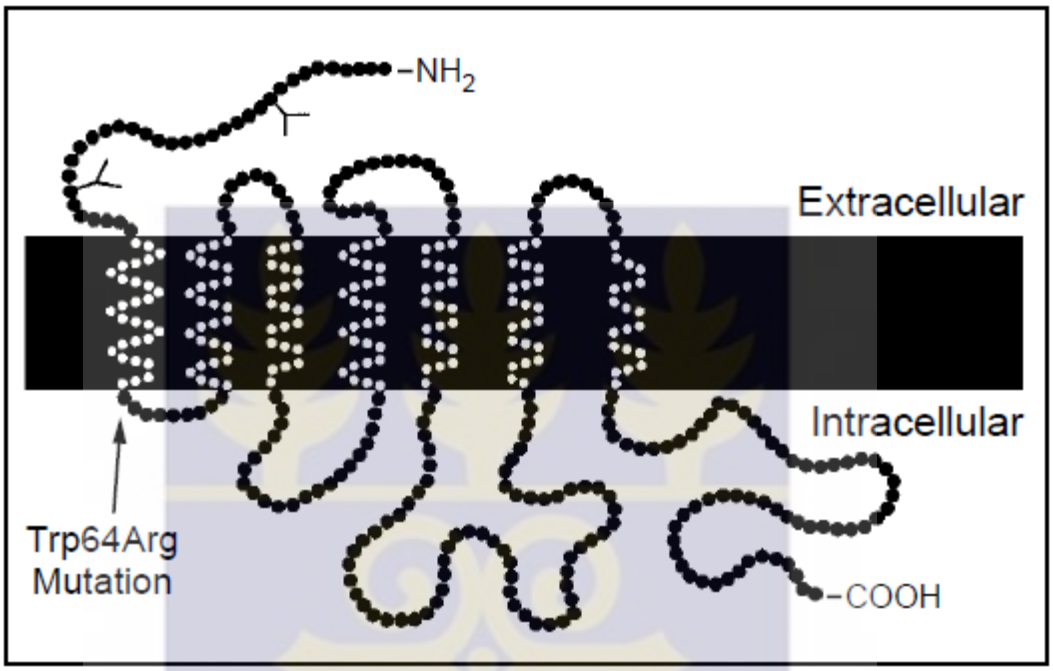


Fig 2: Diagram of the β_3- adrenergic receptor. Each amino acid is shown as a circle. The Trp64Arg mutation appears at the beginning of the first intracellular loop (Walston *et al*. 1995)

2.3.1.1.1 β3-adrenergic receptor polymorphism and obesity

Until recently, 4 single base pair substitutions have been identified in β_3-AR gene: 1856 G/T in the intron; 3139 G/C in the 3'-untranslated region; 381 C/T which is a neutral mutation; and 190 T/C which is the only functional mutation in the β_3-AR gene (Trp64Arg) (Walston *et al*., 1995). The frequency of the Arg64 variant differs between ethnic groups. Three genetic variants of the β3-AR (1856 G/T, 3139 G/C and 190 T/C) were found to be in almost complete association with another and form 2 major haplotypes (Hoffstedt *et al*., 1999).

The relationship between the Trp64Arg polymorphism and basal metabolic rate has also been investigated because β3-AR plays an important role in lipid and glucose metabolism, which might indirectly reflect β3- AR function. Walston *et al*. (1995) first reported a tendency for a lower resting metabolic rate in Pima Indians carrying the Arg64 mutation. Later, a significantly lower resting metabolic rate was reported in obese male and female Finns carrying the Arg64 variant (Arch & Kaumann, 1993). However, other studies have failed to observe such a relationship (Sipiläinen *et al*., 1997).

Much evidence from rodents has shown that the β3-AR is the principal mediator of catecholamine-stimulated thermogenesis in brown adipose tissue and lipolysis in white adipose tissue (Tchernof *et al*., 1999; Lafontan & Berlan, 1993). Clément *et al*. (1995) first reported that the patients with morbid obesity who were Arg64 heterozygotes had an increased capacity to gain weight; however, the allele frequency of the Arg64 mutation was similar in the morbidly obese patients and the normal subjects. Later, association of the Trp64Arg polymorphism in the β3-AR gene with human obesity was extensively investigated. Increased BMI (the most commonly used obesity index) in Arg64 carriers has been demonstrated in different populations (Mo *et al*., 2007; Zhang *et al*., 1996). In these studies only subjects with homozygous Arg64 had an increase in BMI. However, there were still large numbers of studies demonstrating no significant association between the Trp64Arg poly- morphism and obesity (Xinli *et al*., 2001; Garenc *et al*., 2001; Gagnon *et al*., 1996). This discrepancy seems not to be due to the small number of subjects studied, gen der, or different ethnic background, but might be explained by the presence of some other important genetic polymorphisms in the β 3-AR gene or other

genes in this region, or by the combined influence of the β3-AR gene and polymorphisms of other genes.

The interaction between Trp64Arg polymorphism and common variants of other susceptibility genes showed significant correlation with obesity in several studies. Carlsson *et al*. (2001) reported that Gln27Glu and Trp64Arg variants were significantly associated with increased non-esterified fatly acids concentration. The findings of Gagnon *et al*. (2001) also suggested an interaction between the effect of Trp64Arg (β3) and Glu12/Glu9 (α_{2b}) adrenergic receptor polymorphisms on body fatness in Caucasian women.

CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY DESIGN

The research was a case control study.

3.2 STUDY SITES

The study was conducted at St Mark Basic School (Atomic Hills) and Ghana Atomic Energy Commission (GAEC) Basic School (Atomic, Kwabenya).

St Mark Basic School is a catholic school. The school currently provides basic education to about 350 children living within and outside that community. GAEC Basic is the only basic school within the premises of Ghana Atomic Energy Commission, a state organization in Ghana involved with surveillance of the use of nuclear energy in Ghana. GAEC basic school currently has a population of over 1800 pupils.

3.3 PARTICIPANTS

Participants were boys and girls, aged 8-15 years in the two schools who were obese thus BMI at or above the 95th percentile for boys and girls based on WHO growth charts (WHO, 2007). Non obese children boys and girls aged between eight and fifteen years from these two schools were used as control group. Boys and girls below eight or above fifteen years were not included in the study.

3.4 SAMPLE SIZE CALCULATION

The sample size calculated using a simple formula (Daniel, 1999). Using a confidence interval of 95% and a margin of error of 10%, the calculation was as follows:

$$n = \frac{[Z]^2 P(1-P)}{E^2}$$

$$n = \frac{[1.96]^2 (0.15)(1-0.15)}{0.1^2}$$

= 49 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 overall prevalence of overweight and obesity among the children in Ghana (Ghana School Survey, 2013).

3.5 ETHICAL CONSIDERATION

Approval was obtained from the Research Ethics and Protocol Review Board of the School of Biomedical and Allied Health Sciences, University of Ghana before the study began. In addition, written permission was obtained from the head of the schools involved before data was collected. An informed written assent (Appendix I) was signed by parents/guardians of participants after a detailed explanation of the study. Participation in the study was voluntary and participating subject's confidentiality was ensured at all times.

3.6 DATA COLLECTION

3.6.1 Anthropometric Measurements

3.6.1.1 Body mass index (BMI) determination

Height was measured and recorded to the nearest 0.1cm using a portable stadiometer (SECA 213, Hamburg, Germany) which was placed on a firm flat ground to ensure

accuracy. Participants' weight was measured using an Omron floor digital scale (Seca HBF 516, Hamburg, Germany). It was ensured that subject's weight was evenly distributed on both feet. Body mass index was calculated from weight and height (weight/height) in kg/m^2.

Based on such screening, children were identified as obese when their BMI was at or above the 95th percentile for boys and girls based on WHO growth charts (WHO, 2007).

3.6.1.2 Waist and hip circumference measurements

Waist circumference was measured with a non-stretchable tailors' tape measure to the nearest 0.1cm at the level of the umbilicus and hip circumference was measured at the largest horizontal circumference around the buttocks. Subjects stood with body erect, arms at the sides, feet positioned close together with weight evenly distributed across the feet. Waist to hip ratio (WHR) was calculated by dividing the waist circumference by the hip circumference. World Health Organization recommended cut-off points and risk of metabolic complications defined as waist–hip ratio above 0.90 for males and above 0.85 for females was used (WHO, 2011).

3.6.2 Dietary Assessment

A well-structured questionnaire (Appendix III) adapted from Asare-Annan (2011) was administered to all participants. The questionnaire contained a food frequency questionnaire that assessed the frequency of consumption of pastries, sweet drinks, fatty meat, fast food, fruits and vegetables.

3.6.3 Molecular Laboratory Methods

3.6.3.1 DNA extraction

Participants were given 500 ml sachet water and required to rinse the mouth three times, discarding after each rinse to get rid of any food debris. They were asked to fill their mouths 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 50 ml falcon tubes, making sure the 50 ml mark was reached. Samples were placed in a pre-packed ice container and transported to the Molecular Biology Laboratory, Department of Medical Laboratory Sciences, School of Biomedical Allied Health Sciences, Korle-bu.

Samples were spun at 2000 rpm for 20 minutes using a refrigerated table top centrifuge (Eppendorf centrifuge 5810R, Hamburg, Germany). The supernatants were poured off and the pellets then re-suspended in 500 µl 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 1.5 ml Eppendorf tubes, then 30 µl of proteinase K (10 mg/ml) were added to each tube and incubated overnight in a heat block (Thermo Block TDB-120, Warren, United States of America) at 55^{o}C. Samples were retrieved, 200 µl 5 M NaCl was added to each tube and vortexed briefly. The contents were then spun at 14000 rpm for 30 minutes. The supernatants were transferred into 1.5 ml Eppendorf tubes, and 800 µl 100% ethanol added to each tube and rocked gently back and forth.

