DEPARTMENT OF DIETETICS

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CHILD FEEDING BEHAVIOURS, NUTRITIONAL STATUS AND ENERGY INTAKE REGULATION AMONG URBAN GHANAIAN CHILDREN

BY

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DECLARATION

I hereby declare that this thesis is the product of my original independent research conducted in the Ashiedu Keteke sub- Metropolitan District under the supervision of Dr. Matilda Asante, Professor Charlotte Wright, Dr Freda Intiful and Professor Richmond Aryeetey. I affirm that this work has neither been published nor submitted in whole or in part to any institution for any academic award. All references made to other researchers' works have been duly acknowledged.

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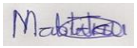

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DEDICATION

I dedicate this work to God Almighty, to my husband Steve and to my children Doxalyn, Adira and Charis. I also dedicate it to my parents, Mr and Mrs Arthur, and my siblings Mavis, Emmanuel, Enoch and Irene.

ABSTRACT

Background: The recent Ghana Demographic Health Survey indicates that infant malnutrition still perpetuates in Ghana. Energy regulation, which is the "internal driving force for search, choice, and ingestion of food", plays a critical role in the causation, prevention, and treatment of malnutrition. Children have an inherent ability to self-regulate energy intake (EI). Successful energy regulation is essential in maintaining a healthy body weight and overall wellness. It is not clear whether undernourished children regulate energy intake similarly to healthy children. This information is important, especially in settings where many children are undernourished. There is also a modicum of knowledge of how energy regulation develops in children and its effect on their nutritional status.

Aim: This study aimed to describe the extent to which undernourished children (with moderate acute malnutrition (MAM) and/or stunted) living in urban Ghana are able to regulate their energy intake and to determine whether genetic, behavioural and environmental factors predict this outcome.

Methodology: This was a two-phase study: a cross sectional study (n=262) and an experimental study (n=41) carried out among child-caregiver dyads (child 1-3 years old) at the Ashiedu Keteke Municipal district in Greater Accra Region-Ghana. The cross-sectional study carried out at selected child welfare clinics involved parental/ caregiver rating of children's dietary intake and eating behaviour using the International Complementary Feeding Evaluation Tool (ICFET). The questionnaire also assessed child's illness, household hunger and socioeconomic factors such housing characteristics, water and sanitation facilities. Saliva samples were collected from children for genetic testing for polymorphisms in genes associated with energy regulation. Nine single nucleotide polymorphisms (SNPS) were genotyped, and a polygenic risk score (PGRS) was generated. The experimental study was a within-subject crossover preload experiment conducted at the homes of randomly selected children from

phase 1 of the study. A standardized satiation methodology was employed to assess the ability to compensate for a drink taken before a meal. For the experiment, caregiver-child dyads were visited twice in their homes. Children were randomly assigned to two conditions: high energy preload drink supplying 10% of daily energy requirements or a low energy drink supplying 9kcal/100ml, after at least 2 hours fast. They were served identical ad libitum lunches 30 minutes after consuming the preload. Calories consumed from preload were calculated and calories from lunch meals were analysed using bomb calorimetry. The proportion of the preload compensated (COMPX) was calculated. The weight and height/recumbent length of all the children in both studies, were measured following standard protocols and converted to Z-scores using WHO Anthro and Anthro Plus software. Data was entered and analysed using SPSS software version 22. Chi square test was used to test associations between categorical variables. T-test and one-way ANOVA were used to compare means across groups. Linear regression and multinomial regression were used to test predictive associations of energy regulation and nutritional status. A p-value <0.05 at a 95% confidence level was considered as threshold for statistical significance.

Results: A total of 262 child-caregiver pairs (average age of child 19.25 ± 6.65 months) participated in the cross-sectional study, of which 75 were wasted (MAM), 53 were stunted, and 134 were classified as having normal anthropometry. There was a significant negative correlation between appetite and food refusal (r= -0.746, p<0.001), forced-feeding (r = -0.382, p<0.001) and caregiver feeding anxiety (r = -0.698, p<0.001). A positive correlation was seen between food refusal and forced-feeding (r= 0.351, p<0.001), food refusal and caregiver feeding anxiety (r= 0.624, p <0.001), as well as forced-feeding and caregiver feeding anxiety (r= 0.297, p<0.001). Wasted children had a significantly lower appetite z-score (-0.27 ± 1.1), a higher z-score for food refusal (0.22 ± 1.1) and a higher z-score for caregiver feeding anxiety (0.49 ± 1.0) compared to both stunted and healthy children (p<0.05). There were no significant

differences in feeding behaviour between the stunted and normal groups. Feeding anxiety was a consistently significant predictor of nutritional status after adjusting for confounders ($p<0.001$). The SNP rs2274333, situated near the CA6 gene, exhibited a higher frequency of homozygosity for the effect allele, AA, in children with wasting compared to their healthy counterparts. There was a significant association between PGRS and appetite ($p=0.046$). There was no significant association between PGRS and nutritional status. There were 41 children (17 MAM, 12 stunted and 12 healthy) in the experimental study. The overall average compensation was 62.43%. More wasted children had either minimal or no compensation (41.2%) or over-compensation (35.3%), and the majority (66.7%) of the stunted children had minimal or no compensation, and none over-compensated: this difference was statistically significant ($p=0.023$).

Conclusion: This study has given insight into the complex interaction between genetics, environmental factors, and nutrition among children. Although a longitudinal study would be more suitable for ascertaining causal directions, the findings from this study have implications for research, practice, and policy, especially regarding appetite, feeding behaviour and attitudes of caregivers. Efforts to prevent or treat undernutrition in children might benefit from focusing on modifiable factors such as dietary factors and feeding behaviours of both children and their caregivers. This also holds promise for precision nutrition as future and larger studies accentuate the genetics of undernutrition.

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LIST OF ABBREVIATIONS

AMA	Accra Metropolitan Assembly
ANOVA	Analysis of variance
BFHI	Baby Friendly Hospital Initiative
BMI	Body mass index
BMIZ	Body mass index z-score
CCK	Cholecystokinin
CEBQ	Child eating behaviour questionnaire
CFQ	child feeding questionnaire
CHO	Carbohydrates
CMAM	Community-Based Management of Acute Malnutrition
CNV	copy number variants
COMPX	Compensation index score
CWC	Child welfare clinic
DNA	Deoxyribonucleic acid
EA	Effect allele
EAH	Eating in the absence of hunger
EC	Energy compensation
ED	Energy density
EI	Energy intake
FTO	Fat mass and obesity associated
GDP	Gross domestic product
GHS	Ghana Health Service
GLP-1	Glucagonlike peptide 1
GSS	Ghana Statistical Service
GWAS	Genome wide association studies
HAZ	Height for age z-score
HED	high energy density
HEP	high energy preload
HES	High energy supplement
ICFET	International complementary feeding assessment tool
IMI	inter-meal interval
Kcal	Kilo calories

LED	Low energy density
LEP	low energy preload
LI	Legislative instrument
LMICs	Lower and low middle income countries
MAD	minimum acceptable diet
MAF	minor allele frequency
MAM	Moderate acute malnutrition
MDD	Minimum dietary diversity
MMF	Minimum meal frequency
NHANES	National Health and Nutrition Examination Survey
PAHO	Pan American Health Organization
PCR	Polymerase chain reaction
PGRS	Polygenic risk score
PML	Princess Marie Louise
RNA	Ribonucleic acid
RUSF	Ready to use supplemental foods
SAM	Severe acute malnutrition
SD	Standard deviation
SDG	Sustainable development goal
SNP	Single nucleotide polymorphism
SUN	Scaling up nutrition
UK	United Kingdom
UNICEF	United Nations International Children's Emergency Fund
WASH	Water, sanitation, and hygiene
WAZ	Weight for age z-score
WHO	World Health Organisation
WHZ	Weight for height z-score

CHAPTER 1

1.0 INTRODUCTION

1.1 BACKGROUND

Optimal nutrition in childhood is not only important at an early age, but also a key determinant of morbidity and mortality in adult years. The onset of multiple diseases in adulthood has been linked to early life events in utero or subsequently in childhood (Nobile *et al.*, 2022; Barker *et al.*, 2002; Kalkwarf *et al.*, 2003; Schack-Nielsen *et al.*, 2005). Childhood is also a period of food habit formation which may continue even in adult years (Huybrechts & De Henauw, 2007). Hence, establishing optimal dietary behaviour and preventing or successfully treating malnutrition during the childhood stage may be a very useful approach to achieving healthy adult years.

Nevertheless, infant malnutrition in all its forms: undernutrition, over-nutrition and micronutrient deficiencies remain a prevalent global health concern. Combating malnutrition is one of the greatest challenges in public health. It is a priority that is specifically pertinent to the United Nations' Sustainable Development Goals (SDGs) goal 2 ('Zero hunger'), which is intended to "end hunger, achieve food security and improved nutrition and promote sustainable agriculture" (United Nations, 2015). Childhood malnutrition remains common in low- and middle-income countries (LMICs). Undernutrition is the most prevailing form of malnutrition among young children and results from insufficient dietary energy and nutrient intake as well as non-dietary factors such as poor hygiene and sanitation as well as poor care. It mainly includes stunting, wasting and underweight, defined as height-for-age z-score (HAZ) < -2, weight-for-height z-score (WHZ) < -2, and weight-for-age Z-score (WAZ) < -2, respectively (WHO Multicentre Growth Reference Study Group, 2006). Globally, in 2020, stunting, wasting and overweight/obesity were, respectively, recorded among 149 million, 45 million and 38.9 million children below five years (WHO, 2021a). In recent years, Ghana has shown marked improvements in rates of malnutrition among children under five years old. However,

according to WHO criteria for public health significance, Ghana still has high rates of underweight (12%), medium rates of wasting (6%) and medium rates of stunting (18%) (Ghana Statistical Service (GSS) and ICF International, 2023; WHO, 2019).

Several factors are associated with undernutrition. The most common ones are inadequate dietary intake, poor dietary diversity, infectious diseases and environmental factors such as poor hygiene and sanitation conditions, poor child care practices, reduced access to proper health services, food insecurity, poverty, low maternal education and nutritional knowledge (Kavosi *et al.*, 2014; Mank *et al.*, 2020; Murarkar *et al.*, 2020; UNICEF, 2013). Current moderate acute malnutrition (MAM) screening methods in LMICS identify a mixed group of children who may be thin (wasted) or short (stunted) or both. Those who are thin are likely to have experienced a recent energy deficit due to illness or family disruption (WHO, 2010). However, those who are short, rather than thin, may not actually be currently malnourished, as much stunting has prenatal origins (WHO, 2023b).

Malnutrition originates from a complex interaction between genes and the environment, which often leads to alterations in hormones as well as metabolism, and behaviour (Freitas *et al.*, 2018; Wells *et al.*, 2021). Energy regulation determines food intake or avoidance and significantly contributes to the causation, prevention, and treatment of malnutrition (Lansigan *et al.*, 2015; Kane *et al.*, 2011; Carnell *et al.*, 2017; Almiron-Roig *et al.*, 2013). There is, however, limited knowledge of how children's ability to manage their energy intake influences their nutritional health, in the context of undernutrition in children (Dodd, 2007). Therefore, a good understanding of energy regulation in children is essential in designing interventions for tackling young child malnutrition.

Energy regulation is "the internal driving force for search, choice and ingestion of food" (De Graaf *et al.*, 2004). Food energy regulation is modulated by environmental, genetic and

physiological factors (de Castro, 2010; Freitas *et al.*, 2018). A combination of these factors defines the eating behaviour of an individual. Eating behaviour is "the attitude and psychosocial factors related to the selection and decision of which foods to eat" (Freitas *et al.*, 2018). Energy regulation represents the relationship between homeostatic and hedonic mechanisms (Lutter & Nestler, 2009; Hopkins, Blundell, Halford, King, & Finlayson, 2000). These two mechanisms are distinct but interconnected in function (Harrold *et al.*, 2012). They function mainly via neurotransmitters, hormones and brain areas to control feeding (Campos *et al.*, 2022). Recognising the interaction between these hormones, neurotransmitters and brain areas will help broaden our understanding of energy regulation and invariably improve interventions to address child feeding problems.

The homeostatic mechanism is regulated biologically to maintain the body's energy stores, increasing motivation for food intake (Freitas *et al.*, 2018). This is simply the drive to eat. The hedonic mechanism is controlled environmentally or socially, in the form of food rewards. This regulates the desire to eat food based on palatability and, therefore, acts mainly based on periods of abundance (Espel-Huynh *et al.*, 2018). This mechanism, therefore, describes the enjoyment of food. The interaction between the homeostatic and hedonic mechanisms maintains a balance between eating based on need and eating based on pleasure. This balance, ultimately, regulates an individual's eating.

It has been postulated that children have an internal food regulation ability (Hughes & Frazier-Wood, 2016). A child's eating self-regulation describes the activities that allow children to initiate and cease eating in a way consistent with maintaining their energy balance (Hughes & Frazier-Wood, 2016). This has been revealed to be genetically controlled (Carnell *et al.*, 2008; Faith *et al.*, 2013). For instance, research from a population-based birth cohort twin study has evidenced a linear relationship between appetite and adiposity (Llewellyn *et al.*, 2010). This reflects the role of genes in appetite regulation with a consequence on nutritional status.

Although the risk factors for obesity are complex, it has been previously revealed that obesity is highly heritable (Llewellyn *et al.*, 2008, 2010) and it is linked to a number of polymorphisms, many of which probably act through appetite regulation. Likewise, thinness has been projected to be heritable and stable across time points (Riveros-McKay *et al.*, 2019).

Despite the genetic influence of appetite, environmental factors, including caregiver feeding behaviour such as forced-feeding, can render a child's response to internal homeostatic stimuli ineffective and leave children focusing on external stimuli (Ha *et al.*, 2002; Dearden *et al.*, 2009). There seems to be a bidirectional relationship between caregiver feeding behaviours and a child's nutritional status. This means caregiver feeding behaviour could influence the child's nutritional status. Conversely, this may also be a reaction to a child's nutritional status (Engle & Lhotska, 1999). For instance, caregivers of children with low weight are more likely to force-feed them in response to the child's weight or because the children are intrinsically reluctant to eat. Studies have evidenced that parental food restriction is associated with concerns about a child being overweight or obese, while parental force-feeding is associated with greater concerns about a child being underweight (Warkentin *et al.*, 2018). These parental feeding practices have been evidenced to possibly lead to reduced interest in food or obesogenic eating behaviour of children (Gregory *et al.*, 2010). Food refusal in young children is common; malnourished children are more likely to refuse food (Mutoro *et al.*, 2020). Research in affluent settings has shown that healthy young children given a high energy drink regulate their energy intake, eating less at subsequent meals (Cecil *et al.*, 2005), but malnourished children regulate less effectively (Kasese-Hara *et al.*, 2002). Apparently, malnourished children may develop food refusal if treated with high energy supplementary (HES) drinks (Kasese-Hara *et al.*, 2002).

A more rigorous explanation and understanding of how children regulate their food intake could be well ascertained through a standardised satiation study (Birch & Deysher, 1985; Birch

& Deysher, 1986). This is a compensation trial that observes and examines children's ability to compensate or adjust for food intake following the administration of a preload (a small amount of food eaten at a fixed interval before an actual meal) and a subsequent ad libitum diet (Fatima *et al.*, 2015; Kane, Wright, Fariza, & Hetherington, 2011; Kasese-Hara, Wright, & Drewett, 2002; Faith, Carnell, & Kral, 2013).

1.2 PROBLEM STATEMENT

The 2022 Ghana Demographic Health Survey reported significant reductions in malnutrition from 2003 to 2022, with stunting decreasing from 35% to 18%, wasting from 8% to 6%, and underweight from 18% to 12% (Ghana Statistical Service (GSS) and ICF International., 2023). In a well-nourished population, the prevalence of WAZ, HAZ, or WHZ below -2 SD would be expected to be only 2.3-2.5%, yet Ghana's national figures are significantly higher. Wasting is more than twice, underweight five times, and stunting eight times more common than in a standard population. This underscores the need to prioritize malnutrition prevention and explore factors influencing infant nutritional status in Ghana.

Malnutrition severely impacts children's lives and has broader societal consequences due to limited human development and economic potential (Bryce *et al*, 2008; UNICEF, 2009). The causes of malnutrition are complex and multifaceted, including poor nutrition, hygiene, infections, ill health, inadequate feeding behaviors, psychosocial care, and poverty (Engle, 1997). Research indicates that malnutrition can result from undereating (Locher *et al.*, 2008) and that food intake and avoidance are centrally regulated by energy mechanisms (Hughes & Frazier-Wood, 2016). Studies on energy regulation describe individuals' ability to eat beyond hunger (satiation), potentially leading to overconsumption and affecting nutritional status, contributing to overweight/obesity (Lansigan *et al.*, 2015). Kane *et al.* (2011) found that healthy toddlers exhibit about 70% energy compensation. Some eating behaviors are heritable

(Llewellyn *et al.*, 2010), and gene polymorphisms associated with thinness might cause inherently low appetite or poor energy regulation, increasing undernutrition risk (Riveros-McKay *et al.*, 2019). Riveros-McKay *et al*. (2019) also suggested that genetic factors largely explain weight variations in a shared environment. Additionally, satiety responsiveness and food cue responsiveness have been found to be heritable (Carnell *et al*., 2008).

Despite previous research, gaps remain in understanding energy regulation and malnutrition. Data on energy regulation among children and its impact on nutritional status are scarce. Studies on eating behavior in young children in low- and middle-income countries (LMICs) are rare, especially regarding undernutrition. There is a lack of recognition of the importance of energy regulation and eating beyond hunger (satiation) in managing infant malnutrition. Existing studies on energy regulation in children primarily focus on those who are overweight or obese in low-infection-risk environments. While obesity is known to be heritable (Llewellyn *et al*., 2010), the genetic factors underlying thinness and shortness are not well understood

The treatment of moderate malnutrition (MAM) and long-term rehabilitation of severe acute malnutrition (SAM) has been underwhelming. Interventions typically involve high energy supplemental (HES) foods and drinks, with substantial global expenditure on these supplements (Heckert *et al*., 2020). However, trial evidence shows that HES foods and drinks have only modest effects on wasting and negligible effects on stunting (Lazzerini *et al.*, 2013). This lack of efficacy is often attributed to non-compliance (Maleta *et al*., 2004), but low appetite and food refusal may also play a role (Wright & Garcia, 2020). Thus, there is a pressing need for research into energy regulation, considering the genetics of thinness/stunting and feeding behavior among healthy and malnourished children in high-infection-risk environments.

1.3 AIM AND OBJECTIVES

1.3.1 Aim

This study aimed to describe the extent to which undernourished children (MAM and/or stunted) living in urban Ghana regulate their energy intake using a standardised satiation study methodology and to determine whether genetic, behavioural and environmental factors predict this.