Deoxyribonucleic acid (DNA) precipitation was visible at this moment. Samples were stored in a -21^{o}C freezer for 3 hours. They were retrieved, allowed to thaw, and spun down at 14000 rpm for 30 minutes. The absolute ethanol was carefully poured off the

pellet, 500 µl 70% ethanol added and spun again at 14000 rpm for 5 minutes. The 70% ethanol was poured off after spinning, tubes were blotted and air-dried. The formed pellets were finally re-suspended in 200 µl TE (10 mM Tris-HCl, pH 8.0; 1 mM EDTA, pH 8.0.) and stored at -21^{o}C.

3.6.3.2 PCR amplification

Partial amplification of the ADRB3 gene was carried out to analyse the Trp64Arg polymorphism using the primers BSTNUP (5'-CGCCCAATACCGCCAACAC-3') and BSTNDOWN (5'-CCACCAGGAGTCCCATCACC -3') [Widén *et al*., 1995]. The PCR reaction mix and conditions were set up as shown in tables 2 and 3. PCR reactions were carried out in a SEEAMP™ SCE1000 thermal cycler (Seegene Inc., Seoul, Korea).

Table 2: ADRB3 PCR reaction mixture

Reagents	**Volume per sample (µl)**	**Final concentration**
Sterile distilled water	4.2	
OneTaq® Quick-Load® 2X Master Mix with Standard Buffer*	10.0	1X
20µM BSTNUP	0.4	0.4µM
20µM BSTNDOWN	0.4	0.4µM
DNA template	5	
Total volume	**20**	

*New England Biolabs Inc. (Ipswich, MA, USA).

Table 3: ADRB3 PCR cycling conditions

Cycle	Temperature	Time	No. of cycles
Initial Denaturation	94ºC	4 minutes	1
Denaturation	94ºC	30 seconds	
Annealing	57ºC	30 seconds	35
Extension	68ºC	50 seconds	
Final extension	68ºC	7 minutes	1

3.6.3.3 Restriction Fragment Length Polymorphism Analysis

After PCR amplifications, the amplified fragments of 210 bp were digested with the restriction enzyme *Mva*1 or *Bst*NI (New England Biolabs Inc., Ipswich, MA, USA). The digestion was carried out as recommended by the manufacturer. The final reaction volume of 20 µl contained 5-10 µl of the amplified products.

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.

3.6.2.4 Agarose gel electrophoresis

After the PCR, 10 µl of PCR product was added to 2 µl of 6 bromophenol blue loading dye and electrophoresed in 2% agarose gels stained with 0.5 µg/ml ethidium bromide. The gels were prepared and run in 1x TAE buffer at 110V for 30-45 minutes and were observed and photographed over UV transilluminator at short wavelength using a Kodak EDAS 290(New York, USA.) gel documentation system. The sizes of the PCR products

wwere estimated by comparing with the mobility of a standard 100bp DNA ladder (New England Biolabs Inc., Ipswich, MA, USA).

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

3.7 STATISTICAL ANALYSIS

Results are presented as mean ± 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 was computed. The level of statistical significance was set at $P < 0.05$. Analyses were performed using IBM® SPSS® Statistics version 22 for Windows and GraphPad Prism version 6.00 for Windows.

CHAPTER FOUR

RESULTS

4.1 SOCIO-DEMOGRAPHIC CHARACTERISTICS OF RESPONDENTS

A total of 68 subjects, 39 cases (obese) and 29 controls (non-obese), were recruited for the study. The average age for the cases was 11.92 ±1.69 years and that of the controls was 13.17 ± 1.77 years (Table 4). There were more females (55.9%) compared to males (44.1%). Majority of the children (52.9%) were in the age range of 12-14 years. (Fig. 3).

Table 4: Socio-demographic characteristics of respondents

Variable	Category	Cases (%)	Controls (%)	Total
N		39 (57.4)	29 (42.6)	68
Sex	Male	13 (43.3)	17 (56.7)	30
	Female	26 (68.4)	12 (31.6)	38
Age (years)				
[Mean ± SD]		11.92 ±1.69	13.17 ± 1.77	

4.2 ANTHROPOMETRIC INDICES

4.2.1 BMI

Fig. 4 shows the BMI values of the children. There was a statistically significant difference (all *ps*< 0.0001) in BMI between cases and the controls for both males and females (Figure 4). For both cases and controls, female children had BMI values higher the male children.

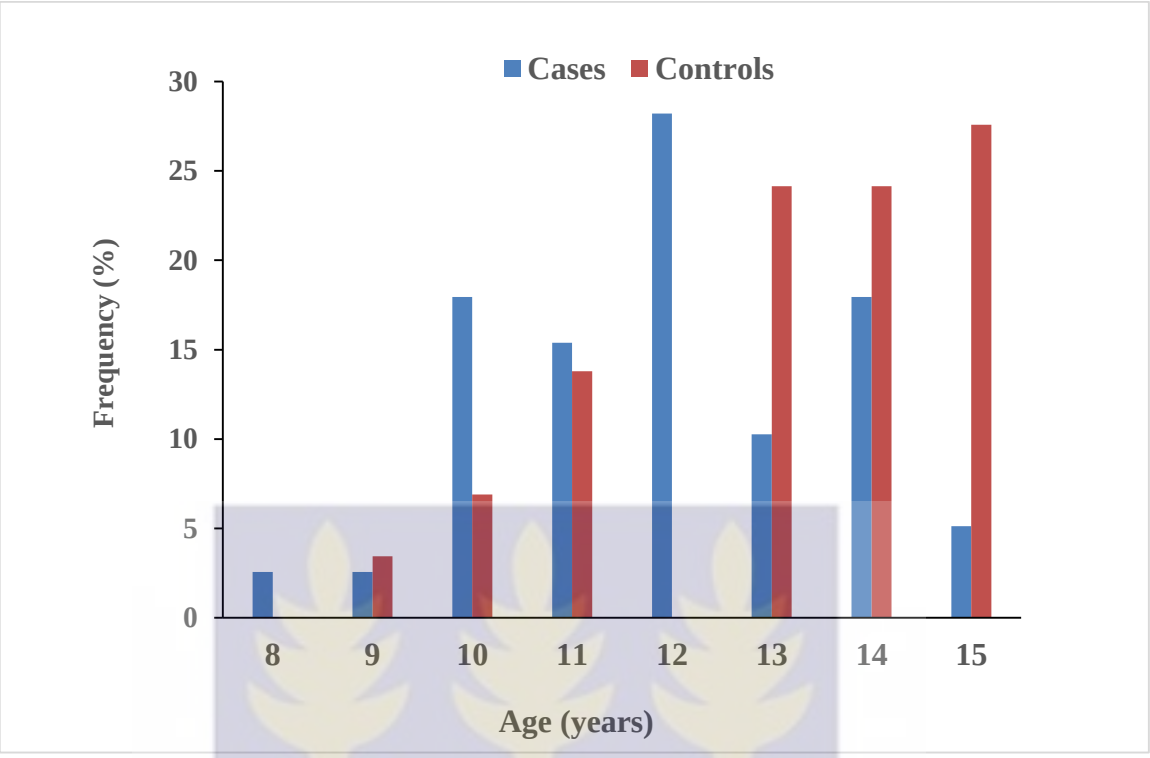
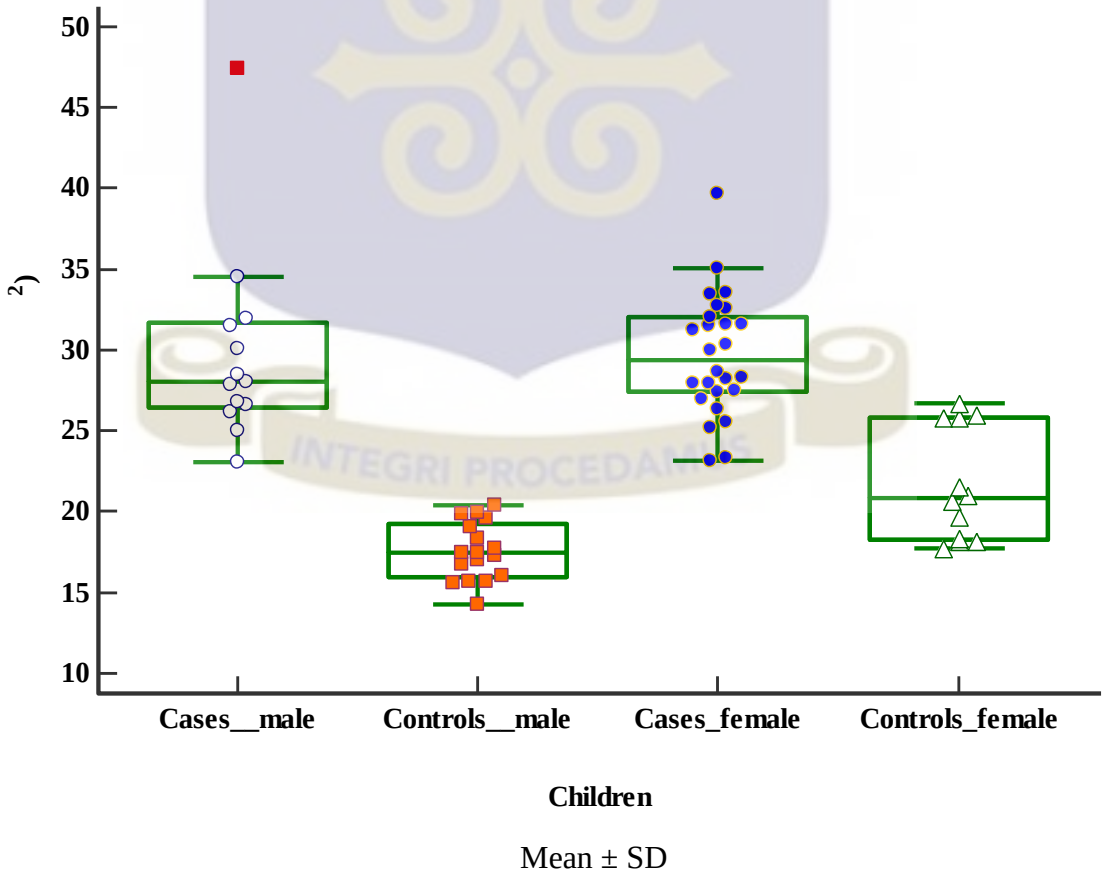


Fig. 3: Age distribution of the children



Mean ± SD

Figure 4. Mean body mass index (BMI) of the children.