1.3.2 Specific objectives

1. To determine the background characteristics of the study children with respect to nutritional status, dietary and socioeconomic characteristics.
2. To determine the association between feeding behaviour and nutritional status of children.
3. To calculate a polygenic risk score (energy regulation SNPs) and determine association with a child's nutritional status and feeding behaviour.
4. To compare energy regulation characteristics between undernourished and well-nourished children using a satiation study methodology.

1.4 RESEARCH QUESTIONS

1. Are undernourished children's feeding behaviour different from that of well-nourished children?
2. Are undernourished children (MAM or stunted) more likely to carry common gene polymorphisms associated with thinness (anti-obesity SNPs) than healthy children?
3. Do undernourished children show satiation patterns comparable to healthy children described in the literature?

1.5 NULL-HYPOTHESES

1. Undernourished children will not have different feeding behaviours than healthy children.
2. Undernourished children will not carry more gene polymorphisms associated with thinness or shortness than healthy children.
3. Children with MAM will not have different satiation patterns compared to healthy children.
4. Satiation patterns of stunted children will not be different from those of healthy children.

1.6 SIGNIFICANCE OF STUDY

The findings from this study will have implications for both practice and policy. Essentially, this study will provide an understanding of the child characteristics predisposing to undereating and how these characteristics operate. This study will also provide evidence to support the expansion of genetics studies on persistent malnutrition by providing evidence on the heritability of thinness and shortness.

The outcome of this study will ultimately provide a fresh and broader insight into infant malnutrition in terms of the genetic and environmental control of energy regulation. It will, therefore, serve as a roadmap for public health experts and clinicians to better manage or treat malnutrition. This is because highlighting the genes and environmental factors that influence malnutrition will help to tailor nutrition interventions towards the unique attributes of the child. More importantly, this study will contribute to expanding African genomics, as the African population is highly underrepresented in genetic studies.

1.7 CONCEPTUAL FRAMEWORK OF THE STUDY

Figure 1.1 presents the conceptual framework of the study. This framework was adopted from the UNICEF conceptual framework on the Determinants of Maternal and Child Nutrition (UNICEF, 2021) and slightly modified for this study. A child's nutritional status is directly influenced by dietary intake and genes that work through appetite regulation (Engle, 1997; Carnell, Haworth, Plomin, & Wardle, 2008). There seems to be a bidirectional association between a child's nutritional status and dietary intake (Engle, 1997). For instance, an obese child may eat more because he is obese or obese because he eats more. There is also a bidirectional association between health and nutritional status (Engle, 1997). Generally, dietary, or nutrient intake is influenced by the availability of food and dietary diversity (Engle 1997), which also influences food energy regulation. The caregiver's style of feeding and the child's attitude during meals also influence how much food is consumed (Dearden *et al.*, 2009; Ha *et al.*, 2002). Dietary intake has also been found to have a genetic predisposition, with some researchers finding the heritability of satiety and food cue responsiveness (Carnell, Haworth, Plomin, & Wardle 2008) as well as taste perception and overall food acceptance (Cont *et al.*, 2019). Ultimately, these factors, child and caregiver feeding behaviour, health status of the child, food availability and genetic factors may influence the child's food energy regulation and, invariably, their nutritional status.

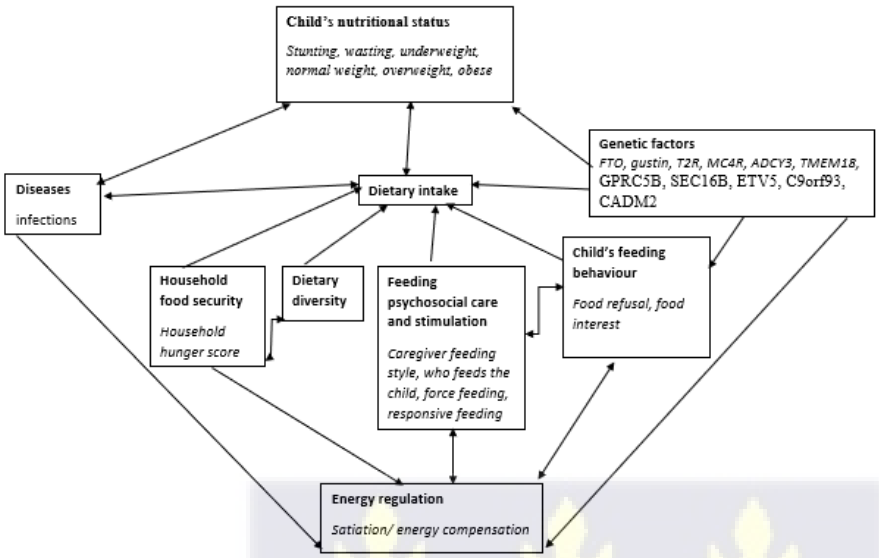


Figure 1.1 – Conceptual framework of the study

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 EPIDEMIOLOGY OF MALNUTRITION

Global estimates of malnutrition among children under five years old show steady progress towards achieving the 2025 World Health Assembly's goal and the 2030 SDG goal 2 (WHO, 2023b). Globally, about 462 million adults are underweight, and 1.9 billion are either obese or overweight (WHO, 2021b). As of 2020, an estimated 149 million children below age five were stunted, 45 million were wasted, and 38.9 million were overweight or obese (WHO, 2021b). Amongst countries worldwide, Africa has the highest prevalence of undernutrition, and this was estimated to be about 20% of the African population (Adeyeye *et al.*, 2023). Undernutrition is more prevalent in sub-Saharan Africa, with data from 2014-2016 revealing that over 200 million people in sub-Saharan Africa were undernourished (Adeyeye *et al.*, 2023). The 2022 Ghana Demographic Health Survey reported a reduction in the prevalence of infant malnutrition from 2003 to 2014. Stunting was reduced from 35% to 18%, wasting from 8% to 6% and underweight from 18% to 12% (Ghana Statistical Service (GSS) and ICF International., 2023). Among the adult population (15 - 49 years), data from 2014 recorded underweight and overweight or obese to be 6.2% and 40.1%, respectively, among women and 10.1% and 16.8% respectively, among men (GSS & GHS, 2015).

The causes of undernutrition are vast and complex. To provide a better understanding of these causes, the United Nations Children's Fund (UNICEF) developed a framework on the determinants of maternal and child nutrition (Figure 2.1) (UNICEF, 2021). The Framework employs an optimistic approach to understanding what promotes good nutrition in children and women. It offers clear concepts about the factors enabling, underlying, and directly influencing adequate nutrition. It also explains how these factors are interconnected vertically and

horizontally. Furthermore, it highlights the positive outcomes in survival, growth, development, learning, and socioeconomic status that arise from enhanced maternal and child nutrition. Details of the framework is presented in figure 2.1.

A case-control study conducted among preschool children aged between three to six years in Udupi district of Karnataka, India, showed that low parental education, childhood illness, short birth interval, open defecation, type of weaning and complementary food given to children were some of the significant determinants of underweight (Ansuya *et al.*, 2018). These findings are comparable to the causes outlined in the UNICEF framework.

Again, a recent review has reiterated that poverty is a major cause of hunger and malnutrition in Africa (Adeyeye *et al.*, 2023). It is well documented that Africa is the poorest continent across the globe, with over 500 million Africans living in abject poverty. Data suggests that most African countries have very low gross domestic product (GDP), with over 28 African countries recorded among the 37 countries across the nations of the world that depend on food aid (Adeyeye *et al.*, 2023; Adeyeye *et al.*, 2017). It is not surprising to have such a high prevalence of undernutrition in sub-Saharan Africa, as undernutrition is also caused by an inability to achieve daily food energy demands over a long period of time, a problem that is common in Africa. Availability and accessibility of food are determined by economic resources as outlined in the UNICEF framework as a basic determinant of malnutrition. This means that most families in Africa are food-poor, hence, the high rate of undernutrition.

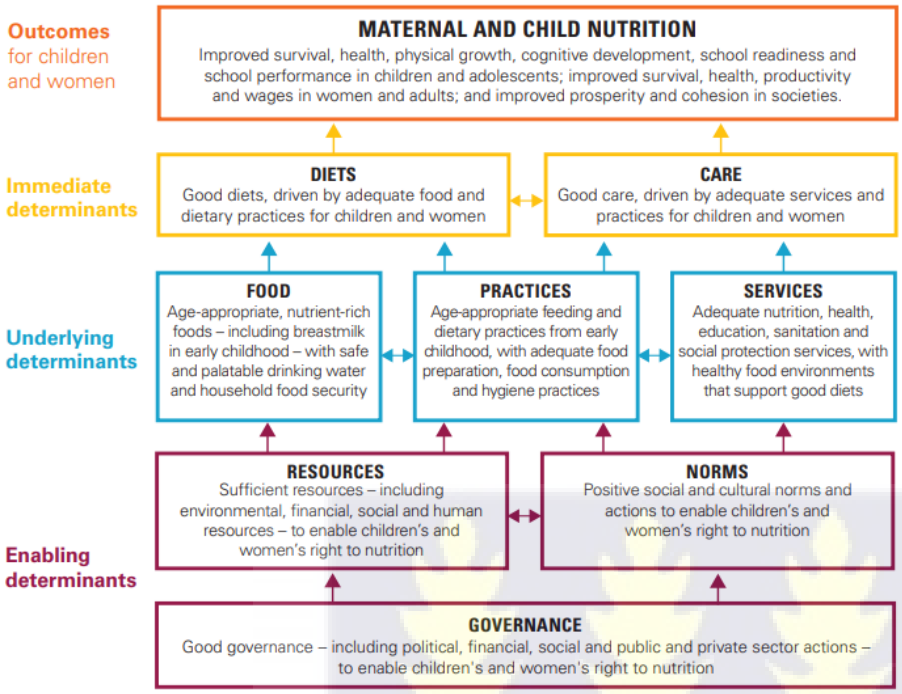


Figure 2.1: UNICEF Conceptual Framework on the Determinants of Maternal and Child Nutrition, 2020 (UNICEF, 2021)

2.2 ENERGY REGULATION

It is posited that children have an inherent drive to control their energy intake. Child-intrinsic characteristics such as neophobia, temperament and sensory perception help them respond to hunger and satiety cues or signals (Hughes & Frazier-Wood, 2016; Blissett & Fogel, 2013). However, it is uncertain why preschool age children exhibit differing abilities to self-regulate their food intake (Huybrechts & De Henauw, 2007). This difference in self-regulation abilities could be attributed to variations in environmental exposure, which may potentially disrupt the innate ability that children possess to self-regulate their dietary habits. Some documented environmental factors that influence children's food intake or acceptance are repeated exposure

to novel foods, exposure to a variety of foods, family food environment, breastfeeding, age of weaning, the taste of food, the energy content of food, parental and peer modelling, and pressure to eat (Blissett & Fogel, 2013; Cooke, 2007; Issanchou, 2017). The phenotypic evidence of energy regulation is related to the complex process of appetite response. Differences in appetite can be detected in infancy and have been seen to be associated with infant growth. Adiposity at ages 1, 2 and 3 years have been demonstrated to be associated with faster sucking in early infancy (Agras *et al.*, 1990; Stunkard *et al.*, 2004). Additionally, excess weight gain during the second 6 months of life has been seen to be associated with infant-initiated bottle-emptying during the first six months (Li *et al.*, 2008).

Food intake or avoidance is centrally determined by energy regulation; the success of it is closely related to an individual's nutritional status. Energy regulation mainly hinges on an interplay between homeostatic and hedonic mechanisms (Lutter & Nestler, 2009; Hopkins, Blundell, Halford, King, & Finlayson, 2000). These two mechanisms are distinct but functionally interconnected (Harrold *et al.*, 2012).

Homeostatic control of food involves psychophysiological processes that initiate appetitive signals to either stimulate or terminate food intake (Campos *et al.*, 2022). These processes are usually hormonally controlled by gut hormones such as ghrelin, insulin and cholecystokinin, as well as hypothalamus peptides such as dopamine (Liu & Kanoski, 2018). These processes control hunger and satiation and, therefore, act in the pre-prandial and prandial phases of food intake (Blundell *et al.*, 2010; Blundell, 1991). This means that the homeostatic mechanism is largely biologically controlled.

Biologically, energy is regulated by hormones such as cholecystokinin (CCK), Glucagon-like peptide-1 (GLP-1), ghrelin and insulin (Freitas, Albuquerque, Silva, & Oliveira, 2018). These hormones may be orexigenic (e.g. CCK, GLP-1, insulin) and, therefore, stimulate appetite and

enhance food intake by signalling the sensation of hunger and the need for food. They may also be anorexigenic (e.g. ghrelin and leptin) by signalling the feeling of satiety and the need to cease or avoid food intake (Watterson *et al.*, 2013). These hormones are essential to keeping a healthy balance between food intake and avoidance. This is because when appetite suppression is blunted, obesity and associated comorbidities ensue, and when suppressed, it causes cachexia (Watterson *et al.*, 2013).

The hedonic mechanism of food is largely controlled by its physical appearance, such as its taste and smell (Saper *et al.*, 2002). The palatability of food, therefore, influences this mechanism. Indeed, the consumption of highly palatable energy dense foods affords some level of excitement that has been found to be explained by the release of neurotransmitters dopamine and serotonin (Lutter & Nestler, 2009). There appears to be a direct association between levels of dopamine and the level of enjoyment experienced from food intake (Small *et al.*, 2003). On the other hand, serotonin controls mood and regulates appetite by promoting the motivation to eat (Saper *et al.*, 2002). Although there are individual differences in responsiveness to palatable foods, they are generally known to activate the reward system. Palatable foods are usually high in sugar and fats. Therefore they have the tendency to disrupt appetite regulation by blunting the response to satiety signals (Erlanson-Albertsson, 2005). This may prolong meal duration and increase food intake.

2.3 ENVIRONMENTAL CONTROL OF ENERGY REGULATION

Generally, environmental factors that influence a child's nutrition are classified under the underlying determinant of a child's survival, growth and development (Engle, 1997). These environmental factors are mainly linked with caregiving, including health services and a healthy environment, care for women, breastfeeding, feeding-related psychosocial care, food processing, hygiene practices, home health practices and household food security.

2.3.1 Caregiving

Inadequate care is classified as an underlying cause of undernutrition that needs to be tackled. Care has been defined as "behaviours and practices of caregivers (mothers, siblings, fathers and child care providers) to provide time, attention, stimulation, emotional support and discipline necessary for a child's healthy growth, self-regulation, cognitive development, language acquisition as well as socio-emotional adjustment" (Maleta, 2006; Engle & Lhotska, 1999b). In the context of infant nutrition, care includes the activities of caregivers that impact the child's nutrient intake, health, growth and development (Engle *et al.*, 1999). According to the UNICEF conceptual framework, these care activities include psychosocial care, food preparation and related practices, young child feeding, household hygiene practices and personal hygiene (Engle, 1997). Care activities extend to infants and young children, as well as women of childbearing age; however, this review focuses on the care activities of infants and young children.

Poor care practices such as early and inadequate breastfeeding, delayed introduction of complementary foods, poor hygiene practices, lack of dietary diversity, the presence of diseases such as diarrhoea, time spent on care activities, child's gender, mother's education level and poor health-seeking behaviour have been associated with poor nutrition outcomes in infants and young children (Baig-Ansari *et al.*, 2006; Demissie, 2013; Garg & Chadha, 2009; Gibson *et al.*, 2009; Teshome *et al.*, 2010; Zhang *et al.*, 2009). These factors are highly variable depending on the setting. For example, in Pakistan, gender, food security and maternal schooling were identified as factors that led to undernutrition (Baig-Ansari *et al.*, 2006). In Ethiopia, an association was reported between stunting and feeding methods (hand and bottle feeding), poor health-seeking behaviour, types of food given to children, and the lack of sanitation facilities (Teshome *et al.*, 2010).

Although informative, these studies only show associations rather than the actual cause. The methods used for data collection in these studies are subject to bias. Under-reporting of negative behaviour and over-reporting of positive behaviours are common in cases where interviews are used as a method of data collection. This may not fully reflect the actual reality as data is collected at only one time point.

Observation studies in homes provide a relatively clear picture of care practices depending on how many observations are carried out. This is, however, very rare, probably due to the difficulties in carrying out studies in people's homes. Nonetheless, a case control observation study carried out in Nepal showed that children who were neglected and not encouraged to eat, as well as children who served themselves, were more likely to be undernourished (Gittelsohn *et al.*, 1998). In this study, researchers spent an entire day in the homestead observing activities in the home. This was done for seven days to ensure that the family was comfortable with the presence of the observer. Poor nutrition status, defined as the presence of vitamin A deficiency, was associated with harsh negative treatment from caregivers. Another observational study that involved videotaping the participants in their homes during feeding revealed that children who received positive comments from their caregivers during feeding were 2.4 times as likely to accept bites compared with children whose caregivers provided no comments (Dearden *et al.*, 2009). Additionally, a study was conducted in Kenya to assess the association between time allocation to child care and the nutritional status of children younger than 2 years old (Kamau-Thuita *et al.*, 2002). The results demonstrated that malnourished (stunted) children had less time devoted to their care, breastfeeding, food preparation and feeding.

These studies are useful, although it should be noted that observation studies are intrusive and equally subject to bias as participants are likely to change their behaviour under research scrutiny. Subsequent studies have attempted to overcome this by making several visits to the participants' homes to make them feel at ease and comfortable (Gittelsohn *et al.*, 1998),

spending the entire day in their homes (Dearden *et al*., 2009) or by conducting surprise visits (Kamau-Thuita *et al*., 2002). The use of mixed methods might be more useful in overcoming the above challenge and providing robust data on behavioural studies.

2.3.2 Responsive care and undernutrition

Childcare should be provided responsively. Responsive care for children emphasizes the caregiver's ability to observe and respond to a child's cues, which are typically gestures, movements or vocalizations (WHO *et al.*, 2018) It also includes responsive feeding, which is particularly crucial for undernourished or ill children (WHO *et al.*, 2018). Lack of responsive caregiving affects a child's growth and development mainly because poor care practices such as non-responsive feeding, poor hygiene and sanitation may increase a child's vulnerability to nutrient deficiencies and common childhood diseases such as diarrhoea. These conditions have a negative effect on metabolism and appetite (Maleta, 2006).

The importance of childcare is evident in studies which have looked at resilience (positive deviance) in disadvantaged populations. Resilience can be described as a process which enables an individual to successfully adapt to and overcome difficult situations and perform well compared to other people living in the same environment (Yousafzai *et al.*, 2013). In the context of nutrition, resilience or positive deviance refers to well-nourished children from impoverished settings (Chek *et al.*, 2022; Berggren & Wray, 2002).

Optimal care is a predictive factor of positive deviance, as some studies have shown that caregivers of positive deviant children have better feeding and hygiene practices as compared to their counterparts (Kanani & Popat, 2012; Mackintosh *et al.*, 2002; Marsh *et al.*, 2004). For example, in a study in rural Ghana, caregivers of positively deviant children were reported to have better household and personal hygiene and feeding practices compared to caregivers of negatively deviant children (Nti & Lartey, 2008). Additionally, in a survey in an urban area in

Accra, Ghana, care practices were strong determinants of the nutrition status of children from poor backgrounds (Ruel *et al.*, 1999). Poor maternal schooling and poverty were shown to influence nutrition status only if mothers were poor carers. This implies that if a child gets positive attention and care, they are likely to grow well despite unfavourable environmental conditions.