4.2.2 WHR

None of the male children was at increased risk for cardiovascular events (Fig. 5a). However, male cases had higher WHRs compared their control counterparts and WHR measurements between cases and controls for male children were statistically significant (p = 0.0145). WHR measurements between cases and controls for female children were also statistically significant (p = 0.0039). A few of the female cases (38.2%) were at increased risk for cardiovascular events (Fig. 5b).

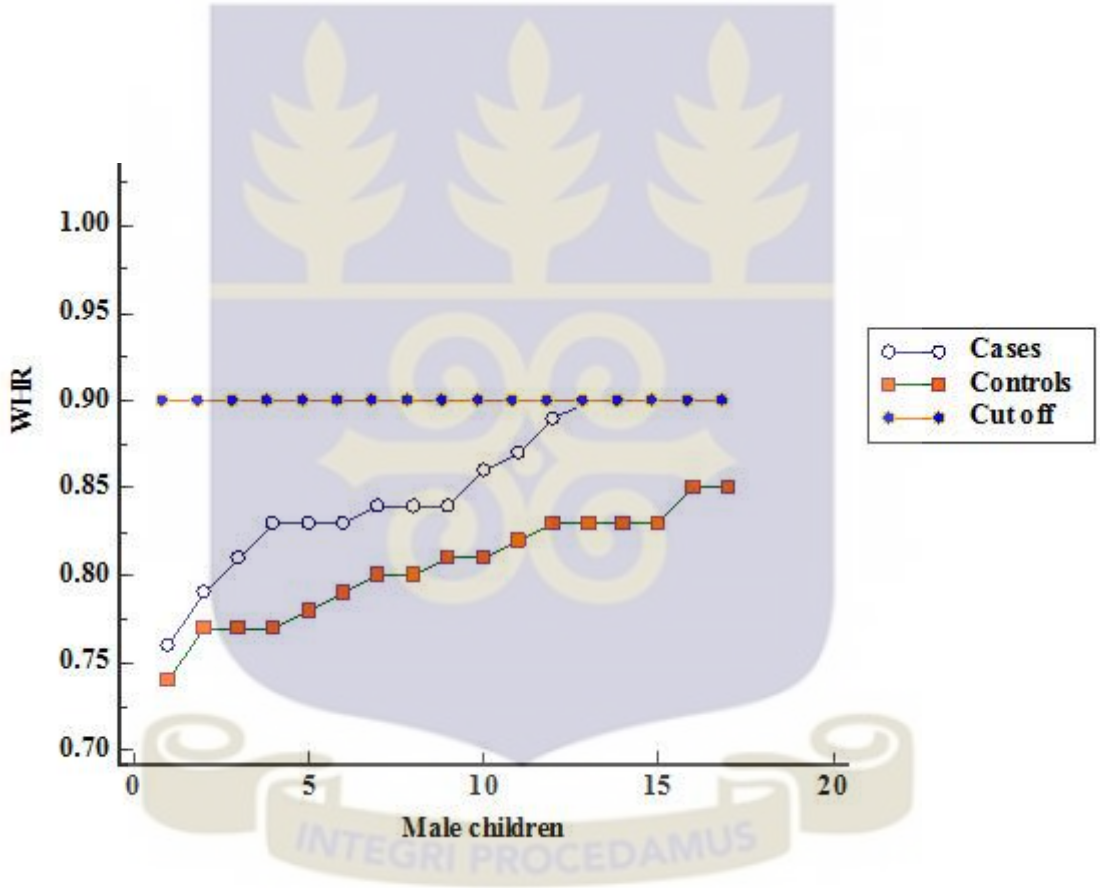


Fig. 5a: WHR of the male cases and controls

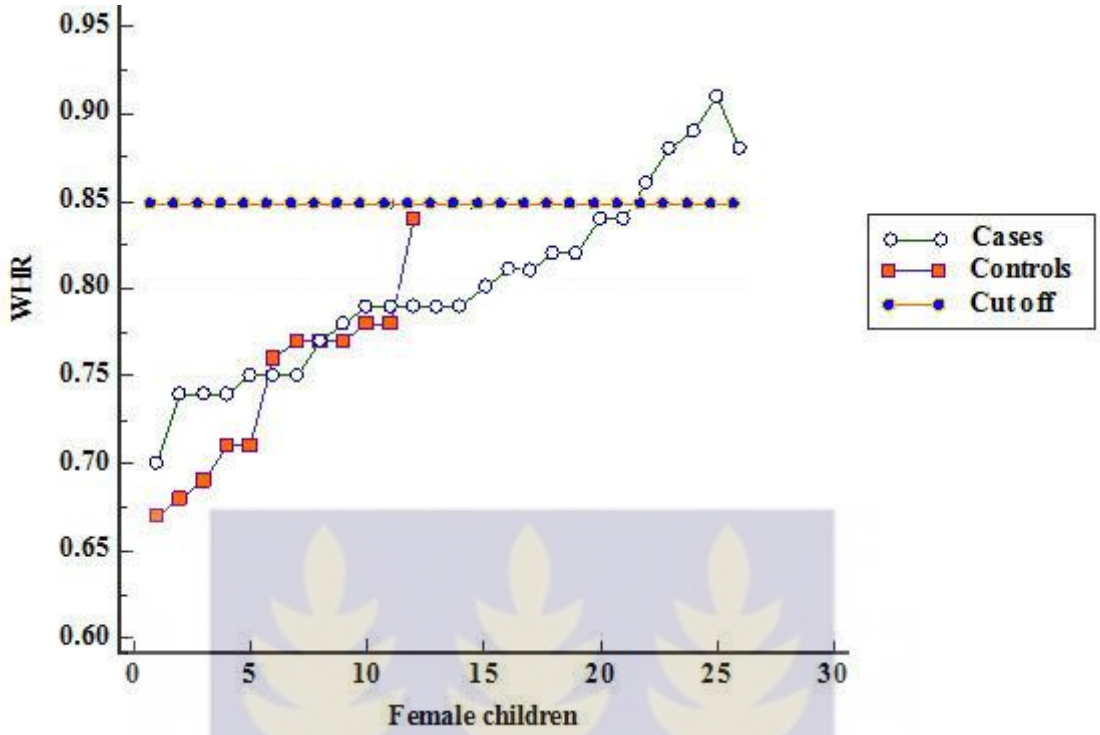


Fig. 5b: WHR of the female cases and controls

4.3 DIETARY PATTERN

Daily consumption of fruits and vegetables were higher in males compared to females (Fig. 6). Again, females consumed sweet drinks and fatty meat more frequently than their male counterparts.

Comparing cases to controls (Fig. 7), sweet drinks and fatty meat were more frequently eaten by obese children (cases). Daily consumption of fruits and vegetables were again, higher in children in the control group compared to their obese counterparts (cases).

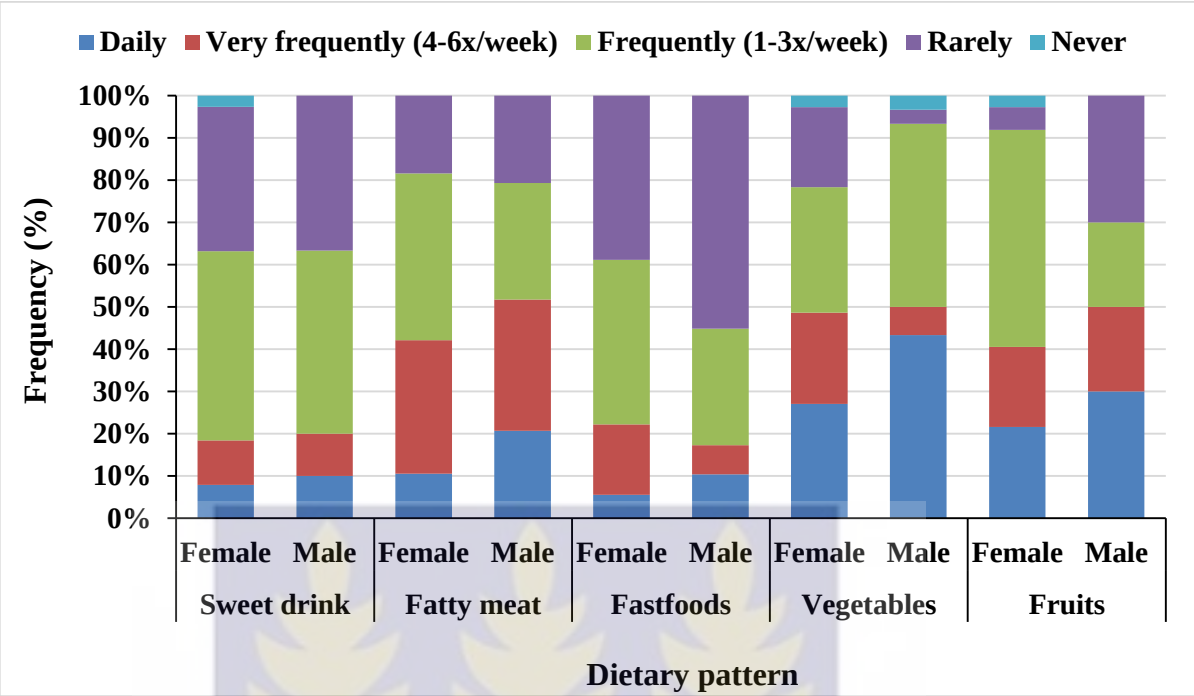


Fig. 6: Dietary pattern based on FFQ in relation to gender

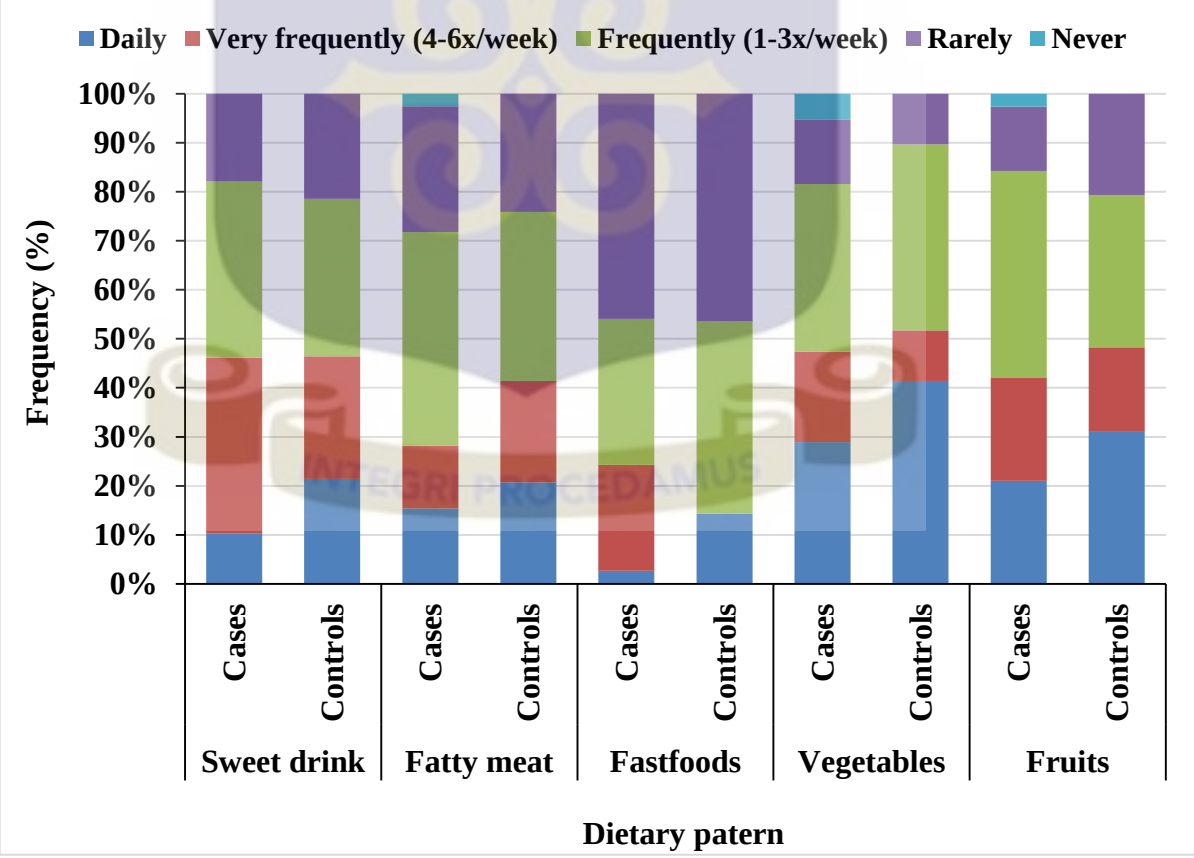


Fig. 7: Dietary pattern based on FFQ of cases and controls

4.4 ADRB3 GENOTYPE POLYMORPHISMS AMONG THE CHILDREN

Amplification was successful in 58 children (35 cases and 23 controls). In each of these, the expected 210 bp fragment of the ADRB3 gene was seen (Fig. 8). Restriction enzyme digestion of the amplicons with *Mva*I (Fig. 9) indicated the presence of the Trp64Trp, Trp64Arg and the Arg64Arg genotypes (Table 5). The frequency of Arg64 allele was 0.629 (0.810 and 0.357 in cases and controls respectively) and higher in the females (0.70). The genotype frequencies were distributed in compliance with Hardy Weinberg equilibrium (χ^2= 2.810, 1 d.f.; p = 0.094). A significant difference emerged for the ADRB3 gene Try64Arg genotype polymorphism frequencies between the cases and the controls (p = 0.0377, OR = 0.1471, 95% CI = 0.02666 to 0.8113).

4.4.1 Relationship between ADRB3 genotype polymorphism and BMI

Details of the ADRB3 genotype polymorphism and BMI is shown in Table 6. No significant differences emerged for the ADRB3 gene Try64Arg genotype polymorphism frequencies between the cases and the controls for females (p = 0.2487, OR = 0.2308, 95% CI = 0.0235 to 2.367) and males (p = 0.2424, OR = 0.1250, 95% CI = 0.0078 to 2.000).

4.4.2 Relationship between ADRB3 genotype polymorphism and WHR

Details of the ADRB3 genotype polymorphism and WHR is shown in Tables 7a and 7b. No significant differences (all ps > 0.05) emerged for the ADRB3 gene Try64Arg genotype polymorphism frequencies between the cases and the controls.

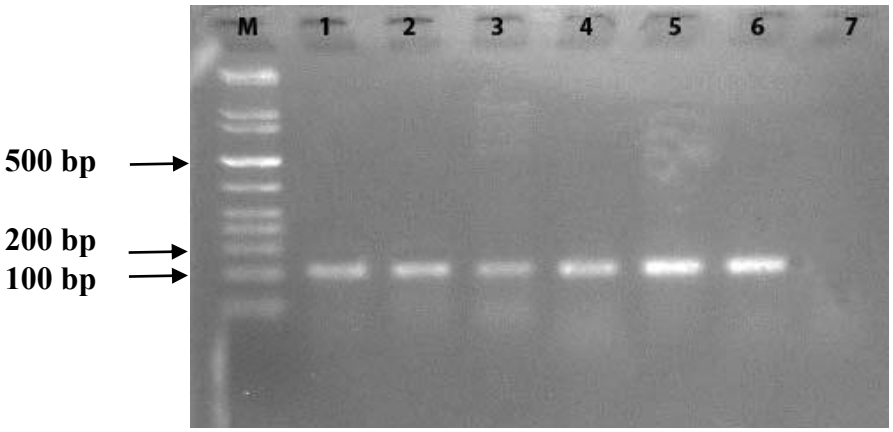


Figure 8. Ethidium bromide-stained 2.0% agarose gel electrophoregram of amplified ADRB3 DNA fragments (210 bp) from the children using BSTUP/BSTDOWN primers.
Lane M = 100 bp ladder; Lanes 1-6 = PCR positives; Lane 7 = negative control

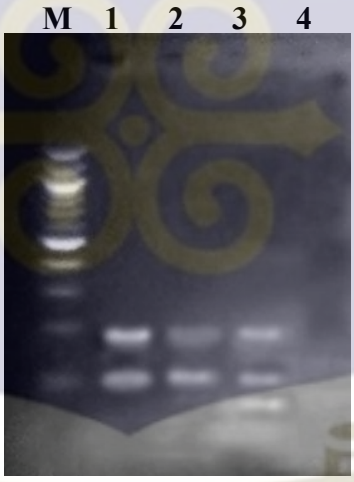


Figure 9: Ethidium bromide-stained 3.0% agarose gel electrophoregram of restriction fragments of ADRB3 amplicons following *Mva* I digestion.
Lane M = 100 bp ladder; Lanes 1 & 2 = Trp/Trp genotypes; Lane 3 = Arg/Trp genotype

Table 5: ADRB3 polymorphism among children

Genotype	Children		Total
	Cases	Controls	
Trp64Trp	3	6	**9**
Trp64Arg	17	5	**22**
Arg64Arg	1	3	**4**
Total	**21**	**14**	**35**

Table 6: Relationship between ADRB3 polymorphism and BMI of children

Genotype	Female		Male	
	Cases	Controls	Cases	Controls
Trp64Trp	2	2	1	4
Trp64Arg	13	3	4	2
Arg64Arg	1	2	0	1
Total	**16**	**7**	**5**	**7**

Table 7a: Relationship between ADRB3 polymorphism and WHR of the female children

Genotype	Cases		Controls	
	Yes*	No	Yes*	No
Trp64Trp	2	0	0	2
Trp64Arg	3	10	0	3
Arg64Arg	0	1	0	2

Yes = more 0.85 (WHO, 2011).

Table 7b: Relationship between ADRB3 polymorphism and WHR of the male children

Genotype	Cases		Controls	
	Yes*	No	Yes*	No
Trp64Trp	0	1	0	4
Trp64Arg	0	5	0	3
Arg64Arg	0	0	0	0

*Yes = more than 0.90 (WHO, 2011)

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSION

The effects of ADRB3 polymorphism were reported in 1995 (Clément *et al.*, 1995; Widén *et al*., 1995). The potential for weight gain among the population having the Trp/Arg mutation of the ADRB3 gene has been suggested (Clément *et al.*, 1995). This study aimed at studying the relationship between ADRB3 polymorphism and childhood obesity among some school children in Ghana.