2.3.3 Infant feeding practices

Feeding practices early in life have been strongly associated with health in adulthood. Optimal feeding in infancy and childhood has both long term and short-term health benefits spanning from childhood into adulthood (Dewey & Begum, 2011; Rolland-Cachera, Akrout, & Péneau, 2016; Yang & Huffman, 2013). The first two years of life present a critical window of opportunity for ensuring children attain optimum growth and development through optimal feeding (Cusick & Michael, 2013). Nutritional deficits within this period can result in long term impairment on the health and development of children. Given this knowledge, the recommendation for infant feeding has been centred on exclusive breastfeeding for six months and continued breastfeeding in addition to appropriate complementary foods until or beyond the 2nd birthday of the infant (UNICEF, 2012; WHO, 2017). This review will focus on feeding practices in children, i.e., breastfeeding and complementary feeding.

2.3.3.1 Breastfeeding

Breastfeeding is critical to infants' health and wellbeing both in the short- and long-term; there are also benefits for the mother (Sattari *et al.*, 2019). The numerous benefits of breastfeeding extend beyond its nutritional content to include the act of suckling at the breast (Sattari *et al.*, 2019). Human milk contains all the macro and micronutrients necessary for the growth and well-being of the infant, especially during the first six months of life (Ballard & Morrow, 2013). Other established benefits of breastfeeding include the physical exercise of the mouth

from suckling, skin-to-skin contact between mother and infant, the provision of antibodies and immune cells for innate immunity in the infant, growth factors responsible for maintaining intestinal integrity as well as other immune modulatory factors (Goldman, 2012; Young, 2017). Breastfeeding also protects against diarrhoea-specific morbidity and mortality throughout the first two years of life (Lamberti *et al.*, 2011). Skin-to-skin contact is essential in newborn temperature regulation and the release of oxytocin needed for breast milk production (Safari *et al.*, 2018).

The benefit of breastfeeding to the infant is not just limited to the composition of breast milk but also includes the mode of feeding. More interestingly, and in the context of this research work, breastfeeding plays a significant role in the regulation of appetite and satiety, with a consequent effect on infants' growth and development (Ramirez-Silva *et al.*, 2021). It has been postulated that children who are directly breastfed have a more efficient appetite regulatory mechanism than those who are bottle-fed (Ramirez-Silva *et al.*, 2021).

In a retrospective study among children aged 3-6 years, researchers Disantis *et al.* (2011) observed behavioural distinctions among children directly fed from the breast and those bottle fed human milk or formula. Specifically, they measured appetite regulation in children using the child eating behaviour questionnaire, focusing on three constructs- satiety response, food responsiveness and enjoyment of food. The results showed that although there were no differences in the growth of the children in any of the feeding categories, children who were directly breastfed had better appetite regulation than those who were bottle-fed human milk. Similarly, an earlier study was conducted to test the association between feeding modes (bottle feeding or direct breastfeeding) and infant milk intake self-regulation. The authors demonstrated that children who were extensively fed from the bottle, regardless of milk type, were more likely to empty the bottle in late infancy than those who were directly fed from the breast (Li *et al.*, 2010). This implies that children who were bottle fed had a lower ability to

self-regulate their milk intake. More pragmatically, Brown and Lee (2012) studied the association between breastfeeding and appetite responsiveness among children. This was a two-phase study that had 298 mothers reporting on breastfeeding practices up to 6 months post-partum in the first phase. In the second phase, the mothers were made to complete the Child Eating Behaviour Questionnaire (CEBQ) with a particular focus on food responsiveness and satiety responsiveness. Breastfeeding for a longer duration was directly linked to increased satiety responsiveness.

The connection between breastfeeding and energy regulation depends on who controls satiety (Disantis *et al.*, 2011). It is suggested that satiety for directly breastfed children is baby-led, as satisfaction during feeding is largely dependent on the infant's ability to stop suckling when full. In this case, the mother has no knowledge of how much milk the baby has taken. She only relies on the infant's external signals for hunger and satiety and responds accordingly. On the other hand, satiety for bottle-fed children is mostly caregiver-led, with the caregiver determining how much is enough for the baby. Hence, satiety for directly breastfed children is internally controlled, with caregivers more reliant on infant cues, while satiety for bottle-fed children is externally controlled, with caregivers reliant on feeding time and schedule and mostly encouraging bottle emptying (Srivastava & Jain, 2023). This assertion has been earlier demonstrated in a study conducted in Australia; the authors reported that directly breastfed infants only ingest 67% of available breastmilk, implying that directly breastfed infants cease milk intake once they reach satiation (Kent *et al.*, 2006).

Although improving globally, the practice of breastfeeding is still suboptimal, contributing to more than 800,000 child deaths annually across the globe (Tongun *et al.*, 2019). In Ghana especially, breastfeeding remains suboptimal (Tampah-naah & Kumi-kyereme, 2013). The rate of exclusive breastfeeding increased from 2% in 1993 to 63% in 2008 (Tampah-naah & Kumi-kyereme, 2013); however, a decline (53%) was reported based on the 2022 Ghana

Demographic and Health Survey (GDHS) (Ghana Statistical Service (GSS) and ICF International., 2023). The mean duration of exclusive breastfeeding reduced from 4.4 months in 2008 to 3.9 months in 2014 (Ghana Statistical Service, Ghana Health Service, & ICF International, 2014). However, 43% of infants were breastfed till 23 months in 2008 compared with 67.2% in 2022 (Ghana Statistical Service & ICF International, 2023; Ghana Statistical Service Ghana Health Service & ICF International, 2008). This continuous decline in breastfeeding duration could be explained by challenges faced by mothers during this period.

Some of the established barriers to breastfeeding are inadequate knowledge and skills on breastfeeding (Mogre *et al.*, 2016), reliance on unprofessional support for breastfeeding (Diji *et al.*, 2017), labour market influence such as shorter maternity leave and inflexible working hours (Danso, 2014), and unhindered access to breastmilk substitutes (Tampah-Naah & Kumi-Kyereme, 2013). These barriers could be reviewed under five levels: individual level, interpersonal level, community level, organizational level, and policy level.

At the individual level, most mothers experience difficulties in practising breastfeeding owing to challenges at the preparatory stages (before delivery) and transition stage (transitioning from breast milk to solid foods). A major challenge hindering the practice of breastfeeding at the individual level is inadequate knowledge and skills in breastfeeding (Mogre *et al.*, 2016). Although the importance and recommendation for breastfeeding are universally known (Asare *et al.*, 2018), most Ghanaian mothers are not adequately equipped with knowledge and skills on breastfeeding during the prenatal phase. Most mothers perceive breastmilk insufficient for a child (Diji *et al.*, 2017; Mogre *et al.*, 2016; Otoo *et al.*, 2009). Evidence suggests that some mothers perceive that exclusive breastfeeding results in a child's refusal of family foods (Aryeetey & Goh, 2013). During complementary feeding, there is complete cessation of breastfeeding because most mothers are not aware of the nutrient contribution of breastmilk. Some are faced with lactation challenges, such as a lack of skills in putting the baby to the

breast, breast engorgement, and nipple soreness, among others (Tampah-Naah *et al.*, 2019), thus affecting their confidence to breastfeed. This ultimately deters them from breastfeeding appropriately. Another challenge to breastfeeding at the individual level is the mother's working status. Self-employed mothers and mothers with part-time jobs are more likely to exclusively breastfeed compared with mothers involved in full time jobs (Danso, 2014; Diji *et al.*, 2017; Nkrumah, 2017). These identified challenges are not exclusively peculiar to the Ghanaian population. They are faced by mothers in other parts of the world, including Ethiopia, Nigeria and China (Agunbiade & Ogunleye, 2012; Asemahagn, 2016; Zhang *et al.*, 2018).

A constraint to breastfeeding at the interpersonal level is socio-cultural pressure. Most mothers rely on friends and family for breastfeeding support after discharge from the hospital. This tends to heighten social pressure placed on mothers to introduce water and food before the baby turns six months. This particular assertion was ascertained in a study by Diji *et al.* (2017), which revealed that socio-cultural pressure is a major challenge to exclusive breastfeeding among mothers in Kumasi South, Ghana. Afaya, Anaman-torgbor, Salia, & Adatara (2017) also disclosed that women in Sagu, Upper West Region of Ghana, rely on the experiences of grandmothers and older women in taking care of their babies, including their feeding. In an exploratory qualitative study, Afaya *et al.* (2017) revealed that family belief systems, such as the superior role of the fathers (family heads) in decision making, extended to the practice of breastfeeding; thus, breastfeeding in accordance to the husbands' orders which often did not support exclusive breastfeeding

At the community level, breastfeeding has been found to be influenced by existing cultural beliefs and practices. Cultural rituals such as bathing and administering certain concoctions to newborns to protect them from diseases and evil people were also discovered in the same study. These rituals interrupted and delayed breastfeeding. Another belief discovered was that spermatozoa could make their way into breast milk when a breastfeeding mother engages in

sexual intercourse, resulting in infants getting diarrhoea and other strange diseases. Mothers were, hence, required to suspend breastfeeding for two weeks after sexual intercourse.

Nevertheless, despite the benefit of breastfeeding through the first two years of an infant's life, there has been less focus on how to support the needs of breastfeeding mothers at the organizational level. Many women have the intention of continuing breastfeeding when they get back to work but are unable to do so due to a lack of policies informing employers on creating an enabling environment (flexible working hours upon resumption of work, lack of a private space or bathroom for expressing milk) for breastfeeding (Salganicoff, 2018). A short maternity leave duration (3 months) coupled with inflexible working hours upon resumption of duties make it difficult for most Ghanaian mothers to continue breastfeeding. This forces mothers to resort to the use of infant formulas and eventually leads to the cessation of breastfeeding at an earlier age than recommended (Danso, 2014).

A lack of support for breastfeeding from health professionals negatively impacts the practice among mothers. This could be attributed to a lack or shortage of staff in a busy maternity service, causing health professionals to be unable to help each mother due to their busyness. The limited number of health care staff makes them more likely to focus on conducting births than attending to postnatal cases. Health education, including breastfeeding education, lacks detail due to work overload (Pemo *et al.*, 2020). Among healthcare professionals, limited professional development on breastfeeding results in a deficit in breastfeeding knowledge and skills, creating barriers to the delivery of best practices, education, and promotion of breastfeeding. Consequently, most new mothers are discharged with little or no knowledge on breastfeeding, reducing their confidence to breastfeed and leading them to seek information from non-health professionals (Pemo *et al.*, 2019).

Other studies have also reported a relationship between the place of delivery and breastfeeding. Tampah-naah & Kumi-Kyereme (2013) reported that the likelihood of breastfeeding was higher among mothers who delivered in public health facilities than those who delivered at home or in private facilities. This could be due to the introduction of the WHO Baby Friendly Hospital Initiative (BFHI), which is more likely to be practised in government hospitals compared to private hospitals.

Although there are government initiatives to promote breastfeeding through the passage of laws, such as the maternity protection right, Ghana Breastfeeding Promotion Regulation 2000 (LI 1667) as well as the Baby Friendly Hospital Initiative, breastfeeding duration continues to decline (Ghana Statistical Service (GSS), Ghana Health Service (GHS), 2015; Ghana Statistical Service (GSS) and ICF International., 2023). The legislative instrument (LI) 1667 code in Ghana, which was adopted to regulate the marketing of breast milk substitutes, has not been without challenges due to the healthcare system being the main promotional point for breastmilk substitutes; the regulation allows informational materials to be delivered to health workers. There is a thin line between information and promotion thus, promotion often gets conveyed in the form of information. Health facilities are also constantly visited by industry representatives who provide gifts and souvenirs for the health workers as a subtle means of promotion (Alabi, 2007).

2.3.3.2 Complementary feeding

Complementary feeding refers to "the timely introduction of safe and nutritious foods in addition to breastfeeding, i.e. clean and nutritionally rich foods introduced at about six months of infant age" (Imdad *et al.*, 2011). At the age of 6 months, most children have good head support and are able to sit up for a short time as well as feed in an upright position (Gerber *et al.*, 2010). Most children are also able to bring their hands to their mouth- an indication that a

child is able to self-feed soft finger foods under close supervision (Gerber *et al.*, 2010). Moreover, delayed introduction of semisolid and solid foods has been shown to hinder the development of psychomotor skills such as chewing and can lead to feeding difficulties in children (Northstone *et al.*, 2001).

In a systematic review, Imdad *et al.* (2011) evaluated the effectiveness of two common complementary feeding strategies: timely introduction of appropriate complementary foods (± nutritional counselling) and maternal education on complementary feeding. They found that both strategies resulted in a significant increase in weight and linear growth. A meta-analysis showed that extra gain in weight and height by 0.25kg (±0.18) and 0.54 cm (±0.38), respectively, in children aged 6-24 months occurred when children were fed with the appropriate complementary foods.

Early as well as late introduction of complementary foods have been associated with poor nutrition status in children (Kulwa *et al.*, 2006; Moore *et al.*, 2006; Teshome *et al.*, 2010; Tessema *et al.*, 2013). Early introduction of complementary foods is a common practice, especially in developing countries where infants as young as two months old are given foods such as gruels, which lack the nutrients and calories required for optimal child growth and development (Ayana *et al.*, 2017; Indongo & Mutorwa, 2019; Qu *et al.*, 2018; Sayed & Schönfeldt, 2020). The risk of malnutrition is further increased if the foods and drinks offered are not prepared in a safe and hygienic way.

Late introduction of complementary foods, i.e., beyond six months, puts a child at risk of poor growth and development. This is because, from the age of six months, breast milk alone is not sufficient to meet the nutrient and energy needs of a growing infant and support their psychomotor development. A longitudinal study carried out in the United Kingdom provides a crucial examination of the implications of the timing of introducing lumpy solids on infants'

dietary patterns and feeding behaviors, utilizing data from the Avon Longitudinal Study of Parents and Children (ALSPAC). The findings suggest that introducing lumpy foods between 6 and 9 months is optimal, as delays beyond 10 months are associated with increased feeding difficulties and a less varied diet at 6 and 15 months. Infants introduced to lumpy solids later were also more likely to develop strong food preferences and aversions. (Northstone *et al.*, 2001). These results underscore the importance of timely dietary transitions to mitigate potential feeding issues and promote dietary diversity in early childhood. Although informative, this study had several limitations; the questionnaire was based on the mother's perception of what lumpy foods meant, as well as feeding problems. The delayed introduction of semisolid and solid foods in developing countries is likely to have a similar effect. In Ethiopia, it was revealed that although the practice of continuous breastfeeding up to 24 months was highly prevalent, the introduction of complementary foods at age 12 months was common and was associated with stunting (Teshome *et al.*, 2010). This corroborates with the findings of Tessema *et al.* (2013), which showed that children who experienced delayed introduction (after six months old) to complementary foods were twice as likely to be stunted. Hence, the timely introduction of complementary food is important for child growth and development.

Apart from the timing, the type and quality of foods introduced need to be considered. The provision of gruel/porridge as the first food is quite common in developing countries (Uvere & Ene-Obong, 2013). These cereal based porridges could be a good source of energy for children if they are well prepared; however, in developing countries where mothers are faced with extreme poverty, low energy dense watery porridge/ gruel that is made from unprocessed cereals is predominantly given and is associated with poor nutrition status due to its energy and nutrient deficits (Tessema *et al.*, 2013).

The energy provided by porridges is as low as 0.6 - 0.8 kcal/g and could be much lower (0.25 kcal/g) if they are prepared entirely from cereals (Michaelsen *et al.*, 2009). Furthermore, due

to their unprocessed nature, these cereal-based products are high in anti-nutritive factors such as phytic acid, which hinder the absorption of micronutrients such as iron, zinc and calcium (Michaelsen *et al.*, 2009) and macronutrients such as proteins especially when flours are made from a mixture of products such as millet, fish, beans and peanut (Gilani *et al.*, 2012; Opiyo & Muita, 2010). It is, therefore, not surprising that children who receive these foods are more likely to be undernourished. Apart from the lack of adequate nutrients, such feeds are usually prepared with unclean water and stored in unhygienic conditions. This puts a child at an increased risk of diarrhoeal diseases, which often leads to poor appetite, nutrient malabsorption and ultimately compromises a child's nutrition status (Wasihun *et al.*, 2018).

2.3.3.3 Dietary diversity

Dietary diversity is an indicator for dietary assessment, and it is positively associated with child growth (Rathnayake *et al.*, 2012; Verger *et al.*, 2021). It is "the number of foods consumed from various food groups over a specified period" and is a valid indicator for dietary assessment (Ruel, 2003). The WHO currently recommends that minimum dietary diversity (MDD) for children should be 5 out of 8 food groups: breast milk; grains, roots and tubers; legumes and nuts; dairy products (milk, yoghurt, cheese); flesh foods (meat, fish, poultry, liver or other organs); eggs; vitamin A-rich fruits and vegetables; and other fruits and vegetables (WHO & UNICEF, 2017).

The diet of children is more energy dense with less nutrient density; only one in three children between 6 - 23 months worldwide achieved the recommendation for MDD (4 out of 7 food groups) according to a UNICEF report in 2016 (UNICEF, 2016). Global evidence in 2016 revealed that MDD was lowest among younger children 6 - 11 months old; the group for which it is most important (UNICEF, 2016). Growing evidence suggests an association between dietary diversity and growth outcomes.

Aboagye *et al.* (2021) found an association between dietary diversity and undernutrition. They studied 48,968 children 6 - 23 months living in 32 countries in sub-Saharan Africa using Demographic Health survey data from 2010-2020. The prevalence of MDD was 25.1%, with prevalence being highest in South Africa and lowest in Burkina Faso. Children who achieved MDD had a 12% less risk of being stunted, 17% less risk of being underweight and 13% less risk of wasting. This study is very informative as it used nationally representative data and a large sample size; however, it used secondary data, and hence, the analysis was limited by the data at hand. The information presented in the dataset is also subject to recall bias as it is mostly subjective, relying on the responses of the participants. Demographic health surveys are cross-sectional and, hence, do not provide a causal inference, and caution must be exercised when interpreting such findings. The results from this study are, however, similar to findings from a study in Nigeria, where stunting and wasting had a significant correlation with MDD (Olumakaiye, 2017). Similarly, in South Africa, a significant association was found between MDD and stunting among preschool children (Modjadji *et al.*, 2020).

In Ghana, Frempong and Annim (2017) used Ghana's Multiple Indicator Cluster Survey to study the association of infant feeding practices with child health. About 47% of 6598 children achieved MDD. The results showed that the prevalence of undernutrition varied with varying consumption levels of the food groups. Overall, about a quarter of the children who achieved MDD (4 out of 7 food groups) were stunted, 5% were wasted, and 12% were underweight. The prevalence of wasting and underweight declined with an increased consumption of different food groups. Likewise, the Tanzania Demographic and Health Survey of 2015–2016 revealed that about 74% of 2960 Tanzanian children did not achieve the minimum dietary diversity (MDD) (Khamis *et al.*, 2019a). This study revealed a direct association between dietary diversity and malnutrition with a decreased risk of stunting, wasting or underweight as the number of food groups consumed increased.