Children recruited into the study were boys and girls between the ages of eight and fifteen years. Cases studied had BMI at or above the 95th percentile for their age and sex using the WHO growth charts. WHO growth charts for BMI (age and sex specific) were used because children are still growing with an accompanying change in body composition therefore BMI cut-offs for adults may not be appropriate (Cameron *et al*, 2006). Though BMI is not a direct measure of body fat (Nihiser *et. al*., 2007), it is still very useful and most frequently used tool for assessment of body fat percentage.

For both cases and controls, female children had significantly higher BMI values than the male children. A significantly higher percentage (above 60%) of females were also obese. Published studies have been inconsistent on the relationship between gender and obesity. For example, a study by Peltzer and Pengpid (2011) among a sample of 5613 Ugandan and Ghanaian children aged 13-15 years found a significant association between gender and overweight/obesity. In their study more girls were found to be more overweight/obese than boys. The sample population of the Ugandan study was largely

adolescents among whom several studies in developing countries have shown a positive relationship between the female gender and overweight and obesity (Armstrong *et al*., 2006).

Contrary to the findings above, studies by McDonald *et al*. (2009) among Columbian children (aged 5 to 12 years) and Schultz (2012) among Australian children (aged 5 to 15 years), showed that overweight and obesity were not associated to gender. According to Toriola *et al*., (2012) there is no significant gender differences in BMI at age groups 10, 11, and 12 years but found that girls at ages 13-16 years unveiled significantly higher mean BMI values compared to the boys.

Waist circumference and WHR are measures of abdominal or central obesity (Dekkers *et al*., 2008). Assessment of central obesity is important since it is associated with high risks of cardiovascular diseases (Mushtaq *et al*., 2011). Though mean WHR was significantly different (p = 0.0045) between both males and females, all the male participants were below values that indicated high risk for cardiovascular and metabolic diseases (Mushtaq *et al*., 2011). However, a few of the female cases (38.2%) were at increased risk for cardiovascular events.

The dietary pattern of the children showed a shift from the traditional diets toward the consumption of high fat and sugar sweetened drinks since all the children took fast foods and sweet drinks which are foods high in calories. The observed trend could be the impact of urbanization, westernization and food market globalization observed in urban areas of developing countries (Prentice, 2006; Popkin & Gordon-Larsen, 2004). Findings from this study revealed that female children had BMI values higher the male children. This could be attributed to their more frequent consumption of sweet drinks, fatty meat

and fast foods than their male counterparts. A positive association has been found between higher frequency of fast food intake and total fat among black and white girls in the US (Schmidt, 2005).

Daily consumption of fruits and vegetables were higher in males compared to females. Daily consumption of fruit and vegetables is an indicator of a healthy diet and contributes to a lower body mass index (Epstein *et al*., 2001). Epidemiological studies have also suggested that consuming more fruits and vegetables is associated with low risk of cancer and protective of cardiovascular diseases, obesity and diabetes (Jimenez-Cruz *et al*., 2002). Comparing cases to controls, sweet drinks and fatty meat were more frequently eaten by obese children (cases). This trend could contribute to the increasing prevalence of childhood obesity (Tucker, 1997; Heaney, 2002). Children in the control group (31%) consumed vegetables and fruits daily in contrast to 21% from the obese group (cases). The consumption of fruits and vegetables may impart protection against conditions like obesity (Jimenez-Cruz *et al*., 2002).

The adrenergic system plays a central role in the regulation of energy balance by stimulating both thermogenesis and lipolysis in brown and white adipose tissues in humans and various animal models (Lafontan & Berlan, 1993). The β_3-AR is located primarily in the small intestine, adipose tissue and vascular endothelium (Berkowitz *et al*., 1995) where it is involved in lipolysis, glucose uptake, cardio-inhibition and relaxation of colon, oesophagus and bladder (Bond *et al*., 2015). SNPs of ADRB3 were identified for the Trp64Arg allele. Seventeen cases and five controls had the Trp64Arg polymorphism. A significant difference emerged for the ADRB3 gene Try64Arg genotype polymorphism frequencies between the cases and the controls (p = 0.0377, OR = 0.1471, 95% CI = 0.02666 to 0.8113). However, no significant difference emerged for

the ADRB3 gene Trp64Arg genotype polymorphism frequency between the cases and the controls for BMI and WHR and thus an association of this polymorphism with obesity could not be established. Similarly, studies by Gagnon *et al*. (1996) and Matsushita *et al.* (2003) found no association between ADRB3 polymorphism and BMI. In contrast, Pietri-Rouxel *et al.* (1997) showed that in human cells with *ADRB3* variant allele, the reduction in cAMP accumulation in response to β3-adrenergic agonists resulted in decreased lipolysis and thermogenesis. Umekawa *et al*. (1999) also showed that *ADRB3* variant was associated with lower lipolytic activities in human omental adipocytes. Therefore, an impairment of *ADRB3* may lead to obesity through the energy expenditure reduction of fat tissue.

Meta-analyses on the association between the Trp64Arg polymorphism and body mass index (BMI) have also been published with inconsistent conclusions. For example, Allison *et al*. (1998) found no significant association using pooled data from 23 studies published before June 1997 including a total of 7399 individuals of different ethnicities. In contrast to the previous meta-analysis, Fujisawa *et al*. (1998) observed a small but significant effect of the Trp64Arg polymorphism on BMI, with a higher BMI for the Arg64-carriers compared to those individuals who were Trp64Trp homozygotes (mean difference¼0.30 kg/m2, 95% confidence interval (CI): 0.13–0.47).

There may also be ethnic-specific differences in the magnitude of the association between Trp64Arg genotype variant and BMI. Association between Trp64Arg genotype and BMI has been found to be statistically significant in East Asians but not in Europeans, although the difference between these two groups did not attain statistical significance (Kurokawa *et al*., 2008).

In the present study there were some limitations. The small sample size might limit the applications of the potential associations detected. Under-reporting or over-reporting of consumed foods by the children could also have occurred.

5.2 CONCLUSION

There were statistically significant differences in BMI and WHR between cases and controls (all *p*s < 0.05). SNPS in the receptors of the ADRB3 gene were detected in the children. No relationships were observed between ADRB3 (Try64Arg) genotype polymorphisms and BMI and WHR of the case and controls. The dietary pattern of the children indicated the consumption of high fat and sugar sweetened drinks which may contribute to childhood obesity. ADRB3 polymorphism may not be associated with obesity in children in Ghana.

It is recommended that

1. More work should be done in the area of genetics of obesity in children to be able to get a clearer picture of childhood obesity in Ghana.
2. A larger sample size should be considered during further studies in this area to establish any potential associations association between ADRB3 and obesity.
3. Children should be limited in their intake of high fat and sugar sweetened drinks.

REFERENCES

Arch JR & Kaumann A J. (1993). Beta 3 and atypical beta-adrenoceptors. *Med Res Rev*. **13** (6): 663-729.

Armstrong, ME, Lambert, MI, Sharwood, KA & Lambert, EV. (2006) Obesity and overweight in South African primary school children -- the Health of the Nation Study. *S Afr Med J* **96**(5): 439-444.

Arner P. (1995). The β_3-adrenergic receptor – a cause and cure of obesity? *N Engl J Med* **333**: 382–383.

Asare-Annan J (2011). Obesity and its determinants among Junior High School children in the Accra metropolis. MSc Thesis. University of Ghana, Legon.

Bagchi D. (ed) (2011). Global *Perspectives on Childhood Obesity: Current Status, Consequences and Prevention.* 1st edition. New York: Elsevier.

Bardou M, Rouget C, Breuiller-Fouché M, Loustalot C, Naline E, Sagot P, Frydman R, Morcillo EJ, Advenier C, Leroy MJ & Morrison JJ. (2007). Is the beta3-adrenoceptor (ADRB3) a potential target for uterorelaxant drugs? *BMC Pregnancy Childbirth*. **1**: 7 Suppl 1:S14.

Barlow SE & the Expert Committee (2007). Expert committee recommendations regarding the prevention, assessment, and treatment of child and adolescent overweight and obesity: summary report. *Pediatrics* **120** Suppl 4: S164-S192.

Berkey, C. S., Rockett, H. R., Field, A. E., Gillman, M. W., Frazier, A. L., Camargo, C. A., & Colditz, G. A. (2000). Activity, dietary intake, and weight changes in a longitudinal study of preadolescent and adolescent boys and girls. *Pediatrics*, *105*(4), e56-e56.

Bell, C.G., Walley, A.J. & Froguel, P. (2005). The genetics of human obesity. *Nature Review Genetics* **6**: 221-234.

Berkowitz DE, Nardone NA, Smiley RM, Price DT, Kreutter DK, Fremeau RT & Schwinn DA. (1995) Distribution of beta 3-adrenoceptor mRNA in human tissues. *Eur J Pharmacol* **289**: 223-228.

Black WK (1992). Cardiovascular risk factors. In B. L. Zaret, M. M. Moser & L. S. Cohen (Eds.), *Yale University School of Medicine Heart Book* (pp. 23-35). New York: Hearst Press.

Bond RA, Bylund DB, Eikenburg DC, Hieble JP, Hills R, Minneman KP, Parra S. (2015). Adrenoceptors: β_3-adrenoceptor. Last modified on 06/07/2015. Accessed

on 08/05/2015. IUPHAR/BPS Guide to PHARMACOLOGY, http://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=30

Bouchard C, Tremblay A, Després JP, Thériault G, Nadeau A, Lupien PJ, Moorjani S, Prudhomme D & Fournier G. (1994). The response to exercise with constant energy intake in identical twins. *Obes Res*. **2**(5):400-410.

Cameron N, Hastings G &and Ellison G (2006). Childhood Obesity: Contemporary Issues. CRC Press, Boca Raton.

Carlsson M, Orho-Melander M, Hedenbro J & Groop LC. (2001). Common variants in the beta2-(Gln27Glu) and beta3-(Trp64Arg)--adrenoceptor genes are associated with elevated serum NEFA concentrations and type II diabetes. *Diabetologia* **44**(5): 629-636.

Carriere G. (2003). Parent and child factors associated with youth obesity. Statistics Canada; 2003.

Carruth BR &, Skinner JD. (2001). The role of dietary calcium and other nutrients in moderating body fat in preschool children. *Int J Obes Relat Metab Disord* **25**:559-566.

Centers for Disease Control and Prevention. Preventing Obesity and Chronic Diseases Through Good Nutrition and Physical Activity Retrieved from: http://www.cdc.gov/nccdphp/pe_factsheets/pe_pa.htm.

Centers for Disease Control and Prevention. (2006). National Health and Nutrition Examination Survey. Retrieved from http://www.cdc.gov/nchs/nhanes.htm

Clement K &and Ferre P. (2003). Genetics and the pathophysiology of obesity. *Pediatr Res* **53**:721–725.

Clément K, Vaisse C, Manning BS, Basdevant A, Guy-Grand B, Ruiz J, Silver KD, Shuldiner AR, Froguel P & Strosberg AD. (1995). Genetic variation in the beta 3-adrenergic receptor and an increased capacity to gain weight in patients with morbid obesity. *N Engl J Med*. **333**(6):352-354.

Curhan GC1, Chertow GM, Willett WC, Spiegelman D, Colditz GA, Manson JE, Speizer FE & Stampfer MJ. (1996). Birth weight and adult hypertension and obesity in women. *Circulation* **94**(6):1310 –1315.

Dallman MF, Pecoraro N, Akana SF, La Fleur SE, Gomez F, Houshyar H, Bell ME, Bhatnagar S, Laugero KD & Manalo S. (2003). Chronic stress and obesity: a new view of "comfort food". *Proc Natl Acad Sci U S A* **100**(20):11696-11701.

Daniel WW. (1999). Biostatistics: A Foundation for Analysis in the Health Sciences. 7th edition. New York: John Wiley & Sons.

Deckelbaum RJ & Williams CL. (2001). Childhood obesity: the health issue. *Obes Res* **9**:S239–S243.

Delva, J., Johnston, L. D., & O'Malley, P. M. (2007). The epidemiology of overweight and related lifestyle behaviors: racial/ethnic and socioeconomic status differences among American youth. *American journal of preventive medicine*, *33*(4), S178-S186.

Dietz WH & Gortmaker SL. (2001). Preventing obesity in children and adolescents. *Annu Rev Public Health*. **22**:337–353.

Donnelly JE, Smith B, Jacobsen DJ, Kirk E, Dubose K, Hyder M, Bailey B & Washburn R. (2004). The role of exercise for weight loss and maintenance. *Best Pract Res Clin Gastroenterol* **18**(6):1009-1029.

Dorn GW 2nd (2010). Adrenergic signalling polymorphisms and their impact on cardiovascular disease. *Physiol Rev* **90**(3):1013-1062

Duke J, Huhman M & Heitzler C. (2002). Physical activity levels among children aged 9-13 Years—United States. *MMWR Morb Mortal Wkly Rep* **52**(33): 785-788.

Duncan, S. et al., (2011). Modifiable risk factors for overweight and obesity in children and adolescents from São Paulo, Brazil. *BMC Public Health* **11**(1): 585.

Eckel RH & Krauss RM. (1998). American Heart Association call to action: obesity as a major risk factor for coronary heart disease. AHA Nutrition Committee. *Circulation* **97**: 2099-2100.

Elgar FJ, Roberts C, Tudor-Smith C & Moore L. (2005). Validity of self-reported height and weight and predictors of bias in adolescents. *J. Adolesc. Health* **37**: 371-375.

Emorine LJ, Marullo S, Briend-Sutren MM, Patey G, Tate K, Delavier-Klutchko C & Strosberg AD. (1989). Molecular characterization of the human B3-adrenergic receptor. *Science* **245**:1118-1121.

Ene-Obong H, Ibeanu V, Onuoha, N & Ejekwu, A. (2012). Prevalence of overweight, obesity, and thinness among urban school-aged children and adolescents in southern Nigeria. *Food Nutr Bull* **33**(4): 242-250.

Epstein LH, Gordy CC, R aynor HA, Beddome M, Kilanowski CK & Paluch R. (2001). Increasing fruit and vegetable intake and decreasing fat and sugar intake in families at risk for childhood obesity. *Obes Res* **9**(3): 171-178.

Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, Perry JR, Elliott KS, Lango H. *et al*. (2007). A common variant in the FTO gene is

associated with body mass index and predisposes to childhood and adult obesity. *Science* **316**: 889–894.

Frederiksen L, Brødbaek K, Fenger M, Madsbad S, Urhammer SA, Jørgensen T & Borch-Johnsen K. (2003). No interactions between polymorphisms in the b3-adrenergic receptor gene and the PPAR-c gene on the risk of the insulin resistance syndrome in the Danish MONICA cohort. *Diabetologia* **46**: 729–731.

Freedman DS, Khan LK, Serdula MK, Dietz WH, Srinivasan SR & Berenson GS. (2005). The relation of childhood BMI to adult adiposity: the Bogalusa Heart Study. *Pediatr* **115**: 22–27.

Frielle T, Daniel KW, Caron MG & Lefkowitz RJ. (1988). Structural basis of beta-adrenergic receptor subtype specificity studied with chimeric beta 1/beta 2-adrenergic receptors. *Proc Natl Acad Sci U S A*. **85**(24):9494-9498.

Gagnon J, Mauriège P, Roy S, Sjöström D, Chagnon YC, Dionne FT, Oppert JM, Pérusse L, Sjöström L & Bouchard C. (1996). The Trp64Arg mutation of the beta3 adrenergic receptor gene has no effect on obesity phenotypes in the Quebec Family Study and Swedish Obese Subjects cohorts. *J Clin Invest*. **98**(9):2086-2093.

Garenc C, Pérusse L, Rankinen T, Gagnon J, Leon AS, Skinner JS, Wilmore JH, Rao DC & Bouchard C. (2001). The Trp64Arg polymorphism of the beta3-adrenergic receptor gene is not associated with training-induced changes in body composition: The HERITAGE Family Study. *J Obes Res*. **9**(6): 337-341.

Ghana School Survey (2013). Report of Ghana School Survey dissemination workshop – Accra September 12, 2013. Department of Nutrition and Food Science University of Ghana, Legon and School of Human Nutrition and Dietetics McGill University, Montreal In collaboration with School Health Education Program (SHEP) Ghana Education Service and Department of Biochemistry Kwame Nkrumah University of Science and Technology.

Ghana Statistical Service (GSS), Ghana Health Service (GHS), and ICF Macro. Ghana Demographic and Health Survey 2008. Accra, Ghana.

Goodman, E. &; Whitaker, R.C. (2002). A prospective study of the role of depression in the development and persistence of adolescent obesity. *Pediatrics* **110**: 497-504.

Gordon-Larsen P, Griffiths P, Bentley ME, Ward DS, Kelsey K, Shields K & Ammerman A. (2004). Barriers to physical activity: qualitative data on caregiver-daughter perceptions and practices. *Am J Prev Med* **27**: 218-223.

Granneman JG1, Lahners KN, Chaudhry A. (1993). Characterization of the human beta 3-adrenergic receptor gene. *Mol Pharmacol*. **44**(2):264-270.

Green SA, Turki J, Innis M & Liggett SB. (1994). Amino-terminal polymorphisms of the human beta 2-adrenergic receptor impart distinct agonist-promoted regulatory properties. *J Biochemistry*. **33** (32): 9414-9419.

Grundy SM. (1998). Multifactorial causation of obesity: implications for prevention. *Am J Clin Nutr* **67**: 563S-572S.

Gundersen C, Mahatmya D, Garasky S. & Lohman B. (2011). Linking psychosocial stressors and childhood obesity. *Obes. Rev*.**12**: e54-e63.

Hallman DM, Srinivasan SR, Chen W, Boerwinkle E & Berenson GS. (2004). The β_2-adrenergic receptor Arg16-gly polymorphism and interactions involving β_2- and β_3-adrenergic receptor polymorphisms are associated with variations in longitudinal serum lipid profiles: the Bogalusa Heart Study. *Metabolism* **53**: 1184–1191.

Hao K, Peng S, Xing H, Yu Y, Huang A, Hong X, Wang Y, Chen C, Wang B, Zhang X, Liu J, Zhu G, Huo Y, Chen D, Zhao X, Ronnenberg A, Wu D, Niu T & Xu X. (2004) . β_3-Adrenergic receptor polymorphism and obesity-related phenotypes in hypertensive patients. *Obes Res* **12**: 125–130.

Heaney RP, Davies KM &, Barger-Lux MJ: (2002). Calcium and weight: clinical studies. *J Am Coll Nutr* **21**:152S-155S.

Hill JO &, Peters JC. (1998): Environmental contributions to the obesity epidemic. *Science* **280**:1371-1374.

Hoffstedt J1, Poirier O, Thörne A, Lönnqvist F, Herrmann SM, Cambien F & Arner P. (1999). Polymorphism of the human beta3-adrenoceptor gene forms a well-conserved haplotype that is associated with moderate obesity and altered receptor function. *Diabetes* **48**(1):203-205.

Institute of Medicine, National Academy of Sciences. (1990). Nutrition During Pregnancy. Washington, DC: National Academy Press.