A systematic review was recently conducted to broadly explore the association between dietary diversity and growth outcomes (stunting, wasting, and underweight) in children aged < 5 years (Molani Gol *et al.*, 2022). The researchers reported that out of 81 articles reviewed, an association of 75%, 56%, and 57% was observed, respectively in relation to stunting, wasting, and being underweight. Further exploration revealed that the association was certain for stunting but uncertain for wasting and underweight. Although systematic reviews present stronger evidence to support claims, it could be safely argued that most (86%) of the individual studies used in this review were cross sectional studies, and, hence, their evidence was not strong enough. A review of studies with stronger evidence, such as randomized controlled trials, will be more substantial.

2.3.4 Water, sanitation, and hygiene (WASH)

Malnutrition can be caused by a lack of nutrient uptake (Cederholm *et al.*, 2015), not just by inadequate intake. This implies that improving dietary intake alone may only form a part of the solution to improve malnutrition rates in developing countries. Research is often focused on the role that diet has in preventing malnutrition; however, dietary interventions covering 99% of children worldwide aimed at modifying all known risk factors are estimated only to reduce stunting prevalence by 33% (Bhutta *et al.*, 2008). Furthermore, researchers have earlier highlighted that inadequate WASH is a key limiting factor for child growth in developing countries (Hasan *et al.*, 2023; Jubayer *et al.*, 2022). Water, sanitation, and hygiene behaviours may be of particular importance in developing countries such as Ghana, where unsanitary environments coupled with malnutrition are common.

Data obtained from the 2008 Ghana demographic health survey was used to explore the potential determinants of malnutrition in children (Aheto *et al.*, 2015). Households lacking toilet facilities were associated with child wasting (β-0.162, 95% CI -0.272,-0.052), and

experience of diarrhoea was associated with underweight (β-0.148, 95% CI -0.253,-0.044) and wasting (β -0.163, 95% CI -0.285, -0.041) (Aheto *et al.*, 2015). One strength of this investigation was the large and representative sample drawn from the national survey, with data from 2083 children (<5 years) analysed from 1641 households across 400 communities throughout Ghana. However, household hand washing facilities and practices, which are essential components of WASH, were not considered in this study.

Improved hand hygiene practices may reduce the risk of faecal ingestion and prevent diarrhoea. It has been evidenced that handwashing accounts for about a 40% reduction in the risk of diarrhoea worldwide (Freeman *et al.*, 2014). This was also demonstrated by Ngure *et al.* (2013), who followed 23 infant-caregiver pairs from Zimbabwe for over 130 hours in order to identify routes of faecal-oral bacteria transmission. Infants' and mothers' hands were highlighted as primary sources of bacteria transmission. After changing their child's diaper, mothers only washed their hands 7% of the time with soap or 44% without, and the infant's hands entered their mouths an average of 38.0 (±38.9) times, with them visibly dirty on 75% of these occasions. In addition, *Escherichia coli* bacteria were present on 82% of kitchen floors, indicating faecal contamination within the household and risk of exposure to children freely crawling in these areas.

There is a bidirectional relationship between diarrhoea and malnutrition, with poor nutritional status increasing diarrhoea risk, whilst recurrent diarrhoea contributes to malnutrition via reduced appetite, reduced food and nutrient intake as well as malabsorption (Wasihun *et al.*, 2018). Children with severe malnutrition have about three times the risk of death from diarrhoea than healthy children (Tickell *et al.*, 2020). A pooled analysis of nine longitudinal studies demonstrated that for every five diarrhoeal episodes in infants (<24 months), there was a 13% increased risk of stunting (95% CI 1.07–1.19), and that 25% of all stunting was attributed to having five or more diarrhoeal episodes (95% CI 8–38%) (Checkley *et al.*, 2008). This study

used data that covered daily diarrhoea morbidity over a 20-year period in five countries. However, this study was potentially biased due to expert opinion being sought to identify research conducted by experienced investigators only. Nevertheless, this study indicates that repeated bouts of diarrhoea play a role in the onset of stunting in infants from developing countries. Further research advocates for the importance of prolonging diarrhoea-free periods to allow for "catch-up" growth and minimise malnutrition risk (Richard *et al.*, 2014).

The GDHS highlights that over a two-week period, 13% of infants under five years old were reported to have experienced diarrhoea (Ghana Statistical Service (GSS) and ICF International., 2023). Although this information only represents a snapshot over a short time frame, the large number of infants reported (n= >10,000) suggests that diarrhoea is common in Ghanaian infants and could, therefore, be a significant contributor to childhood malnutrition. Diarrhoea may occur due to inadequate hygiene practices, with 30.3% of households having non-improved toilet facilities and 12.3% having non-improved sources of drinking water (Ghana Statistical Service (GSS), Ghana Health Service (GHS), 2015).

The common cause of diarrhoea is pathogens, with the association between diarrhoea and pathogens being strengthened by acute malnutrition. For instance, Tickell *et al.* (2020) demonstrated that children with acute malnutrition had a 2.08 odds of diarrhoea associated with typical enteropathogenic Escherichia coli compared with children with better nutritional status (OR-0.97). A similar trend was seen for diarrhoea associated pathogens such as *Shigella* spp, norovirus, and sapovirus.

The pathway between malnutrition, pathogens and diarrhoea (Figure 2.2) has been clearly described by Selimoglu *et al.* (2021). It suggests that malnutrition alters gastrointestinal function, leading to problems in digestion and absorption and consequent diarrhoea. This leads to nutrient losses and deficiencies, immune suppression that increases the risk of infection and

ultimately further deepening malnutrition. Again, poor hygienic practices, which often lead to exposure to disease causing pathogens and infections, are associated with intestinal inflammation that results in atrophy of intestinal villi and consequent malabsorption of nutrients, which ultimately results in malnutrition. This pathological state is often termed as environmental enteropathy (EE), which is mainly seen among individuals chronically exposed to poor hygiene and sanitation.

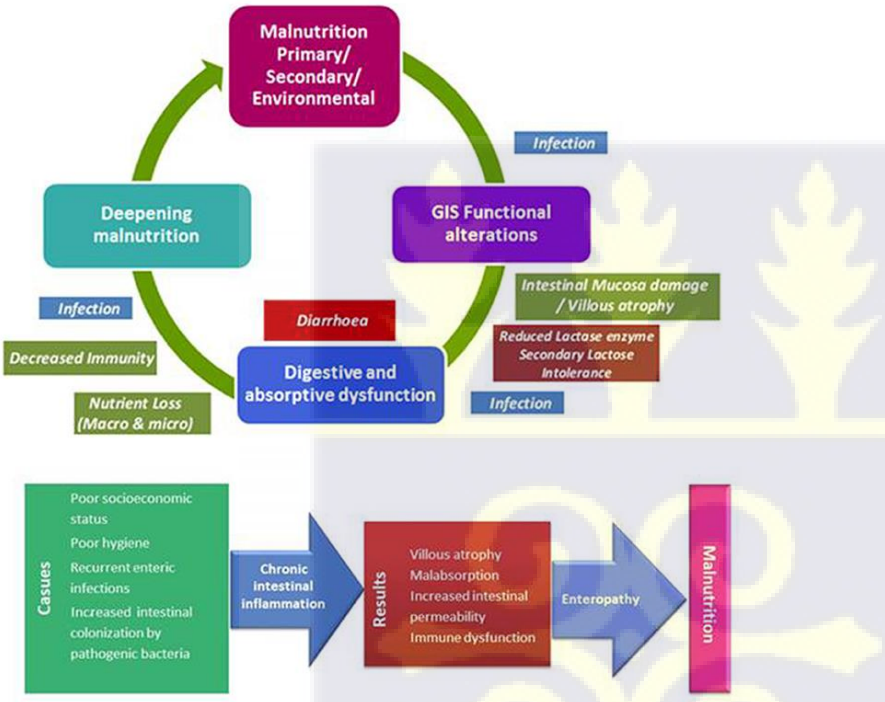


Figure 2.2- Interplay between malnutrition and gastrointestinal functional alterations; environmental enteric dysfunction- (Selimoglu *et al.*, 2021).

2.4 FEEDING BEHAVIOUR AND STIMULATION

Feeding behaviour refers to the practices and interactions of the child and caregiver during feeding events. Feeding behaviour is influenced by the caregiver who feeds the child, the child's own developmental state, the feeding style exhibited during feeding episodes and the environment where feeding takes place (Wood *et al.*, 2020).

The caregiver directly involved in feeding the child often influences food intake based on his or her opinion of the appropriate feeding style (Wood *et al.*, 2020). The primary caregiver, in most cases, is the mother of the child. However, in families, other individuals such as the father, older siblings, grandparents, and friends may also offer caregiving support. The caregiver's capacity and relationship with the child have been shown to be key determinants of the quality of care given to a child (Engle & Lhotska, 1999). For instance, an older sibling feeding the child has been associated with poor nutrition status (Baig-Ansari *et al.*, 2006). This could be because appropriate recognition and response to a young child's needs or signals may not be fully present in cases where older siblings act as caregivers (Pelto *et al.*, 2003). In addition, if a child has a preferred caregiver, he or she is likely to react negatively to feeding by another person. In such a case, the caregiver needs to be patient with the child and provide much encouragement to get the child to eat.

2.4.1 Feeding Styles

Food acceptance keenly depends on feeding style during feeding times (Dearden *et al.*, 2009; Ha *et al.*, 2002). Caregiver's style of control during feeding shapes a child's food preferences and consumption pattern (Van Der Horst & Sleddens, 2017; Cullen *et al.*, 2000; Gable & Lutz, 2000; Bentley *et al.*, 2011) and hence, an important contributing factor to a child's growth and nutrition. While most research and interventions focus on what to feed to infants, some studies have discovered the role of caregiver feeding styles on infant nutrition. Until 1995, there were

conflicting definitions for feeding styles, making comparison among studies difficult. The common types are controlling, Laissez-faire and responsive feeding styles (Birch & Fisher, 1995).

2.4.1.1 Control feeding (forced-feeding)

In controlled feeding, the caregiver exercises complete control over the time food should be eaten, the quantity as well as the kind of food a child eats (Birch & Fisher, 1995). This style is characterised by the use of intimidation, excessive pressure, punishments, guilt invoking phrases and distraction, and physical restraint during the meal. In extreme cases of controlled feeding, aggressive methods such as blocking a child's nose and forcing liquids down the throat have been previously reported in Nigeria (Oni *et al.*, 1991). This method of feeding has been linked with increased food rejection (Ha *et al.*, 2002).

2.4.1.2 Laissez-faire feeding

In contrast, the laissez-faire feeding method allows children to exercise direct control over their food intake; they decide when to eat and the kind and quantity of food to eat based on the perception that "if a child is hungry, he will eat" (Dettwyler, 1989). This style reflects minimal interaction between child and caregiver. It is characterised by little encouragement or physical help given by a caregiver to get the child to feed, irrespective of the child's nutritional status and whether there is a need for special attention. This feeding style is detrimental when a child has a low appetite and requires encouragement during feeding (Dettwyler, 1989). This style of feeding was reported in Nicaragua, where over 60% of caregivers did not encourage feeding (Engle & Zeitlin, 1996). In rural Bangladesh, the laissez-faire feeding was also observed among mothers of 8-24-month-old children (Moore *et al.*, 2006). Such feeding methods can have a negative impact on a child's feeding habits and affect the development of a child's hunger and satiety cues (Birch & Fisher, 1998).

2.4.1.3 Responsive feeding

Responsive feeding, on the other hand, is where the caregiver has close interaction with the child during feeding, offers physical help, monitors and responds to the child's verbal and non-verbal cues in a reasonable time. This style has been recommended by WHO (2005) as the appropriate style for feeding children. It encourages feeding the child in a warm, affectionate manner as well as letting older children to feed themselves. It takes into consideration the child's hunger and satiety cues as well as his/her psychomotor abilities. It recognizes low appetite and encourages the child to eat. In responsive feeding, both the child and caregiver control the feeding (Engle, 2002). A detailed review of responsive feeding has been provided in later pages in this chapter.

Although these feeding styles or behaviours seem to be an old concept, they are still relevant currently and have been broadly conceptualised as demandingness and responsiveness (Hughes *et al.*, 2005). Demandingness is the level of control, maturity, demand and supervision shown by the caregivers, whereas responsiveness refers to the level of affective warmth, acceptance, and involvement of the caregiver. Hence, demandingness refers to the degree to which a parent encourages a child to eat, and responsiveness refers to how a parent encourages the child to eat.

Some researchers have linked parenting styles to feeding styles (Black & Aboud, 2011; Goodman *et al.*, 2020; Harbron *et al.*, 2013; Hubbs-Tait *et al.*, 2008; Van Der Horst & Sleddens, 2017). Subsequently, different groupings have been made based on parenting styles and the degree of responsiveness or demandingness exhibited by caregivers during feeding episodes. Hughes *et al.* (2005) utilised a cross-sectional study among 231 African American and Hispanic parents of 3-5-year-old children enrolled in Head Start facilities in Houston, Texas. Parents completed a questionnaire (Child feeding questionnaire, parenting dimensions inventory, caregivers feeding style questionnaire) assessing their feeding behaviours with the

child and parenting style. Four feeding styles were identified based on parenting styles exhibited in the study: authoritative, authoritarian, indulgent and Laissez-faire (Table 2.1).

Table 2.1: Feeding style based on parenting style (Hughes et al., 2005)

DEMANDINGNES	RESPONSIVENESS		
		+	-
	+	authoritative	authoritarian
	-	Indulgent	Laissez-faire

Among these four styles, the authoritative style has been shown to elicit a more positive impact on a child (Arlinghaus *et al.*, 2018; Goodman *et al.*, 2020). To further consolidate this, Patrick *et al.* (2005) reported on the same study used by Hughes *et al.* (2005) among African Americans and Hispanics in Houston, Texas. Using regression analysis, they established that the authoritative feeding style had a significant positive association with the availability of fruits and vegetables, while authoritarian was negatively associated with the availability of fruits and vegetables. Furthermore, the authoritative style had a positive significant association with attempts to feed dairy, fruits, and vegetables and with children's consumption of dairy and vegetables. Authoritarian style, however, had a significant negative association with the consumption of vegetables. The researchers also tested for the robustness of the association by adjusting for potential covariates such as the caregiver's ethnicity, the child's BMI, the caregiver's BMI, the child's sex, and the caregiver's level of education. The results remained statistically significant. These results signify that caregivers who are more authoritative are more likely to get their children to practice healthy eating. This study is informative but limited by the study design employed. It was a cross-sectional study and, hence, only gives an association rather than a causal inference. It was also conducted among only African-Americans and Hispanics. The results can, therefore, not be generalised as they were conducted

among a well-nourished population and did not set out to associate feeding style with undernutrition. The findings may also only be true for that group of ethnic minorities. Although a validated questionnaire was used, it was still flawed as some of the questions only measured a particular meal of the day, such as dinner, while others measured meals throughout a whole week. Despite these limitations, the study still provides good evidence for the benefits of an authoritative feeding style over an authoritarian. A recent study by Goodman *et al.*, (2020) was conducted among 2-7 year old children to explore whether mindful feeding mediates the relationship between parenting style and child eating behaviours. The researchers discovered that mindful feeding mediated all forms of parenting styles (authoritative, authoritarian, and permissive parenting) and child feeding behaviour ((i.e., food fussiness, problematic mealtime behaviours, and emotional overeating). Specifically, there was a significant association between authoritative parenting and higher rates of mindful feeding, while authoritarian and permissive parenting was associated with lower rates of mindful feeding.

Certain researchers have also viewed Ethnicity- a proxy for cultural differences, as an important cause of differences in feeding care practices. For instance, in Enuga-Yoruba, Nigeria, caregivers force-feed children by forcing food into the mouths of children, distracting them or nose pinching them mainly due to weight related reasons (Ndu *et al.*, 2016). This practice is also prevalent among mothers in Nairobi-Kenya, as discovered by Mutoro *et al.* (2020). A systematic review of feeding styles in China among children under six years old, revealed that the most common feeding style was "active response". This style is similar to authoritative parenting. The most commonly reported feeding practices were praise, encouragement of a balanced diet, encouragement to try new foods, and encouragement of healthy eating (Guo *et al.*, 2022).

Specifically, in Ghana, a study by Kakpovbia (2010) to examine caregivers feeding behaviour in two regions (Brong Ahafo and Northern regions) showed that generally, caregivers did not

explicitly exhibit the distinct groups of feeding styles, controlling, laissez-faire and responsive. They displayed a range of behaviours; hence, they did not use any one style in isolation. For instance, some would sit with the child and allow him/her to self-feed while watching but would not give any verbal or non-verbal encouragement. Other caregivers also employed the use of different techniques when a child refuses food. The common response to food refusal was to first respond verbally and then subsequently remove food if the refusal persists. In view of this, the researcher gave scores to both negative and positive responsive behaviour, with 3 being the maximum score. Responsive feeding behaviour was then determined by subtracting the total scores of negative behaviours from the total scores of positive behaviours. Resulting scores were categorised as low (-1 to +1), medium (score of 2) or high (score of 3) responsive feeding behaviour. The results showed that 41% of the mothers used in the study used high responsive feeding practices, with the Akans/Effutu tribe being more responsive.

When looking at findings of feeding behaviour, one must exercise caution when interpreting results as there is a probability of reverse causality where the caregiver's response is largely influenced by the child's behaviour (Engle & Lhotska, 1999b). For example, caregivers are likely to invest more attention and resources in children who are healthy, happy, and growing well. Some are also more likely to forcefully feed children who are perceived to be undernourished and refusing food. More research is needed to provide information on feeding practices in different settings especially in African countries where there is a general lack of information and yet a high burden of undernutrition.

2.4.2 Responsive Feeding

The PAHO/ WHO (2001) infant feeding guidelines advocate for responsive feeding using the principle of psychosocial care;

1. Feed infants directly and assist older children when they feed themselves, being sensitive to their hunger and satiety cues.

2. Feed slowly and patiently, and encourage children to eat, but do not force them.

3. If children refuse many foods, experiment with different food combinations, tastes, textures, and methods of encouragement.

4. Minimize distractions during meals if the child loses interest easily.

5. Remember that feeding times are periods of learning and love - talk to children during feeding, with an eye-to-eye contact.