Jimenez-Cruz A, Bacardi-Gascon M & Jones EG. (2002). Consumption of fruits, vegetables, soft drinks, and high-fat-containing snacks among Mexican children on the Mexico- US. Border. *Arch Med Res* **33**: 74-80.

Kimani-Murage, EW, Kahn, K, Pettifor, JM, Tollman, SM, KlipsteinGrobusch, K & Norris, SA. (2011). Predictors of adolescent weight status and central obesity in rural South Africa. *Public Health Nutr* **14**(6): 1114-1122.

Kopelman PG. (2000). Obesity as a medical problem. *Nature* **404**: 635-643.

Kramoh KE, N'goran YN, Aké-Traboulsi E, Boka BC, Harding DE, Koffi DB, Koffi F &and Guikahue MK. (2012). Prevalence of obesity in school children in Ivory Coast. *Ann Cardiol Angeiol* **61**(3):145-149.

Krief S, Lönnqvist F, Raimbault S, Baude B, Van Spronsen A, Arner P, Strosberg AD, Ricquier D & Emorine LJ. (1993). Tissue distribution of □3-adrenergic receptor mRNA in man. *J Clin Invest* **91**:344-349.

Kurokawa N, Nakai K, Kameo S, Liu ZM &, Satoh H. (2001). Association of BMI with the b3-adrenergic receptor gene polymorphism in Japanese: meta-analysis. *Obes Res* **9**:741–745.

Lafontan M & Berlan M. (1993). Fat cell adrenergic receptors and the control of white and brown fat cell function**.** *J Lipid Res* **34**(7):1057-1091.

Liggett SB. (1997). Polymorphisms of the beta2-adrenergic receptor and asthma. *Am J Respir Crit Care Med.* **156**(4 Pt 2): SI56-162.

Link K, Moell C, Garwicz S, Cavallin-Stahl E, Bjork J, Thilen U, Ahren B & Erfurth EM. (2004). Growth hormone deficiency predicts cardiovascular risk in young adults treated for acute lymphoblastic leukemia in childhood. *J Clin Endocrinol Metab*, **89**:5003-5012.

Liu C, Mou S & Cai Y. (2013). FTO gene variant and risk of overweight and obesity among children and adolescents: a systematic review and meta-analysis. *PLoS One* **8**(11): e82133.

Liu ZQ, Mo W, Huang Q, Zhou HH (2007). Genetic polymorphisms of human beta-adrenergic receptor genes and their association with obesity. *Zhong Nan Da Xue Xue Bao Yi Xue Ban*. **32**(3):359-367.

Loos RFJ. (2009). Recent progress in the genetics of common obesity. *Br J Clin Pharmacol* **68**(6): 811-829.

Lowell BB & Bachman ES (2003) Beta-Adrenergic receptors, diet-induced thermogenesis, and obesity. *J Biol Chem* **278**(32): 29385-29388

Lustig R.H. (2011).Obesity Before Birth: Maternal and Prenatal influences on the offspring. p. 104. Springer Science + Business Media, New York.

Maffeis C, Zaffanello M, Pinelli L &, Schutz Y. (1996). Total energy expenditure and patterns of activity in 8-10-year-old obese and nonobese children. *J Pediatr Gastroenterol Nutr* **23**:256-261

Mahan KL, Escott-Stump S & Raymond (2012). Food and the Nutrition Care Process. 13th Edition, USA, Frazier Publishing Services. p.169

Mason DA, Moore JD, Green SA & Liggett SB. (1999). A gain-of-function polymorphism in a G-protein coupling domain of the human beta1-adrenergic receptor. *J Biol Chem* **274** (18): 12670-12674.

Matsushita Y1, Yokoyama T, Yoshiike N, Matsumura Y, Date C, Kawahara K & Tanaka H. (2003). The Trp(64)Arg polymorphism of the beta(3)-adrenergic receptor gene is not associated with body weight or body mass index in Japanese: a longitudinal analysis. *J Clin Endocrinol Metab* **88**(12):5914-5920.

McDonald, CM, Baylin, A, Arsenault, JE, Mora-Plazas, M &, Villamor, E. (2009). Overweight is more prevalent than stunting and is associated with socioeconomic status, maternal obesity, and a snacking dietary pattern in school children from Bogota, Colombia. *J Nutr* **139**(2): 370-376.

McVeigh, J. A., Norris, S. A., Cameron, N., & Pettifor, J. M. (2004). Associations between physical activity and bone mass in black and white South African children at age 9 yr. *Journal of Applied Physiology*, *97*(3), 1006-1012.

Mo W, Zhang GG, Yang TL, Dai XP, Li HH, Zeng H, Liu J, Tan YM, Zhou HH & Liu ZQ. (2007). The genetic polymorphisms of beta3-adrenergic receptor (AR) Trp64Arg and beta2-AR Gln27Glu are associated with obesity in Chinese male hypertensive patients. *Clin Chem Lab Med*. **45**(4):493-498.

Mogre V, Gaa PK, Nagumsi R & Abukari S. (2013). Overweight, Obesity And Thinness And Associated Factors Among School- Aged Children (5-14Years) In Tamale. *Eur Sci J* **9**(20):160-175.

Mohammed H and Vuvor F. (2012). Prevalence of childhood overweight/obesity in basic school in Accra. *Ghana Med J*. **46**(3): 124–127.

Musa DI, Toriola AL, Monyeki MA and Lawal B. (2012). Prevalence of childhood and adolescent overweight and obesity in Benue State, Nigeria. *Trop Med Int Health*. **11**:1369-1375.

Mushtaq, M.U., Gull, S., Abdullah H.M., Shahid, U., Shad, M.A. & Akram, J. (2011). Waist circumference, waist-to-hip ratio and waist-height ratio percentiles and central obesity among Pakistani children aged five to twelve years. *Biomed Central Pediatrics* **11**: 105.

Mutch DM & Clément K. (2006). Unraveling the genetics of human obesity. *PLoS Genetics* **2**:1956–1963.

Muthuri SK, Francis CE, Wachira LJ, Leblanc AG, Sampson M, Onywera VO and Tremblay MS. (2014). Evidence of an overweight/obesity transition among school-aged children and youth in Sub-Saharan Africa: a systematic review. *PLoS One*. **9**(3):e92846.

Nahmias C, Blin N, Elalouf JM, Mattei MG, Strosberg AD & Emorine LJ. (1991). Molecular characterization of the mouse beta 3-adrenergic receptor: relationship with the atypical receptor of adipocytes. *The EMBO Journal* **10**(12):3721-3727.

Narayan, K. V., Boyle, J. P., Thompson, T. J., Sorensen, S. W., & Williamson, D. F. (2003). Lifetime risk for diabetes mellitus in the United States. *Jama*, *290*(14), 1884-1890

NCMP (2014). National Child Measurement Programme: England, 2013/14 school year. Available at http://www.hscic.gov.uk/catalogue/PUB16070/nati-chil-meas-prog-eng-2013-2014-rep.pdf

Nicklas TA. (1995). Dietary Studies of Children - the Bogalusa Heart Study Experience. *J Am Diet Assoc* **95**:1127-1133.

Nihiser AJ, Lee SM, Wechsler H, McKenna M, Odom E, Reinold C, Thopson D. & Grummer-Strawn L. (2007). Body mass index measurement in schools. *J Sch Health* **77** (10): 651-671.

Ogden, C.L. & Carroll, M.D. (2010). Prevalence of overweight, obesity, and extreme obesity among adults: United States, trends 1976–1980 through 2007–2008. NCHS Health E-Stat. Hyattsville, MD: National Center for Health Statistics.

Overgaard D, Gamborg M, Gyntelberg F & Heitmann BL. (2004). Psychological workload is associated with weight gain between 1993 and 1999: analyses based on the Danish Nurse Cohort Study. *Int J Obes Relat Metab Disord*. **8**:1072-1081.

Peltzer, K &, Pengpid, S. (2011). Overweight and obesity and associated factors among school-aged adolescents in Ghana and Uganda. *Int J Environ Res Publ Health* **8**(10): 3859-3870.

Popkin BM & Gordon-Larsen P. (2004). The nutrition transition: worldwide obesity dynamics and their determinants. *Int J Obes* **28**:S2-S9.

Powell LM, Szczypka G & Chaloupka FJ. (2007). Adolescent Exposure to Food Advertising on Television. *Am J Prev Med* **33**(4S): S251-S256.

Prentice AM & Jebb SA. (1995) Obesity in Britain: gluttony or sloth? *Br. Med J.* **311**: 437- 439.

Prentice AM & Poppitt SD. (1996). Importance of energy density and macronutrients in the regulation of energy intake. *Int J Obes Relat Metab Disord* **20** (Suppl 2):S18–23.

Prentice AM. (2006). The emerging epidemic of obesity in developing countries. *Int J Epidemiol* **35**: 93-99.

Rankinen T, Rice T, Teran-Garcia M, Rao DC & Bouchard C. (2010). FTO genotype is associated with exercise training-induced changes in body composition. *Obes (Silver Spring, Md.)***18**(2): 322–326.

Saunders CL, Chiodini BD, Sham P, Lewis CM, Abkeich V, Adeyemo AA, de Andrade M, Arya R, Berenson GS, Blangero J, Boehnke M, *et. al*. (2007). Meta-analysis of genome-wide linkage studies in BMI and obesity. *Obes (Silver Spring)* **15**: 2263-2275.

Scherrer U, Randin D, Tappy L, Vollenweider P, Jéquier E & Nicod P. (1994). Body fat and sympathetic nerve activity in healthy subjects. *Circulation* **89**(6): 2634-2640.

Schmidt M, Affeinto SG, Striegel-Moore R, Khoury PR, Barton B, Crawford P, Kronsberg S, Schreiber G, Obarzanek E & Daniels S. (2005). Fast-food intake and diet quality in black and white girls. *Arch Pediatr Adolesc Med* **159**(7): 626-631.