The term responsive feeding was first defined by Birch & Fisher, (1995) as "when the caregiver is in close proximity to their young child during the meal and responds to the child's hunger cues in a reasonable time". Here, the caregiver makes meaning of the child's verbal and non-verbal cues and responds in a manner that is consistent with the intended meaning of the signal. Responsive feeding, therefore, reflects a mutual relationship between mother and child characterised by the child signalling feelings of hunger and satiety through verbal and non-verbal cues followed by the caregiver's correct interpretation of the cues and a prompt response. The response is often characterised by offering child-appropriate nutritious food while monitoring and ensuring a healthy feeding environment. Responsive feeding has been conceptualized as a 4-step process (Black & Aboud, 2011b);

1. Establishment of a structured interactive routine with known expectations

2. Verbal and non-verbal expression of hunger and satiety by infant

3. Caregiver's supportive, contingent, prompt and appropriate response to infant's signals.

4. Ability of infants to perceive caregiver's responses

Children signal their hunger and satiety by crying, turning their heads away from undesirable foods, spitting out food when they are full etc. (Harbron *et al.*, 2013). The correct interpretation of these signals is essential for an appropriate response. The development of feeding behaviour and responsivity is progressive with regard to the infant's age. Younger children use more non-verbal cues, while older children use some level of both verbal and non-verbal signals depending on their level of growth and development. This requires that caregivers' responses to children should conform to the child's developmental stage and mode of signalling. Responsive feeding is an active feeding of food to a child where the caregiver acts positively with the child while encouraging and bearing in mind the child's interests and needs at mealtime. Examples of some positive behaviours are having a conversation about food, demonstrating good feeding habits (healthy choices), playing food games, and providing verbal encouragement. Actions such as force-feeding, threatening, holding the child's nose, and shaking the child., are non-responsive and negative feeding behaviours.

Evidence suggests that responsive feeding is associated with the child's development of eating behaviour, self- regulation of food intake, optimal nutrient intake and long-term regulation of weight (Harbron *et al.*, 2013). It has been revealed to be positively associated with infant energy/nutrient intake (Vazir *et al.*, 2013; Nti & Lartey, 2008; Kakpovbia, 2010), child food acceptance (Aboud *et al.*, 2009; Nti & Lartey, 2008; Ha *et al.*, 2002), child's food interest (Mwase *et al.*, 2016) and child self-feeding (Aboud *et al.*, 2008, 2009). Non-responsive feeding is, on the other hand, associated with undernutrition and over nutrition (Fisher & Birch, 1999; Fisher & Birch, 2002; Harbron *et al.*, 2013).

A pilot cross-sectional study was conducted by Mwase *et al.* (2016) in Nairobi-Kenya to assess feeding, hygiene practices and nutritional status of 33 children. The researchers found a high prevalence of malnutrition in this group of children (stunting (33%), wasting (12%), underweight (33%) and undernutrition (39%)). They further observed one lunchtime meal episode for 11 children in five day-care centres as well as a general observation of the feeding event and found that children who had their mothers visit to feed them had more mealtime and more responsive behaviour from the mothers compared to children who were fed by the caregivers. This was explained by the fact that the ratio of caregivers to children was very high and hence, caregivers spent less time encouraging eating, used threats more often and paid little attention to children's signals. Generally, there was a high rate of non-responsive feeding practices in these centres, which could have contributed to the high prevalence of malnutrition. Although informative, this study is limited by the small sample size and the fact that only one meal event was observed, which may not be a true reflection of their usual practices.

The impact of responsive feeding on a child's nutritional status, such as weight, length, WAZ, HAZ and BMIZ, is conflicting and hence, requires increased depth of exploration. For instance, Vazir *et al.* (2013) and Aboud *et al.* (2009) did not find any significant impact of responsive feeding on children's anthropometry, whereas Aboud *et al.* (2008) did find a significant impact. These three studies are experimental studies. Similarly, cross sectional studies by Engle and Zeitlin (1996) and Kakpovbia (2010) also found no significant association between responsive feeding and anthropometry. These disparities may be because there are not many experimental or longitudinal studies on this topic. The differences in exposure and subjects used in the various studies could also account for the disparities, as some of the studies were carried out in populations already receiving education on infant nutrition and development, and some also provided information to control groups. Notwithstanding, the measures used to determine feeding styles and a child's dietary intake are mostly subjective and rely on caregivers' recall

and truthfulness. It is worth exploring deeper in this area using more robust tools for measurements.

2.4.3 The feeding environment

The setting for feeding, such as the presence of a television, family eating together, and so forth,. has been evidenced to impact on feeding behaviour and adiposity. In a survey, researchers examined the relationship between television watching, energy intake, physical activity, and obesity status in US boys and girls aged 8 to 16 years (Crespo *et al.*, 2001). They used a nationally representative cross-sectional survey with an in-person interview and a medical examination, which included measurements of height and weight, daily hours of television watching, weekly participation in physical activity, and a dietary interview. They discovered that increasing hours of television watching was positively associated with the prevalence of obesity and total energy intake, especially among girls.

Similarly, Bassul *et al.* (2020) demonstrated that children's dietary intake was influenced by the home environment. This study revealed that the sort of food items available in the home did influence what was consumed by the children. For instance, there was an increased consumption of fruits among children living in homes where a variety of fruits were available (OR 1.35 95% CI 1.09–1.68, $p = 0.005$). They also found an increase in consumption of confectionary and sugar sweetened beverages as well as a reduction in consumption of vegetables when children watched television beyond 1 hour per day.

Evidence from a systematic review suggests that the environment in which feeding takes place does influence eating in the absence of hunger (EAH), especially among girls (Lansigan *et al.*, 2015). Specifically, the researchers opined that regarding EAH behaviours among boys and girls, external factors might exert a greater influence. They explained that girls may be more aware of and sensitive to behavioural expectations regarding their dietary behaviours and thus,

engage in greater levels of EAH in private based on an increased pressure to exert dietary restraint. It was further realised that EAH could be a learned behaviour because EAH was common among children whose parents used food to regulate their emotions as well as children with parents who had disinhibited eating style, a style characterised by eating in response to external cues.

2.5 GENETIC CONTROL OF ENERGY REGULATION

Genes control almost every activity in the body that is often expressed physically. Evidence suggests that eating behaviours are a mediation between genes and BMI. Twin studies have revealed that genes are involved in a wide range of dietary phenotypes, such as dietary patterns, energy and macronutrient intakes and specific food group intakes (Pallister *et al.*, 2014). Development of energy regulation has also been explained to have a genetic predisposition as some genetic polymorphisms have been shown to play a major role in the etiology of child eating self-regulation. In a bid to ascertain this, Carnell *et al.* (2008) hypothesised that genetic influences on weight could function through both behavioural and metabolic pathways. They assessed the relative contribution of genes and environment for two aspects of appetite that have been implicated in obesity. This was done among 8–11-year-old twins. Their results revealed a 63% heritability of satiety responsiveness and 75% heritability for food cue responsiveness.

Similarly, Llewellyn *et al.* (2010) concluded from a cohort study of 2404 twin-pairs that genetically determined variability in appetitive traits may be one of the pathways through which genes influence the growth rate in infancy and that appetite regulation is genetically controlled, especially during the very earliest period of feeding. This study is one of the first studies to assess the heritability of appetitive traits in infancy. The researchers used data from the Gemini Study, a population-based sample of twins born in England and Wales in 2007.

Children's eating behaviour during the first three months of life was described following four subscales of the Baby Eating Behaviour Questionnaire (BEBQ): "enjoyment of food," "food responsiveness," "slowness in eating," and "satiety responsiveness." The researchers also used quantitative genetic model fitting to estimate the heritability of the various eating behaviours.

The results showed significant heritability for all four appetite traits, with higher heritability revealed for slowness in eating (84%) and satiety responsiveness (72%). Heritability was moderate for food responsiveness (59%) and enjoyment of food (53%). While it may be argued that this study was among twins and hence, findings cannot be generalised to include singletons, other researchers have revealed that twin study is a unique epidemiological tool to study the heritability of traits and to quantify the effect of a person's shared environment (family) and unique environment (the individual events that shape a life) on a trait (Guo, 2001; Sahu & Prasuna, 2016). Others have further explained that twin studies are advantageous over classical epidemiological studies in studying the genetic control of traits because twins have shared upbringing, matched genes and sex (in the case of monozygotic twin pairs), and age (Pallister *et al.*, 2014).

It could again be criticised that the researchers employed the use of only subjective means to arrive at their outcome. Indeed, objective measures such as laboratory tests for genes that control these appetitive traits could have further strengthened this study. However, twin studies have been confirmed to be a useful exploratory tool by many molecular genetic studies and besides the researchers employed the use of advanced statistical techniques and implemented many controls to further strengthen the results of this study. The researchers acknowledged that the validity of BEBQ was uncertain among younger infants and, hence, could be a limitation of this study. Although this is a larger study with a huge sample size, it cannot be ascertained if it was powered enough to draw the conclusions presented by the researchers due to the lack of information on the power calculation. It is worth noting that this is an observational study

and, hence,cannot give any inferential conclusion.

Fisher *et al.* (2007) assessed the heritability of eating in the absence of hunger (EAH) among 801 5–8-year-old children from 300 Hispanic families. The authors revealed that EAH was significantly heritable among the participants. They also reported heritability for food energy regulation hormones such as fasting levels of ghrelin, amylin, insulin, and leptin. Their findings suggested that there were genes that regulated the expressions of such hormones, with significant genetic correlations seen between the hormones and eating behaviour.

Specifically, the FTO (fat mass and obesity related) gene has been implicated in EAH and obesity. This gene is predominantly expressed in the hypothalamus and has been found to be associated with the "satiety responsiveness" and "food responsiveness" of children (Wardle, Llewellyn, Sanderson, & Plomin, 2009; Wardle *et al.*, 2008). Wardle *et al.* (2009) tested the association between FTO genotype status and food intake. They genotyped the FTO single nucleotide polymorphism rs9939609 among 131 children aged 4–5 years in the United Kingdom. The results demonstrated a significant association between the three different genotypes (AA, AT and TT) and EAH. More specifically, children with TT genotype ate less during the free access meal than those with AT and AA genotypes. Hence, the T allele seems to have a more protective effect against EAH by enhancing responsiveness to internal satiety cues.

More recently, food preferences for basic tastes of salt, bitter, sweet, umami and sour have been discovered to also have a genetic predisposition. Indeed, food taste preferences are largely related to food intake and avoidance. Several taste receptors have been identified within the taste cell membranes on the surface of the tongue. This includes the T2R family of bitter taste receptors, the T1R receptors associated with sweet and umami taste perception, and the ion channels PKD1L3 and PKD2L1 linked to sour taste (Garcia-Bailo, Toguri, Eny, & El-Sohemy,

2009). Variability in individual taste perception, food preferences and dietary habits within a population may be explained by common polymorphisms in genes involved in taste perception (Garcia-Bailo, Toguri, Eny, & El-Sohemy, 2009). Cont *et al.* (2019) assessed how infants' acceptance of their first complementary foods was associated with TAS2R38 bitter taste genotype. The children were genotyped at birth and classified as "bitter-insensitive" (genotype AVI/AVI) and "bitter-sensitive" (genotypes AVI/PAV or PAV/PAV). Parents were instructed to feed their babies a first complementary meal of 150 mL at 4 to 6 months of age. The results revealed that more bitter-insensitive infants (31%) than bitter-insensitive infants (13%) consumed the whole complementary meal at the first attempt. These findings suggest that the *TAS2R38* bitter taste gene might be involved in eating behaviour at weaning.

2.5.1 Mendelian weight regulation

Body mass index is heritable, as revealed by major studies. Studies of monozygotic twins raised apart indicate that between 50-70% of the phenotypic variation in BMI can be explained by genetic variation (Allison *et al.*, 1996). This phenotypic variation can be seen from as early as five months, as demonstrated in cross-sectional twin studies (Dubois *et al.*, 2012). A study by Riveros-McKay *et al.*, (2019) concluded that for people living in the same resource setting, variations in weight is largely attributed to genetic factors.

2.5.1.1 Genetics of thinness

It has been previously revealed that obesity is highly heritable (Llewellyn *et al.*, 2010) and it is linked to a number of polymorphisms, many of which probably act through appetite regulation.

While a large body of researchers has delved deeply into the genetics of obesity, with over 700 polymorphisms identified (Orthofer *et al.*, 2020), very few studies have looked at the genetic regulation of thinness in order to prevent any assumption that the opposite of genetics of obesity

is true for thinness. Despite the significant growth in obesogenic environment worldwide, some people remain thin. It is not apparent why some people resist weight gain despite the implementation of proven workable nutrition interventions. Therefore, It is worth exploring the genetics of thinness, as few studies have been done and discovered that thinness is just as heritable and stable across time points as obesity is (Riveros-McKay *et al.*, 2019).

Genome wide association studies (GWAS) are the gold standard for studying polymorphisms involved in complex human traits; however, very few studies have been conducted focusing on thinness. A large number of the existing GWAS focused on the opposite ends of the BMI spectrum, with thin individuals often perceived as healthy controls (Berndt *et al.*, 2013; Hinney *et al.*, 2007). Most GWAS have been performed among cohorts of European descent (Hinney *et al.*, 2007; Hoffmann *et al.*, 2018; Winkler *et al.*, 2015; Riveros-McKay *et al.*, 2019) with very few studies focusing on cohorts of African descent (Martin, Teferra, Hoal, *et al.*, 2018) A study by Riveros-McKay *et al.* (2019) focused on persistent healthy thinness. After analysing 2000 thin UK adults of European descent, they found 15 nominally significant loci for thinness, some of which were shared with obesity. Some of these loci showed a stronger association with underweight than obesity. Thus suggesting that the genetic overlap between these traits is incomplete.

Several copy number variants (CNVs) have been used to explain the variations in BMI, with some revealed to be associated with thinness. These CNVs have been shown to elicit a large phenotypic effect by altering the dosage of many genes via deletion or duplication of a chromosomal region (Walters *et al.*, 2010; Hasstedt *et al.*, 2015). Findings indicate that CNV within genes at 16p11.2 and 20q13.3 may be associated with both appetite and energy regulation. Specifically, a deletion at chromosome 16p11.2, spanning 28 genes, was associated with hyperphagia (Walters *et al.*, 2010). Similarly, the reverse of this effect (a duplication of chromosome 16p11.2) was found to be associated with an 8-fold increased risk of carriers being

clinically underweight (Jacquemont *et al.*, 2011). A genome-wide search for CNVs associated with obesity was also performed on subjects with severe obesity or healthy thinness. The researchers demonstrated that duplication or deletions of a 77kilobase CNV on chromosome 20q13.3 were respectively associated with obesity or thinness (Hasstedt *et al.*, 2015).

Several other researchers have also discovered the contribution of single nucleotide polymorphisms in BMI and thinness. A SNP in a serotonin receptor gene (*HTR2C)* (assumed to be involved in the influence of serotonin on food intake), leading to the substitution of cysteine for serine, has been shown to be associated with low weight in Swedish teenage girls (Westberg *et al.*, 2002). Two SNPs in the MC4R gene, rs571312 and rs17782313, were found to be significantly associated with a greater decrease in body weight and BMI in a study that involved a behavioural intervention for weight loss (Holzapfel *et al.*, 2021).

In addition, researchers have in recent times, identified variants within the ALK gene to be associated with thinness. A GWAS was carried out among groups within the various BMI spectrum using the Estonian biobank (Orthofer *et al.*, 2020). An association was found between thinness and a variant in an uncharacterised long non-coding RNA, AG013652.1. Two variants, rs79938778 and rs568057364, were located within candidate genes DEPTOR and the first intron of ALK. The ALK variant rs568057364 was discovered to be in strong linkage disequilibrium with another thinness-associated ALK variant rs202021741. These two ALK variants were seen to be more frequent among Europeans, Asians, and admixed Americans but less among Africans. The researchers further carried out GWAS data mining to further ascertain their findings of the link between ALK variants and thinness using Drosophila and mice. Several metabolic traits, including thinness, BMI, lipid, and glucose homeostasis, were found to be associated with several variants of ALK. Genetic deletion of ALK increased resistance to diet-induced weight gain in adult mice. Indirect calorimetry showed a high energy expenditure among ALK deleted mice. This is suggestive that excess catabolism is a possible

cause of the resistance to weight gain among ALK deleted mice. This study is more robust and promising. The data used (Estonian biobank) is unique with a wide age range and it is one of the largest and strongest phenotypic datasets worldwide. This allowed the researchers to put in more vigorous control measures and more sophisticated analytical procedures. The dataset allowed the researchers access to a population with a true metabolically healthy thinness state. The lack of access to such a population is one of the reasons why genetics of thinness has been largely understudied.

2.5.2 Polygenic Risk Score (PGRS)

A polygenic risk score (PGRS) is " a single value estimate of an individual's common genetic liability to a phenotype, calculated as a sum of their genome wide genotypes, weighted by corresponding genotype effect size estimates (or Z-scores) derived from summary statistic GWAS data" (Choi *et al.*, 2020). It is used to quantify the genetic liability of common phenotypic traits. A PGRS is generally calculated as a weighted sum of all risk alleles of SNPs to a particular phenotype (Torkamani *et al.*, 2018). Although GWAS has identified several SNPs associated with certain traits, the predictive power of these SNPs is largely limited (Choi *et al.*, 2020). Besides, several different SNPs may be associated with a single trait. These SNPs, however, may have a different and unequal predictive power of the phenotypic trait, with some SNPs having stronger effects than others. An aggregation of these SNPs based on the weight of their contribution has been found to be more beneficial in explaining certain phenotypic variations (Choi *et al.*, 2020). Hence, a PGRS is set to play a crucial role in research and medicine. For instance, Yang *et al.* (2010) revealed that the heritability of height is best explained by evaluating the effect of all the SNPs associated with height. Unlike linkage disequilibrium regression, PGRS provides an estimate of genetic liability at the individual level. While some researchers have questioned the validity of a PGRS (Janssens, 2019), others have considered it suitable for multiple genetic applications. For instance, PGRS has been used

to test for genome-wide gene-by-environment and gene-by-gene interactions (Agerbo *et al.*, 2015; Mullins *et al.*, 2016). It has been used to perform Mendelian randomization studies to infer causal relationships (Agerbo *et al.*, 2015; Mavaddat *et al.*, 2019; Natarajan *et al.*, 2017). Thus, although PGRS is an individual-level proxy of genetic liability to a trait, predictions from PGRS are often aggregated and used for research purposes, investigating diseases, testing hypotheses and planning interventions.

A PGRS is calculated by computing the sum of risk alleles that an individual has, weighted by the risk allele effect sizes as estimated by a GWAS on the phenotype (Choi *et al.*, 2020). The unit of a PGRS is, hence, determined by the unit of the GWAS effect sizes. The predictive power of PGRS is enhanced by not just using SNPs with genome-wide significance in GWAS but by using a large number of SNPs (Agerbo *et al.*, 2015; Mavaddat *et al.*, 2019; Purcell *et al.*, 2009). The genotypes used in PGRS are those of common biallelic SNPs. That is a minor allele frequency above 0.01 in GWAS (Choi *et al.*, 2020).

There is a recognised difficulty in calculating PGRS because the results are based on base data (GWAS). Unfortunately, there is usually a high level of heterogeneity in GWAS as well as the data does not allow for individual characterization due to privacy legalities. Some effect alleles may not be well described or established and there may be mismatched, ambiguous or misleading SNPs. Hence, it is suggested that the base data to be used should have good quality control and a sample size of not less than 100 individuals (Choi *et al.*, 2020).

2.6 ENERGY COMPENSATION

Energy compensation is defined as "the adjustment of energy intake provoked by the previous ingestion of a given stimulus (preload), whether it's a meal, a snack, or a beverage"(Almiron-Roig *et al.*, 2013). Successful energy compensation is essential for maintaining a healthy weight and overall wellness (Carnell *et al.*, 2017). It has been revealed from several studies

that over compensation and under-compensation lead to negative and positive energy balance, respectively (Almiron-Roig *et al.*, 2013; Carnell *et al.*, 2017). Studies that quantify the nature of the relationship between energy compensation and nutritional status have shown linear associations between appetite and adiposity across the entire weight spectrum (Carnell & Wardle, 2008; Llewellyn *et al.*, 2008).

2.6.1 Energy compensation and satiation

There is an overwhelming heterogeneity in study methodologies that assess energy compensation. This has resulted in a more complex quantification of energy compensation and satiation. While some studies have used eating in the absence of hunger (EAH) to assess energy regulation or compensation, the most common methodology is the use of the preload paradigm. This model, first described by Birch and Deysher in 1985, is used for the assessment of the impact of a previous meal on a subsequent meal. The method involves the supply of a preload meal or drink, followed by a period of waiting and then an *ad-lib* consumption of a meal. Calories consumed in both preload and ad-lib meals are calculated, and compensation is assessed. Most studies that have employed this method have differed in the time allocated between preload and ad-lib meals, mostly ranging between 20 minutes to 4 hours (Almiron-Roig *et al.*, 2013; Rogers *et al.*, 2016). They have also differed in the type of preload offered: liquid, semi-solid, solid and composite meals (Almiron-Roig *et al.*, 2013). In some studies, the sensory properties of preloads are manipulated independently of nutrient composition, while in others, both nutrient content and organoleptic properties of the preload vary (Almiron-Roig *et al.*, 2013). Indeed, two foods of equal energy may have distinct effects on satiety if their macronutrient compositions differ (Chambers *et al.*, 2015). A common approach, therefore, is to manipulate factors that influence satiety, such as macronutrient content, appearance, taste, flavour and texture. Specifically, these factors have been explained in the satiety cascade developed over two decades ago and modified by Mela (Blundell *et al.*, 2010; Mela, 2006).

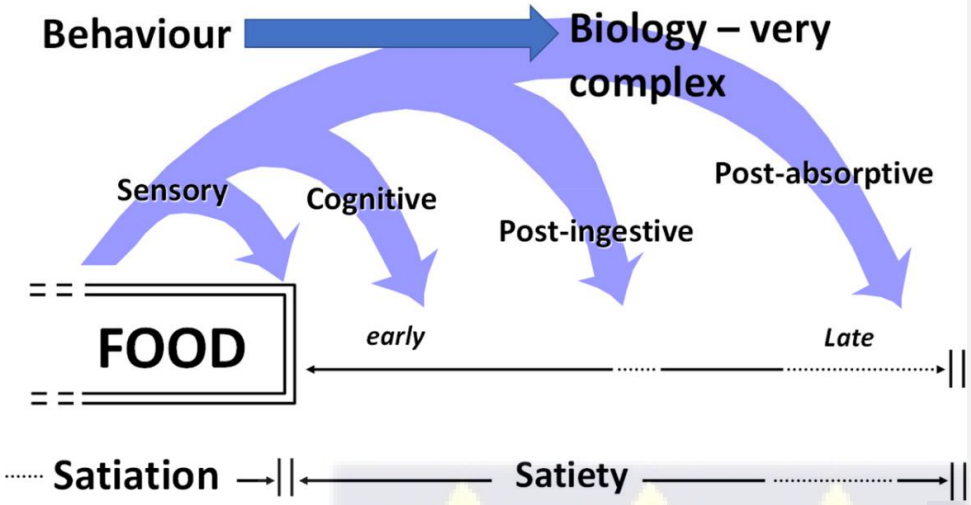


Figure 2.3 : Satiety Cascade- Source: (Mandalari, 2020).

The Satiety Cascade (Figure 2.3) provides a conceptual framework for examining the impact of foods (such as the preload meals) on satiation and satiety. It conceptualizes that satiety can be viewed as an emergent property of complex gastrointestinal (GI) physiology, arising through the integration of numerous factors, including cognitive, sensory, post-ingestive and post-absorptive signals (Mandalari, 2020). In essence, the cascade shows that sensory, cognitive, post-ingestive and post-absorptive signals will jointly determine the feeling of satiation and satiety. Technically, it implies that macronutrient composition, energy density, physical structure and sensory qualities all contribute to the modulation of satiation and satiety (Blundell *et al.*, 2010). Hence, the satiating power of food depends on the amount and types of proteins, fats and carbohydrates (Chambers *et al.*, 2015).

There are evidence that protein is the most effective macronutrient that offers a high level of satiety and that the expectations of satiating capacity are increased by oral exposure cues of protein-rich foods (Morell & Fiszman, 2017). It has been proposed that there is a macronutrient

satiety hierarchy, with proteins being more satiating than carbohydrates, which in turn are more satiating than fat (Fraser *et al.*, 2012). Fraser *et al.* (2012) explored this proposition by investigating the effects of macronutrients on satiety when energy density is matched. About 10 women were made to consume a manipulated preload drink on three different occasions. Each preload contained 1.4 MJ of energy with an energy density (ED) of 6.4 kJ/g and was enriched with 50g of whey protein isolate, 48g of maltodextrin or 16g of corn oil. The volumes and taste of the preload were closely matched. The participants were offered an ad-libitum lunch 60 minutes after preload, and caloric intake was measured. Participants rated their hunger, fullness, and desire to eat immediately pre and post the preload and pre and post the lunch meal. The results revealed that only protein consumed at a dose of 765 kJ in a volume of 235 ml at an energy density of 6.4 kJ/g may exert a weak effect on hunger but not on energy intake. The researchers concluded that at such dosages and volumes, the macronutrient satiety hierarchy was not demonstrated.

Another experimental study using a randomized cross-over design, which involved offering a high-protein yoghurt (14 g protein/25 g CHO/0 g fat); high-fat crackers (0 g protein/19 g CHO/9 g fat); and high-fat chocolate (2 g protein/19 g CHO/9 g fat) revealed similar results (Ortinau *et al.*, 2014). Specifically, the study aimed to determine whether a high-protein afternoon yoghurt snack improves appetite control and satiety and reduces subsequent food intake compared to other commonly consumed, energy dense, high-fat snacks. Three isocaloric snacks (i.e., yoghurt, crackers, and chocolate) that varied in macronutrient composition and physical characteristics were compared. The yoghurt was high in protein and low in energy and fat whereas the crackers and chocolate were both low in protein but high in energy and fat. Twenty healthy premenopausal women of an average age of 27 years were recruited for the study. They were offered the various snacks to consume at home or work for three consecutive days.

On the 4th day of each snack sequence, they made a visit to the laboratory about an hour prior to their lunch meal. They were made to consume a standard 300 kcal breakfast meal (with a similar macronutrient composition) at home before turning up at the study. The study was set up for a period of 8 hours. They were offered a 500-kcal lunch meal to consume, followed by a 3-hour delay and then the respective snack, which was also meant to be eaten within 15 minutes. The participants were asked to rate their appetite sensation after the snacks and at every 30 minutes throughout the afternoon until they voluntarily requested dinner. Dinner was served and meant to be eaten ad libitum until the participants had eaten to their fill. The number of meals consumed at dinner was then assessed.

The results revealed that consumption of the yoghurt snack led to greater reductions in afternoon hunger compared to chocolate ($p < 0.01$). The yoghurt snack also delayed eating initiation by approximately 30 minutes compared to the chocolate snack ($p < 0.01$) and approximately 20 minutes compared to crackers ($p = 0.07$). The yoghurt snack led to approximately 100 fewer kcals consumed at dinner compared to the crackers ($p = 0.08$) and chocolate ($p < 0.05$). In their discourse, the authors explained that less energy dense, high-protein foods like yoghurt improve appetite control and satiety and reduce short-term food intake in women. Even though the snacks varied in their organoleptic properties, their varied macronutrient composition, as against the results, gives credence to the existence of a macronutrient hierarchy. The researchers further discussed that the differences in hunger, satiety, or subsequent food intake detected between the crackers and chocolate could be because the higher saturated fatty acid content of the chocolate probably negated the negative effects of the simple carbohydrates. They, therefore, recommended the need to control not only the macronutrient content but the type/quality as well. Although the evidence is strong due to the design used, a more robust method in assessing hunger and satiety, such as testing for

hormonal controls in addition to the subjective method used, could have been more beneficial and further strengthened this evidence.

There is also a broad consensus that the energy density (ED) of foods affects energy intake (EI) and that lowering the energy density of food without any manipulation of the macronutrient content further enhances satiety and reduces overall energy intake at a subsequent meal (Rolls, 2009). Energy density is the amount of energy in a particular weight of food, often presented as the number of calories per gram of food (kcal/g) (Rolls, 2009). Energy density is directly influenced by macronutrient composition as well as the moisture content of foods or beverages. Therefore, foods that are high in fat will have high energy density as 1g of fat is equivalent to 9 kcal, while 1g of carbohydrate or protein also contains 4 kcal. High moisture content lowers the energy density of foods, even those high in fat, because water contains zero calories but contributes to the overall weight of the food. Energy density ranges from 0 kcal/g to 9 kcal/g (Rolls, 2009).

This suggests that if an individual takes in a consistent amount (weight or volume) of food, any adjustment to the energy density of the food can either improve or reduce calorie intake. For instance, increasing the moisture content of a preload without adjustment to its macronutrient and quantity will lower overall calories consumed from the preload. Rolls (2009) gave a clear example that on a typical day, an adult might consume 1200 g of food with an overall energy density of 1.8 kcal/g, giving an energy intake of 2160 kcal. If the average energy density of the diet were decreased by 0.1 kcal/g while the same weight of food was consumed, then the individual would ingest 2040 kcal. Thus, a relatively small change in the overall energy density of the diet would reduce energy intake by 120 kcal per day. In a review, Rolls (2009) opined that compared to macronutrient composition, the energy density of a preload offers a greater influence on subsequent meal intake.

A randomised crossover study was carried out to examine whether energy density and taste quality have an impact on energy intake and postprandial blood glucose response (Tey *et al.*, 2018). Using a preload design, 32 healthy lean males were asked to consume a sweet ("Cheng Teng") or a savoury (broth) preload soup in high energy density (HED; around 0.50 kcal/g; 250 kcal) or low energy density (LED; around 0.12 kcal/g; 50 kcal) in mid-morning and an ad libitum lunch was provided an hour after the preload. An assessment of lunch meals consumed ad libitum showed a higher intake of sweet LED and savoury LED compared to sweet HED and savoury HED. Consumption of HED preloads resulted in a larger spike in postprandial blood glucose response compared with LED preloads, irrespective of taste quality ($p < 0.001$). The researchers suggested that energy density rather than taste quality plays an important role in energy compensation and postprandial blood glucose response.

In a systematic review, Almiron-Roig *et al.* (2013) analysed the relative contributions of two key variables, preload physical form and inter-meal interval (IMI), to differences in energy compensation. They assessed 48 publications from which percent energy compensation (%EC) data were extracted for 253 interventions (121 liquid, 69 semisolid, 20 solid, and 43 composites preloads). The findings indicated a large range of energy compensation across the studies. Energy compensation ranged from -370% (overconsumption, mostly of liquids) to 450% (overcompensation). The researchers further carried out a meta-regression analysis of studies that reported positive energy compensation. They discovered that inter-meal interval (IMI) was a predominant factor for energy compensation and IMI, together with preload physical form and energy, contributed significantly to energy compensation differences, accounting for 50% of the variance, independently from gender and BMI. They found that energy compensation was maximized when the preload was in semisolid/solid form, and the IMI was 30–120 min.

Specifically, the study revealed that energy compensation decreased in all types of preloads when IMI was kept longer. Compensatory behaviour also appeared to decrease faster over time

with semisolids and solids than with liquids. Liquid preloads were associated with incomplete energy compensation (%EC < 100) more frequently than other preloads. The researchers suggested that under controlled laboratory conditions, the IMI is a strong determinant of energy compensation differences irrespective of gender, BMI, preload physical form, and preload weight, energy, and energy density. Following IMI as the strongest contributing variable is the physical form of the preload and its energy density. This is a systematic review that also used a more comprehensive approach to include different studies and used robust statistical methods to analyse the results and, hence, present stronger evidence.

Of course, the evidence above suggests that the type of preload selected for an appetite study as well as the time interval between preload and meals, could influence the outcome. The macronutrient composition, food matrix, energy density and organoleptic properties as well as the individual's previous experience with the preload may influence energy compensation. the effect of any food or food component could be modified by the context in which it is eaten, whether it is eaten in the laboratory or in the natural environment. Evidence is, hence, quite equivocal with regard to energy regulation among humans.

2.6.2 Energy compensation and nutritional status

Current research shows that healthy toddlers have about 70% energy compensation (Kane *et al.*, 2011). Kane *et al.* (2011) demonstrated from an experimental pilot study that children who have been previously artificially fed have a similar energy compensation to healthy children. The authors used a formal satiation study to assess energy compensation in children who have been artificially fed. Eleven children of a median age of 4.5 years were involved in this study. Using a cross-over design, on two separate days, the children were randomly assigned to consume a high energy preload (HEP) and low energy preload (LEP) drink, followed after a 30-minute delay by a multi-item test lunch. The median (range) COMPX of the participants

was 70%, with boys (n=8) compensating more than girls (n=3). Indeed, this was a small study among a highly heterogeneous group but enough for a pilot work and informative for designing a larger study.

Kasese-Hara *et al.* (2002) also used an energy compensation procedure to test the hypothesis that young children who fail to thrive lack a normal sensitivity to internal hunger or satiation cues. Twenty-seven children with failure to thrive (weight gain in the lowest 5% for their age) were matched by age and sex with 26 normal weight healthy controls resident in the same local geographical area. Using a double-blind cross-over design, participants were assigned to receive either a high energy (402 kJ) drink or a low energy (1 kJ) drink followed by a 25 minute delay and then lunch meals.

The study was carried out in the participant's own home. Pre-loads were 150 ml in volume. The low energy preload contained 1 kJ, and the high energy pre-load 402 kJ (1 kcal ¼ 4.184 kJ). The high energy pre-load was prepared by adding 50 ml of Liquid Maxijul to 30 ml of low energy orange or blackcurrant squash and diluting it with 70 ml of water. The low energy pre-load comprised 30 ml of squash diluted with 120 ml of water. The test meals were similar (energy (KJ) per 100g provided in parenthesis) and consisted of potato chips and rings and cheese singles; sandwiches made up with white, medium-sliced bread, low-fat spread, and spreads made of tuna and mayonnaise, cheese, or Marmite; carrot sticks and cucumber slices; fromage frais and jelly; orange drink and blackcurrant drink.

The mean energy intake of the meal on the control day (meal after low energy preload) was significantly lower in the case children than the controls ($p < .001$). After the high energy drink, controls reduced their energy intake at the meal by a mean (SD) –257.3 (383.3) kJ (similar to the amount provided in preload) while the cases showed a slight average increase of +78.1 (365.9) kJ; t ¼ 3.26, df 51, $p < .001$. Compensation was precise in the controls, with one kJ of

pre-load on average reducing subsequent intake by 1.18 kJ. It was, however, completely absent in the cases who, on average, took 0.80 kJ more at the test meal for each kJ consumed in the pre-load. The controls compensated as expected for their high energy load at the subsequent meal, but the case children did not, showing that they lack the normal responses to internal hunger/satiation cues.

In a recent experimental study, Carnell *et al.* (2017) tested whether heavier children demonstrated poorer caloric compensation across a range of conditions and further explored whether compensation failure was the result of inadequate adjustment of overall intake or specific over-consumption of highly palatable, high energy-density 'junk' foods. Two preload conditions were tested in this study. The two-preload sequences (low and high energy preload) for test A were organoleptically similar and only differed in carbohydrate content (orange juice-0kcal and 200kcal). Preloads for test B were, however, organoleptically different and differed in terms of macronutrient composition. Specifically, water was used as the low caloric preload and strawberry flavoured milkshake were used as the high caloric preload. Participants for this study were 4–5-year-old preschool children and the study took place in their school during lunch time and under normal lunch conditions.

The children were fed with a multi-item ad libitum lunch meal consisting of junk foods (chocolate cookies, cheese-flavoured crackers) and core foods (fruits and vegetables, bread rolls, protein foods) 30 minutes after preload. The mean COMPX score for Test A was 70 ± 77% SD with a wide range of scores (−87% to 234%) was substantial, indicating wide variation in compensation ability between individuals. There was no significant association between weight status and energy compensation in test A. However, further analysis revealed that overweight and obese children ate relatively more junk foods than normal-weight kids. For test B, which involved the use of water and strawberry milkshake as preloads, the mean COMPX score was lower than test A (51 ± 58% SD, with a range of −131 to 200%). This difference

was, however, not statistically different. There was also no significant association between energy compensation and weight status for test B. However, when both tests were considered together, results revealed that the mean COMPX score averaged across Test A and Test B was 61 ± 51% SD, with a range of −57 to 181%. There was a significant negative correlation between mean compensation and adiposity (BMI score) (r=−.26; p=.049) such that overweight/obese children showed the least compensation (41%), children over 50th centile the next least (59%), and children under the 50th centile (80%) the most.

These findings suggest that, indeed, obese/overweight children have poor energy compensation and even normal weight children who are heavier also compensate poorly than their colleagues putting them at risk of obesity. The researchers opined that the increased consumption of junk foods among obese/overweight children in test A is indicative that under certain conditions, overweight/obese children's preferences for obesogenic/junk foods may overwhelm intake regulation mechanisms within multi-item meals. This study did not support the prediction that when children are given preloads that are organoleptically similar, as in test A, they tend to focus on internal satiety signals and when supplied with preloads that are different as in test B, they focus on external cues. Compensation scores were lower for test B (Water vs milkshake condition) than test A (orange juice), although this was not statistically significant.

It could be argued that the study was not powered enough to detect a difference between the two test groups. This study is robust in the type of design used and the combination of two different preload conditions. Unlike most energy compensation studies that take place in the laboratory setting, this experiment took place in the children's natural environment and, hence, supplies some level of ecological validity compared to other laboratory-based studies. However, eating together with peers can also influence how much food is consumed as well as the food choices. Therefore, the results may not be generalizable to other free-living eating

situations, e.g., eating at home. The total sample size for the combined test was small and, therefore, limited the power of the study.

In a comprehensive and systematic review of 19 cross-sectional and prospective studies, Lansigan *et al.* (2015) aimed to identify individual, familial, and societal level correlates of eating in the absence of hunger (EAH) among 3-12 years old children. The results showed an observable EAH across all age groups and gender. There were large variations among studies, with individual studies examining different aspects of EAH ranging from individual characteristics of the child to characteristics of the child's parent. There was also a lack of uniformity in the operationalization of EAH. Irrespective of this heterogeneity, it was consistently evidenced that EAH was positively associated with increased weight status, with EAH being significantly observed among overweight and obese children from both cross sectional and prospective studies. The review also revealed that absolute levels of EAH increased with age and with maternal feeding styles were associated with EAH among girls.