Schultz R. (2012). Prevalences of overweight and obesity among children in remote Aboriginal communities in central Australia. Rural Remote Health 12: 1872.

Sekine, M., Yamagami, T., Handa, K., Saito, T., Nanri, S., Kawaminami, K., ... & Kagamimori, S. (2002). A dose–response relationship between short sleeping hours and childhood obesity: results of the Toyama Birth Cohort Study. *Child: care, health and development*, *28*(2), 163-170.

Senekal, M., Steyn, N. P., & Nel, J. H. (2003). Factors associated with overweight/obesity in economically active South African populations. *Ethnicity & disease*, *13*(1), 109-116.

Seth A & Sharma R. (2013). Childhood obesity. *Indian J Pediatr* **80**(4):309–317.

Shuldiner AR & Sabra M. (2001). Trp64Arg b3-adrenoceptor: when does a candidate gene become a disease-susceptibility gene? *Obes Res* **9**: 806–809.

Sipiläinen R1, Uusitupa M, Heikkinen S, Rissanen A & Laakso M. (1997) Polymorphism of the beta3-adrenergic receptor gene affects basal metabolic rate in obese Finns. *Diabetes* **46**(1):77-80.

Skinner JD, Bounds W, Carruth BR & Ziegler P. (2003). Longitudinal calcium intake is negatively related to children's body fat indexes. *J Am Diet Assoc* 103:1626-1631.pg

Snell, EK, Adam, EK &, Duncan, GJ. (2007). Sleep and the body mass index and overweight status of children and adolescents. *Child Dev* **78**(1): 309- 323.

Spraul M, Ravussin E, Fontvieille AM, Rising R, Larson DE & Anderson EA (1993). Reduced sympathetic nervous activity. A potential mechanism predisposing to body weight gain. *J Clin Invest* **92**(4): 1730-1735.

Strauss, RS &, Pollack, HA. (2001). Epidemic increase in childhood overweight, 1986-1998. *JAMA* **286** (22): 2845-2848.

Swinburn B &, Egger G. (2002). Preventive strategies against weight gain and obesity. *Obes Rev* **3**:289-301.

Tchernof A, Starling RD, Walston JD, Shuldiner AR, Dvorak RV, Silver K, Matthews DE & Poehlman ET. (1999). Obesity-related phenotypes and the beta3-adrenoceptor gene variant in postmenopausal women. *Diabetes* **48**(7):1425-1428.

Thomas B & Bishop J. (2007). Manual of Dietetic Practice. 4th ed. pp. 567-571. Blackwell Scientific Publishing Ltd, London.

Toriola AL, Moselakgomo VK, Shaw BS & Goon DT. (2012). Overweight, obesity and underweight in rural black South African children. *S Afr J Clin Nutr* **25**(2): 57-61.

Trogdon, JG, Finkelstein, EA, Feagan, CW & Cohen JW (2012). State- and payer-specific estimates of annual medical expenditures attributable to obesity. *Obes (Silver Spring)* **20** (1):214-220.

Troiano RP, Briefel RR, Carroll MD & Bialostosky K. (2000). Energy and fat intakes of children and adolescents in the United States: data from the national health and nutrition examination surveys. *Am J Clin Nutr* **72**(5 Suppl):1343S-1353S.

Tucker LA, Seljaas GT & Hager R.L. (1997). Body fat percentage of children varies according to their diet composition. *J Am Diet Assoc* **97**:981-986.

U.S. Department of Health and Human Services (2006). Healthy people.

Umekawa T, Yoshida T, Sakane N, Kogure A, Kondo M & Honjyo H. (1999). Trp64Arg mutation of beta3-adrenoceptor gene deteriorates lipolysis induced by beta3-adrenoceptor agonist in human omental adipocytes. *Diabetes* **48**(1):117-120

USDHHS, (2013). NIH Public Access. **13**(1): 61–69.

van Spronsen A, Nahmias C, Krief S, Briend-Sutren MM, Strosberg AD & Emorine LJ. (1993). The promoter and intron/exon structure of the human and mouse b3-adrenergic-receptor genes. *Eur J Biochem* **213**:1117-1124.

Walston J, Silver K, Bogardus C, Knowler WC, Celi FS, Austin S, Manning B, Strosberg AD, Stern MP, Raben N, *et al.* (1995). Time of onset of non-insulin-dependent diabetes mellitus and genetic variation in the beta 3-adrenergic-receptor gene. *N Engl J Med*. **333**(6):343-347.

WHO (2007). Growth reference 5-19 years. Available at http://www.who.int/growthref/cht_bmifa_girls_perc_5_19years.pdf http://www.who.int/growthref/who2007_bmi_for_age/en/

Widén E, Lehto M, Kanninen T, Walston J, Shuldiner AR & Groop LC. (1995) Association of a polymorphism in the beta 3-adrenergic-receptor gene with features of the insulin resistance syndrome in Finns. *New England Journal of Medicine* **333**(6): 348-351.

Wiecha JL, Peterson KE, Ludwig DS, et al. (2006). When Children Eat What They Watch: Impact of Television Viewing on Dietary Intake in Youth. Arch Pediatr Adolesc Med **160**(4): 436-442.

Willett W. (1998). Food Frequency Methods. In Nutritional Epidemiology Volume 5. 2nd edition. p74. Oxford University Press..

Williams CL. (2001) Can childhood obesity be prevented? In: Bendich A, Deckelbaum RJ, eds. Primary and Secondary Preventive Nutrition. Totowa, NJ: Humana Press. pp. 185–204

World Health Organization (2015). Fact Sheet: Obesity and Overweight. Fact sheet N°311. http://www.who.int/mediacentre/factsheets/fs311/en/

World Health Organization. (2000). Waist Circumference and Waist–Hip Ratio: Report of a WHO Expert Consultation Geneva, 8–11 December 2008. Geneva, Switzerland: World Health Organization.

World Health Organization. (2011). Obesity: Preventing and Managing the Global Epidemic. World Health Organization Technical Support Series No. 894. Geneva, Switzerland: World Health Organization

World Health Organization. World Health Organization Child Growth Standards. 2006.

Xinli W, Xiaomei T, Meihua P & Song L. (2001). Association of a mutation in the beta3-adrenergic receptor gene with obesity and response to dietary intervention in Chinese children. *Acta Paediatr* **90**(11): 1233-1237.

Zhang Y, Wat N, Stratton IM, Warren-Perry MG, Orho M, Groop L & Turner RC. (1996). UKPDS 19: heterogeneity in NIDDM: separate contributions of IRS-1 and beta 3-adrenergic-receptor mutations to insulin resistance and obesity respectively with no evidence for glycogen synthase gene mutations. UK Prospective Diabetes Study. *Diabetologia* **39**(12):1505-1511.

APPENDICES

Appendix I

PARTICIPANT INFORMATION SHEET

STUDY TITLE: ADRENOCEPTOR BETA 3 (ADRB3) GENE VARIANTS AND CHILDHOOD OBESITY

Childhood obesity is on the rise in Ghana and also predisposes a person to obesity in adulthood.

Genes and environmental factors contribute to obesity. Obesity is also a very important risk factor for several non-communicable diseases such as diabetes, cardiovascular diseases, hypertension, cancer, psychological problems and sleep apnea. These diseases have adverse consequences which can be life threatening. This study seeks to investigate the role of Fat mass and obesity associated gene, one of the major genes associated with obesity, in children.

Your child's participation in the research is entirely voluntary. You may decide to allow your ward partake or withdraw from the research at any point in time without anyone objecting. You would provide some information about yourself. Mouth rinse would be the sample to be collected and it is not invasive or harmful.

All information collected will be kept confidential. The forms will not bear personal identities but rather numbers or codes. Questionnaires will be used in this study. Body measurements will also be taken.

Results will be made known to you on request or otherwise held confidential. Your wards' participation in this study will be useful for the elucidation of the role of FTO in the pathogenesis of obesity and allow to separate patients with the tendency for higher body weight, in the development of new therapeutic options for this epidemic of the 21st century.

For further enquiries you can contact:

Dr. Charles Brown (University of Ghana, School of Biomedical and Allied Health Sciences) and Dr. Matilda Asante (University of Ghana, Head of Nutrition and Dietetic Department), supervisors of study.

Appendix II

ASSENT FORM

I have fully explained to _____ the nature and purpose of the above described research, its procedures, risks and benefits. I have allowed the subject to ask questions and will answer all questions relating to the study.

I _____ have read (or have had read to me in a language that I fully understand) and understand the nature of the proposed study. I am aware of the fact that my child can withdraw from the study at any point in time without receiving any objection. My signature or thumbprint below indicates that I have given the assent for my child to participate in this study.

Name of Researcher	Signature	Date

Name of Parent	Signature	Date

Appendix III

NAME OF PARTICPANT……

AGE…… GENDER……

ANTHROPOMETRIC MEASUREMENT

WEIGHT (kg)…… WAIST CIRCUMFERENCE (cm)……..

HEIGHT (cm)…… HIP CIRCUMFERENCE (cm)……

BMI…… WHR……

FOOD FREQUENCY QUESTIONNAIRE

TICK (√) IN THE APPROPRIATE BOX THE APPLICABLE

FOOD ITEM	DAILY	VERY FREQUENTLY	FREQUENTLY	RARELY	NEVER
Pastries (e.g.. cake, pie)					
Sweet drinks (e.g. Fanta, coke)					
Fatty meat (e.g. sausage, khebabs)					
Fastfood (e.g. fried rice, burger)					
Vegetables (eg onions, tomatoes)					
Fruits (e.g. orange, mango)					