2.7 PUBLIC HEALTH INTERVENTIONS TO COMBAT MALNUTRITION: GLOBAL CONTEXT AND OPPORTUNITIES IN GHANA

Apart from death, malnutrition during early childhood adversely affects adult size, intellectual ability, economic productivity, and reproductive performance (De Sanctis *et al.*, 2021; Hoddinott *et al.*, 2013; Mwene-Batu *et al.*, 2020). Furthermore, it also increases the risk of non-communicable diseases (Barker *et al.*, 2002; Black *et al.*, 2008). The high prevalence of malnutrition and its related short term and long-term effects on an individual and the population warrant a need for effective evidence-based interventions targeting affected populations.

The WHO has championed nutrition interventions to help combat malnutrition. Some of such interventions are behavioural interventions which focus on the adjustment of personal practices and habits, e.g. breastfeeding, complementary feeding, caffeine intake in pregnancy, nutrition counselling during pregnancy, increasing fruits and vegetable consumption to reduce

noncommunicable diseases, portion size control, and reduced intake of sugars (WHO, 2013). They have also championed fortification programmes to fortify staple crops, iodize salts, and provision of multiple micronutrient powders for point-of-use fortification of foods taken by children, infants, and pregnant women. There have also been conducting health related actions and situational actions such as deworming of children, adolescents and pregnant women, optimal timing of cord clamping for prevention of iron deficiency anaemia in infants, use of insecticide treated nets, water, sanitation, and hygiene interventions to prevent diarrhoea (WHO, 2013). WHO also has regulations on the marketing of foods, sugars, and non-alcoholic beverages to children, as well as the marketing of breastmilk substitutes (World Health Organization, 2023). Nutrient supplementation, such as folic acid, iron, vitamin A, iodine, zinc, and multiple micronutrients, has also been among the WHO's initiatives to curb malnutrition, especially among vulnerable groups (WHO, 2013). Most of these intervention programs are still ongoing in various parts of the world, with some being transformed into health policies. The efficacy and effectiveness of these interventions, however, need a wider exploration.

In Ghana, the 2022 Demographic Health Survey revealed that the prevalence of infant malnutrition had reduced marginally from 2003 to 2022 (Ghana Statistical Service (GSS) and ICF International., 2023). These reductions may possibly be due to the implementation of several strategies starting in 1951 with the aim of eliminating malnutrition (Ghartey, 2010). Such strategies include nutrition education (to improve knowledge and attitudes), food demonstrations, supplementation, fortification, behaviour change communication in Infant and Young Child Feeding, the United Nations Scaling up Nutrition (SUN) initiative, the Baby Friendly Hospital Initiative, legislation on salt iodization and uncontrolled exposure to infant formula, etc. (Ghartey, 2010; Gongwer & Aryeetey, 2014)

Despite these interventions, the problem persists as reported in the recent Demographic Health Survey (Ghana Statistical Service (GSS) and ICF International., 2023). Gongwer and Aryeetey (2014) in their study to assess district-level capacity and commitment for accelerating the implementation of effective nutrition interventions to address the high burden on maternal and child malnutrition, discovered that nutrition interventions are underfunded and are challenged by low prioritization of nutrition, inadequate skilled personnel, and insufficient job aids. This shows that the implementation of nutrition interventions in Ghana is quite weak and requires strengthening.

2.8 EFFECTIVENESS OF EARLY CHILDHOOD NUTRITION INTERVENTIONS

There has been very little established on the Standard treatment of eating in the absence of hunger (EAH). A pilot study by Boutelle *et al.* (2011) examined two treatment methods on overweight and obese children who exhibited EAH behaviour under an experimental condition. In the first treatment method (food cue exposure training), children underwent eight weekly training sessions to build self-efficacy to avoid consuming food in the absence of hunger when exposed to food cues. In the second treatment method (appetite awareness training), children were trained over eight weekly training sessions to increase self-awareness of internal states of hunger and satiety and trained on how to monitor such sensations. These children were also taught coping skills to manage the urge to engage in EAH. The results showed a significant decrease in EAH in children who received the first treatment method immediately following the 8-week treatment and six months post-treatment. However, no significant changes in EAH over eight weeks or six months were observed among children in the second treatment method. Although the study was limited by the small sample size and the absence of a true, non-treatment control group, the results are still promising. The study suggests that behavioural training based on building self-efficacy to control food cravings may reduce EAH among overweight and obese children.

2.9 SUMMARY OF LITERATURE REVIEW

The reviewed literature shows that infant malnutrition is still a global challenge. There are many existing interventions to help curb malnutrition. However, the problem persists because the cause of malnutrition is complex. There are both dietary and non-dietary causes of malnutrition. Some of the dietary causes reviewed in this session were breastfeeding, complementary feeding, dietary diversity, and feeding behaviour. The non-dietary causes reviewed were infant caregiving, water and sanitation (which is seen from the literature to cause diarrhoea and other infections). No matter the cause, the ultimate goal is to ensure successful energy regulation, that which mainly controls food intake or avoidance. Energy regulation is influenced by biological factors such as hormones and genetics as well as environmental factors such as caregiving feeding practices encompassing breastfeeding, complementary feeding and dietary diversity. Energy regulation is also influenced by feeding behaviour and stimulation, mainly, feeding styles adopted during feeding episodes as well as the feeding environment. Energy regulation is also genetically controlled, with certain genetic polymorphisms seen to be associated with appetite and body weight regulation. It has been recently discovered that just like obesity, thinness may also be genetically driven. Energy regulation has been generally studied by measuring energy compensation. This is often described as an individual's ability to eat in the absence of hunger, i.e., eating even after satiety has been achieved. The study of energy compensation has been commonly done by using the preload paradigm to assess how much food is eaten after a preload meal or drink has been taken. Most studies have adopted different ways of using the preload concept, with differences seen in the type of food item used as well as the duration between preload intake and test meal intake. This chapter ended with a review of some of the existing public health interventions to combat malnutrition and the effectiveness of early childhood nutrition interventions. An overview of the literature revealed a paucity of work on;

- Energy regulation among malnourished children living in resource constrained environments.
- Energy regulation among preschool age children.
- The genetics of thinness.

This research work therefore sought to fill these knowledge gaps.

CHAPTER 3

3.0 METHODOLOGY

3.1 STUDY DESIGN

This study comprised two phases. Phase one (1) was a cross-sectional study, and phase two (2) was an experimental study (cross-over study).

3.2 STUDY SITE

This study was carried out in selected Child Welfare Clinics (CWC) in Ashiedu Keteke sub-metropolitan district in the Greater Accra Region, Ghana. The Greater Accra Region was selected because it is cosmopolitan, and being the capital region in Ghana, it hosts a wide variety of people with different ethnic backgrounds. Clinics in Jamestown, Bukom, Agbogbloshie, Ussher Town, Princess Marie Louise (PML) Children Hospital and the Nutrition Rehabilitation Centre of PML were selected for the reasons discussed below.

The Ashiedu Keteke sub-metropolitan district is the smallest among 11 sub-metro districts in Accra and is in the central part of Accra, where most economic activities happen (Sarpong *et al.*, 2015). The estimated population as of 2021 was 88,633, of which 40423 were males and 48210 were females and comprised a total number of 33572 households (Ghana Statistical Service, 2021). The district is home to the central business area of the Greater Accra Region Accra, hosting major markets such as Markola, Agbogbloshie, and *Kwasiadwaso*. Numerous other economic and social activities include banking, education, hospitals, light industrial activities and tourist sites (Kwame Nkrumah mausoleum). The district, therefore, attracts over 2 million people daily who travel from all around Ghana and beyond for administrative, educational, industrial, recreational and commercial purposes (Accra Metropolitan Assembly (AMA), n.d.). The heavy influx of people on a daily basis helps to enhance economic activities

within the Metropolis; However, this puts pressure on already heavily burdened infrastructure facilities, coupled with sanitation challenges.

The Ashiedu Keteke sub-metropolitan district was one of the two districts in the Greater Accra Region where the Ghana Health Service first established the Community-Based Management of Acute Malnutrition (CMAM) learning sites in 2008. It is a major hub of malnutrition and covers major slums in Accra (Bahwere *et al.*, 2010). The PML Children's Hospital is located within the Ashiedu Keteke sub-metro district. The main services provided in the hospital are medical care, reproductive and child health services, family planning and nutrition services (Sarpong *et al.*, 2015). The PML Children's Hospital was selected because it serves as a key facility in the Greater Accra Region for the treatment of malnutrition (Sarpong *et al.*, 2021). The Nutrition Rehabilitation Centre mainly operates to prevent, promote, restore, and improve the nutritional status of nutritionally vulnerable children through nutrition specific and nutrition sensitive interventions (Princess Marie Louise Children's Hospital, 2022). These child welfare clinics and Nutrition Rehabilitation Centre were, hence, purposely selected for the study to allow access to the required number of children with moderate acute malnutrition and stunting.

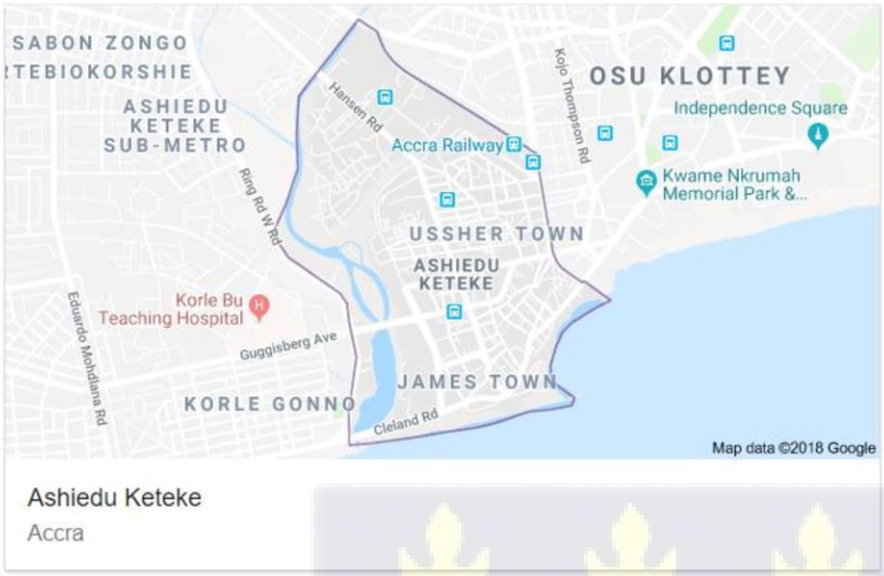


Figure 3.1- Map of the study site; Ashiedu Keteke sub-metropolitan district

DATA COLLECTION PROCEDURE

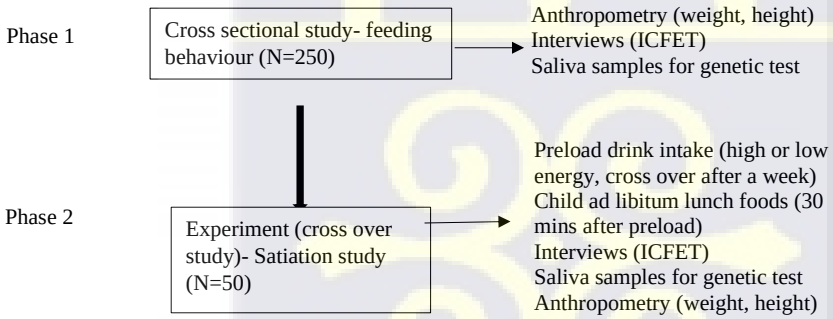


Figure 3.2: Flow chart of data collection

3.3 METHODOLOGY- PHASE 1

3.3.1 Study design

A cross sectional study was employed for this phase of the study.

3.3.2 Study population

This phase involved caregivers of / and preschool children aged 1-3 years attending CWC in the selected study sites (Jamestown, Bukom, Agbogbloshie, Ussher Town, Princess Marie Louise (PML) Children Hospital) in the Greater Accra Region.

3.3.3 Variables: Dependent and Independent

Based on the specific objectives, the dependent and independent variables are

OBJECTIVE	DEPENDENT VARIABLES	INDEPENDENT VARIABLES
1	Nutritional status- wasting, stunting or normal weight	-Dietary characteristics (breastfeeding, complementary feeding, minimum dietary diversity, meal frequency, self-feeding) -sociodemographic characteristics (age, gender, employment status, marital status, birth order, relationship with child, availability of child's father, education, wealth quintile, ethnicity)
2	Nutritional status- wasting, stunting or normal weight	Feeding behaviour (food interest, food refusal, forced feeding)
3	-Nutritional status (wasting, stunting or normal weight) -Feeding behaviour (food interest, food refusal, forced feeding)	Polygenic risk score

3.3.4 Sampling

Purposive sampling was used to select the communities and child welfare clinics to allow access to the required number of malnourished children. At the CWC, healthy children were selected using systematic sampling from a random start, while all eligible malnourished children attending the clinic during the data collection period were allowed to take part in the study.

3.3.5 Sample size

$n= (z\alpha/2)^2 (P\times Q) /E^2$ (Cochrane formula)

$(z\alpha/2)^2 = 1.96^2$

P= 19%

Q= 81%

E= 0.05

n=236.5

$(z\alpha/2)^2$ – Absolute value of a two tailed test at 95% confidence interval

(P=Proportion of population exhibiting what is being studied, Q= Proportion of population not exhibiting what is being studied, n= sample size and E= accepted error margin.)

The estimated prevalence of chronic malnutrition (stunting) among children 0-5 years in Ghana based on the 2014 Ghana Demographic Health survey is 19%, hence p=19%, and the prevalence of wasting is 5% (GSS & GHS, 2015). Using the Cochrane's formula for sample size determination (calculation shown above), a sample size of 236.5 (approximately 250) was needed for the cross-sectional study.

A pilot study was conducted among 65 wasted children with feeding problems in the UK. The mean polygenic risk score for this sample was 11.22 (± 1.8) out of a possible 20. This was significantly higher by 1.53 points (95% CI: 0.75-2.32) than the simulated mean polygenic risk score of 9.77 (± 1.91) for a healthy UK population (independent t-test, t=4.04, p=0.001). This result indicates a potentially meaningful variation in risk scores that could relate to health outcomes such as being wasted. Notably, 52% of the sample had polygenic risk scores more

than 1 SD above the expected mean, and 13% had scores more than 2 SD above, suggesting a significant deviation in the distribution of risk scores.

Based on the pilot study, it was hypothesized in this current study that children with moderate acute malnutrition would have higher polygenic risk scores than healthy children. Epi Info StatCalc was used to determine the sample size needed to achieve 80% power to detect a difference between the two study groups. Considering feasibility, including logistical, financial, and temporal constraints such as the availability of participants, the cost of genotyping, and the study's overall duration, it was determined that a sample size of 250 children (125 malnourished and 125 healthy) would be adequate.

The power calculation indicated that with this sample size, this study would have 80% power to detect a risk difference of 0.17 between the two groups, assuming that 47% of malnourished children would have a higher polygenic risk score compared to 30% of healthy children. This power calculation ensured that the study was adequately powered to detect a significant difference in the proportion of children with high polygenic risk scores between the two groups. Given the significant differences observed in the pilot study and the specific hypothesis of this current study, the power calculation guided the design of this robust and feasible study to test this hypothesis.

3.3.6 Sampling procedure

At the selected study sites, a systematic sampling from a random start was used to select healthy participants at the child welfare clinics. Using the Randomiser App v.10.00 (softexe.net), the first two healthy children in the queue were randomly allocated to either be approached or not. From that, every first mother/child pair in the queue was approached when the researcher was free to do the next interview. However, all malnourished children were approached. Hence, all

eligible malnourished children attending the clinic on the data collection days were selected. This was done until the required numbers of healthy and malnourished children were achieved.

3.3.7 Inclusion and exclusion criteria

Criteria for inclusion in this study were children aged 1-3 years attending child welfare clinics at the selected enumeration areas. The children should be accompanied by the main caregiver actively involved in cooking for and/or feeding the infants.

Group 1 (healthy children) had WHZ >-2SD and/ or HAZ >-2SD and group 2 were wasted (WHZ between -2SD and -3SD) and/or stunted (HAZ < -2SD) children.

Children with congenital disorders, disabilities and diseases requiring specialised care and hospitalisation were excluded from the study. Children with severe malnutrition with complications that required inpatient care and tube-fed children were also excluded.

3.3.8 Data collection procedure- Phase 1

3.3.8.1 Assessment of feeding behaviour.

The international complementary feeding evaluation tool (ICFET) questionnaire (Appendix 3) was used to interview caregivers to provide parental ratings of avidity (appetite), food refusal and force feeding. The ICFET is a structured interviewer administered questionnaire. Most of the questions are on infant and caregiver feeding behaviour and interactions. The questions were obtained based on information from a pilot study in August 2014 in Mukuru slum area in Nairobi-Kenya, a responsive feeding intervention study carried out in rural Bangladesh (Aboud, Shafique and Akhter *et al.*, 2009; Aboud, Moore and Akhter *et al.*, 2008) and the alive and thrive initiative (Nizame *et al.*, 2013). The questionnaire was further piloted in Pakistan among five households and in an urban informal settlement in Nairobi –Kenya. The final questionnaire has been tested for face validity, internal consistency, and predictive validity

among six different languages or regions, i.e. UK, Cyprus, Indonesia, Pakistan, Kenya and Guatemala (Wright *et al.*, 2020; Wright *et al.*, 2020). The questionnaire has been found to be a valuable tool for the identification of poor feeding and eating practices among children.

The tool comprises standard questions on meal frequency and self-feeding, and a 5-point score for enthusiasm for eating (avidity/ appetite), food refusal (avoidance), force feeding and caregiver feeding anxiety (Wright *et al.*, 2020). The questionnaire also assesses foods eaten within 24 hours as well as dietary diversity, child illnesses, child development, household hunger score and wealth quintile. The participants were interviewed in either English or two commonly spoken Ghanaian languages: *Twi* or *Ga*. These are the predominant languages in the selected study areas. Each interview lasted for about 30 minutes.

3.3.8.1.1 Determination of feeding behaviour

Feeding behaviours were assessed for both the child and caregiver. There were specific questions which were rated on a 5-point Likert scale from "1" being "not at all" to "5" being all the time. For the child's feeding behaviour, an assessment was made for the child's appetite (avidity) and food refusal. Caregivers' behaviours assessed were force-feeding and feeding anxiety. Questions asked for each behaviour are presented in the table below.

Table 3.1 - Assessment of feeding behaviour

Appetite (eating avidity)	Food refusal	Force-feeding	Feeding anxiety
Would you say your child- Likes food Interested in food Enjoys food Enjoys eating variety Eats quickly Finishes meals	*How often does your child do the following when offered food?* Turns away Pushes food Cries Spits out Meals last for 1 hour	*What sort of things do you do if your child refuses to eat?* Restrains Pours food into mouth Forcefully opens mouth	Do you worry that your child is not eating enough Does your child's feeding cause you significant anxiety *Are you concerned about* The variety of foods your child eats Your child's behaviour at mealtimes Your child's lack of interest and/or enjoyment of food Your child's eating speed

3.3.8.2- Determination of food intake and dietary diversity

A 24-hour dietary recall was conducted. This method of dietary assessment is common and retrospectively collects data on all foods and drinks consumed 24 hours prior to the time of assessment (Huang *et al.*, 2022). Although easy and common, this form of assessment is subject to recall bias. The caregivers in this study were asked to list the foods given to the children in the last 24 hours. The foods listed were classified under six categories: plated foods, dry finger foods, moist finger foods, milk drinks, other drinks, and ready-to-use foods.

Again, a food frequency questionnaire was also used to determine the frequency of consumption from ten different food groups. The food groups were starch foods, meat/fish/poultry, dairy products, legumes/nuts, fruits, leafy vegetables, savoury snacks, sweet snacks, and food cooked in oil. Caregivers rated the frequency of foods as being consumed by the child more than once daily, once daily, once a week but not daily, once a month but not weekly or never/rarely.

Dietary diversity was determined using the data collected from the 24-hour recall. Foods consumed were classified under the following groups;

1. Starchy foods (grains, roots, and tubers)
2. meat/fish/poultry
3. eggs
4. dairy products
5. legumes/nuts
6. vitamin A rich fruits and vegetables
7. other fruits and vegetables
8. breastmilk

For this study, minimum dietary diversity was considered to be 5 out of 8 food groups for children 12-23 months and 4 out of 7 food groups for children 24-36 months (Keyata *et al.*, 2022; WHO, 2023a). A score of one was given if the food was consumed and zero when not consumed.

3.3.8.3 Determination of household hunger score

The household hunger scale was used to determine food poverty within the study population. This scale is a simple tool used to assess food deprivation within populations and hence, serve

as a proxy for food security (Ballard *et al.*, 2011). This tool has been designed for use across different cultures and is most useful in areas with significant food insecurity. Caregivers were made to rate their responses using a 4-point Likert scale from '0' being 'never' to '3' being 'Often (more than 10 times in the past 30 days)'. The questions asked are listed below.

1. Was there ever no food to eat of any kind in your house because of lack of resources to get food?
2. Did you or any household member go to sleep at night hungry because there was not enough food?
3. Did you or any household member go a whole day and night without eating anything because there was not enough food?

3.3.8.4 - Determination of household wealth

Questions on ownership of household assets were also included in the questionnaire for assessing the wealth index and subsequent categorisation into wealth quintiles. The wealth index, created by assessing ownership of household assets and other variables, is widely used in larger national surveys such as the Ghana Demographic Health Survey (Ghana Statistical Service (GSS), Ghana Health Service (GHS), 2015) and especially in developing countries as a measure of economic status (Smits & Steendijk, 2015).

3.3.8.5 Anthropometric measurements

Children's weight and recumbent length or standing height were measured following standardized protocols (World Health Organization, 2008; NHANES, 2007). For precision, duplicate measurements were taken, and the average was recorded.

Children who could stand on their own were weighed in minimal clothing and barefooted using a Seca weighing scale (Seca 354). Children who could not stand on their own were weighed

naked or in a clean, dry diaper using a Seca baby scale (Seca 354). They were weighed either sitting or lying in the tray of the scale. For children who refused to be weighed, their caregivers were made to stand on the scale and their weight was tared. The child was then given to the caregiver while standing on the scale. Weight measurements were made and corrected to the nearest 0.1 kg.

Standing height was measured with a portable Seca stadiometer (Seca 213) which was placed on a firm, flat ground to ensure accuracy. Measurements were taken without shoes/footwear. Each child was made to stand upright with hands by the sides and back towards the measuring pole while the head was held in the Frankfurt plane. The headpiece of the stadiometer was lowered to the head. Reading was taken when the child had taken a deep breath. For children who could not stand on their own, recumbent length was measured using a Seca mobile measuring mat for babies and toddlers (Seca 210). The infantometer was placed on a flat surface. With the help of the mother, the infant was made to lie supine on the mat. The child's head was gently supported in a Frankfort position and in contact with the head piece by applying gentle traction. The child's feet were also supported with gentle pressure on the knees by the researcher while pulling up the footpiece to be perpendicularly and firmly aligned with the child's sole (feet pointing upwards). In situations where a child was fussy, only one leg was used. The measurements were taken to the nearest 0.1cm.

3.3.8.6 Genetic testing

These genetic tests were done at the Duedu laboratory at the University of Health and Allied Sciences, Ho, Ghana.

3.3.8.6.1 Sample collection, transport and storage

Saliva samples for genetic analysis were collected using ORAcollect for Pediatrics (OC-175), an easy-to-use DNA collection kit (DNA Genotek Inc., Canada), following the manufacturer's

instructions (Appendix 11). This kit is a swab that is used to collect saliva from the mouth of children. It contains a stabilising reagent that inhibits the growth of bacteria from the time of sample collection to processing. The kit is designed to allow the DNA to remain stable at ambient temperature (between 15° to 25°C (59° to 77°F) or up to 30°C) for one year.

The saliva samples were transported in insulated cooler bags from the study sites to the laboratory, where they were stored at room temperature on the bench until DNA extraction. The extracted DNA samples were stored in a laboratory freezer at -20^0c until analysis.

3.3.8.6.2 DNA extraction and genotyping

DNA extraction was performed using a DNA Genotek Oragene kit, and genotyping was done using Taqman PCR. The extraction and genotyping process were carried out following the laboratory protocol described by Moran, Hunter and Innes (2013) (Appendix 12). DNA concentration, A260/A280 and A260/A230 ratios for integrity and quality were measured using the NanoDrop spectrophotometer (ThermoFisher, Switzerland). Genotyping and polygenic risk scores were done for the following SNPs due to their role in energy regulation.

Table 3.2- Genes and SNPs

GENES	ROLE IN ENERGY REGULATION	SNPS
MC4R	Body weight regulation gene	rs6567160
ADCY3	Energy homeostasis	rs10182181
FTO	Fat mass and obesity-associated gene	rs1558902
TMEM18	Appetite and body weight regulation	rs13021737
SEC16B	vesicular transport of secretory molecules from the endoplasmic reticulum (ER) to the Golgi apparatus	rs543874
ETV5	Regulation of insulin secretion	rs1516725
CADM2	BMI-associated gene variant	rs4740619
CA6 (Gustin)	PROP taste sensitivity and papillae density gene	rs2274333
TAS2R38	Bitter taste receptor gene	rs10246939

(Carnell *et al.*, 2008; Garcia-Bailo, 2019; Riveros-McKay, 2019)

3.3.9 Data and statistical analysis

3.3.9.1 Data analysis

The WHO Anthro (Plus) survey analyser was used to convert the anthropometric measurements into z-scores. This is an online tool that analyses and reports anthropometric indicators: length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age for children under five years of age. Using the WHO cut-offs, children were categorised as healthy (WHZ >-2SD and/ or HAZ >-2SD, wasted (Weight for height z

score (WHZ) <-2SD), stunted (Height for age z-score (HAZ) <-2SD) and underweight (weight for age z-score (WAZ) <-2SD) (WHO, 2008).

A simple polygenic risk score was calculated for each child to determine their genetic liability to malnutrition. This was done by summing up all risk alleles present for each child. A score of 1 was assigned for heterozygote risk alleles, and a score of 2 for homozygous risk alleles. Therefore, a possible highest score of 18 was, hence, expected for the 9 SNPs tested, assuming a child was homozygous for the risk alleles of all 9 SNPs.

The child and caregiver feeding behaviour variables were grouped into appetite, food refusal, force feeding and maternal anxiety scores (Mutoro *et al.*, 2020) using the variables shown in Table 3.1. The means of the scores were then used to create categories reflecting high, Amoderate, and low occurrence.

Table 3.3 - Child and caregiver feeding behaviour scores.

Scores	**Components**	**Scores Assigned to Each Response**	**Score Range**	**Score Interpretation**
Appetite Score	Likes food Interested in food Enjoys food Enjoys eating variety Eats quickly Finishes meals	Not at all=1 All the time=5	6 to 30	6-14= low 15-22= moderate 23-30= high
Refusal Score	Turns away Pushes food Cries Spits out	Not at all=1 All the time=5	5 to 25	5-11= low 12-18= moderate 19-25= high

	Meals last for 1 hour			
Force Feeding Score	Restrains Pours food in mouth Forcefully open mouth	Not at all=1 All the time=5	5 to 15	5-8= low 9-11= moderate 12-15= high
Anxiety Score	child not eating enough child's feeding cause significant anxiety child's variety of foods child's behaviour at mealtimes child's lack of interest and/or enjoyment of food child's eating speed	Not at all=1 All the time=5	6 to 30	6-14= low 15-22= moderate 23-30= high

A score of 1 was given for household ownership of a mobile phone, radio, television, motorcycle, bicycle, refrigerator, and car. Scores of 1 were also given for improved household characteristics, which included having water piped into their house, having a flush toilet, not sharing toilet facilities, not paying for toilet use, owning their house, and having a home which is a permanent construction. In all other cases, scores of 0 were allocated. A principal component analysis (PCA) was used to compute the wealth index score from categories of asset ownership and the household characteristics of the study participants. The PCA computed scores were then ranked into five quintiles representing five categories of wealth: poor, lowest middle, middle, upper middle and richest (Ghana Statistical Service (GSS), Ghana Health Service (GHS), 2015) as shown in table 4.1.

3.3.9.2 Statistical analysis

All data were entered into and analysed with the Statistical Package for Social Sciences version 22. Data cleaning was done by running descriptive and frequency analyses to check out for missing values and wrongly coded values. All missing values and wrongly coded values were filled out by going back to the filled questionnaires. A Shapiro-Wilk test was carried out to test for normality of the data. Basic descriptive and frequencies were run for the various child and caregiver variables to describe their background characteristics. The table below summarises the statistical tests done for each specific objective depending on the normality distribution of the data.

Table 3.4: Statistical analysis for phase 1

OBJECTIVES	VARIABLES	TEST
1. To determine the background characteristics of the study children with respect to nutritional status, dietary and socioeconomic characteristics.	Nutritional status – WAZ. HAZ, WHZ Dietary characteristics Sociodemographic characteristics	Descriptive (Mean, frequency) One-way ANOVA, Scheffe post hoc, Linear regression, Kruskal Wallis, Chi Square
2. To determine the association between feeding behaviour and nutritional status of children.	Feeding behaviour (appetite, food refusal, force feeding) Nutrition status	T-test, multinomial regression

3. To calculate a polygenic risk score (Energy regulation SNPs) and determine any association with child's nutritional status and feeding behaviour.	Polygenic risk score (PGRS) Nutritional status (WAZ, HAZ, WHZ) Feeding behaviour	Chi square One-way ANOVA Scheffe post hoc Pearson correlation Relative risk

3.4 PHASE 2 METHODOLOGY

3.4.1 Study design

This was an experimental study (cross-over study).

3.4.2 Study population

The experimental study involved healthy (WHZ >-2SD and/ or HAZ >-2SD), moderately malnourished (WHZ between –3 and –2 Z-scores) and stunted (HAZ<-2SD) children aged 1-3 years who were attending child welfare clinics in the selected communities.

3.4.3 Variables: Dependent and Independent

Based on the specific objectives 4, the dependent variable is compensation index (COMPX) and the independent variables are nutritional status (wasting, stunting or normal weight)

3.4.4 Sampling

Simple random sampling was used to select an equal number of stunted and moderately malnourished children from phase 1 for the experimental study.

3.4.5 Sample size

Current research shows that healthy children exhibit approximately 70% energy compensation (Kane *et al.*, 2011). If it is assumed that undernutrition is caused by poor energy compensation, then wasted children are more likely to have imprecise compensation than healthy children, either overcompensating or undercompensating beyond or below 70%. On the other hand, stunted children who are not wasted are also not acutely malnourished and, therefore, are expected to compensate more similarly to healthy children. Hence, in this study, a group of stunted, non-wasted children could function as a proxy for healthy controls and be of interest in their own right.

An experimental study by Carnell *et al.* (2017) that involved overweight/obese children, children above or under the 50th centile showed an average compensation of 70% with a standard deviation of 77%. This mean compensation and standard deviation (SD), a sample size was calculated for two independent samples of moderately wasted and stunted children using SPSS. A sample size of 42 (21 moderately wasted and 21 stunted) would supply an 80% power to detect an effect of 0.9 SD at 0.05 significant level; however, such a large standard deviation (77%) would only allow us to detect a very large difference, which is not likely to be found among this group of children. Besides, such large differences will also allow the average to mask more interesting differences in the distribution of values. Previous studies tended not to show the mean and standard deviation for energy compensation precisely.

However, a pilot study conducted among four healthy and five malnourished children with feeding problems in Glasgow generated a mean COMPX of 91% and an SD of only 40%, while the COMPX varied from 71% for healthy to 106% for the sick children; and thus a risk difference of 35% was recorded. Using this SD in a power calculation with SPSS software, a sample of 38 (19 per group) would provide 80% power to detect a mean difference of this size between the two groups.

The alternative approach was to carry out a power calculation based on proportions. With the assumption that in the wasted cohort, there might be more children who under- or over-compensate, with fewer children showing normal compensation, the Epi Info StatCalc software package was used to calculate the sample size. A sample of 20 children in each of the two groups (moderately wasted and stunted) offered 80% power to detect a difference in the percentage who under-compensated or over-compensated of 30% versus 74% or 10% versus 50%. These multiple scenarios were made to help assess the sensitivity of the study design and sample size calculation to different possible situations. It helps understand how changes in the assumed proportions of interest might affect the statistical power of the study. In this case, the power calculation is performed under these different scenarios to ensure that the study has an adequate chance of detecting meaningful differences in compensation patterns, regardless of the specific conditions that may exist in the population.

Therefore, we aimed to work with a total of 50 children (25 per group) for the experimental study because this number seems feasible and sufficiently powered to detect possible differences.

3.4.6 Sampling procedure

A simple random sampling technique was employed for the experimental study (Phase 2). These participants were selected among the cases from phase 1 of the study. The names of all eligible participants were inputted into the Randomizer App v 10.00 to randomize healthy children, moderately malnourished children and stunted children. Caregivers of eligible children were contacted and invited into the phase 2 study after thoroughly explaining the study protocol. Children of consenting caregivers were recruited for the study. This was done until the required numbers for each arm of the study were achieved.

3.4.7 Inclusion and exclusion criteria

Criteria for inclusion in this study were children aged 1-3 years attending child welfare clinics at the selected enumeration areas. The children should be accompanied by the main caregiver actively involved in cooking for and/or feeding the infants.

Children who were included in the experiment were healthy controls (WHZ >-2SD and/ or HAZ >-2SD), wasted (WHZ between -2SD and -3SD or HAZ < -2SD) and/or stunted (HAZ < -2SD).

Children with congenital disorders, disabilities and diseases requiring specialised care and hospitalisation were excluded from the study. Children with severe malnutrition with complications that required inpatient care and tube-fed children were also excluded. The experiment required children to have fasted for at least 2 hours prior to the experiment. Children who were both stunted and moderately malnourished were not eligible for the experimental study.

3.4.8 Data collection procedure- satiation study (cross-over design)

Two satiation studies (high /low preload) were conducted for randomly selected children. Participants were visited in their homes between mid-morning and lunchtime after they had fasted for at least 2 hours. The test was rescheduled for children who were unable to fast for at least two hours prior to the study or children who were ill on the test day. Before the experiment began, a history of breakfast meals, snacks, and breastfeeding on the day of the study was collected. The history included time for meals and snacks, quantities of meals eaten (estimated using handy measures and food models), as well as recipes. The caregiver was asked to feed the same breakfast meal and snacks on the next study day.

Children were randomly assigned (using the randomizer app) to preload the sequence (high or low energy on test day 1 and the alternate version on test day 2). The two (2) preload drinks differed in carbohydrate content and energy density but not in flavour or appearance. The low energy preload was a sugar-free squash (Vimto) that supplied 19 kcal per 100 ml diluted product. The high-energy drink was the same squash plus a weighed amount of Super Soluble Maxijul. Super Soluble Maxijul is a commercial powdered carbohydrate energy source, which can be mixed with sweet or savoury foods/ liquids. It is safe for use in both children and adults who require fortification with a high or readily available carbohydrate. It is flavourless and tasteless, making minimal to no impact on the taste, flavour or texture of the food being added to. It supplies 380 kcal energy per 100g powder. Maxijul was used by Kasese-Hara et al .(2002) to test for energy compensation among children 12-24 months.

The high energy preload supplied 10% of the individual child's daily energy needs (kcal/kg/day). For instance, a 3-year-old child weighing about 16 kg requires approximately 1300 kcal/day. The high energy preload, hence, supplied 130 kcal. The preload drinks had the same volume, and there was at least 100 kcal difference between the low and high energy preloads. All preload drinks were supplied in transparent but coloured cups. The drink allocation (first visit) was performed by a research assistant and the researcher was blinded to the type of preload offered to the child to avoid bias. The volume of drink before and after intake was recorded.

Participants were given a standardised, weighed buffet lunch (chosen in advance to suit the child) 30 minutes after preloading. Caregivers of eligible children were contacted in advance to give a list of preferred lunch meals of the child. The procurement of the meals was also agreed on with the caregiver, i.e. either the caregiver or researcher supplied the meals for the two study days. The type of meals was as preferred by the child and hence, was not the same

for all participants. The meal component was thoroughly described in terms of recipe and quantities. This process was repeated after at least 1week and at most four weeks in a cross-over manner. The variations in this wash-out period were considered in the analysis. Participants who received a low energy preload drink the week before, was given a high energy preload drink on the second visit. The same selection of lunch meals was offered on both study occasions. The caregivers were asked to feed the same breakfast meals and snacks for the two study days.

Feeding episodes were in accordance with normal parental feeding assistance or encouragement (such as spoon feeding, cutting up or handing food, depending on developmental stage), and the meals ended when children demonstrated signs of satiation as determined by the parents, such as stopping eating, refusing to be fed further or saying that they were full. Food items were weighed before and after intake using a Seca digital kitchen scale (Seca 852), with spillages accounted for by gathering and weighing where possible.

3.4.8.1 Analysis of the energy content of foods using Bomb calorimetry

3.4.8.1.1 Collection, transportation, and storage of food samples

The energy component of the lunch meals was tested using a bomb calorimeter. Bomb calorimetry is a more accurate way of determining calories and it is considered the gold standard. It was, hence,used to determine the amount of energy consumed by the children to accurately assess their energy compensation. About 50-100g of food samples were collected into labelled zip-lock bags, put on ice packs to prevent decay and transported to the Department of Dietetics research laboratory. The samples were transferred into 50 ml falcon tubes and stored in a -20^{0}C freezer on the same day of collection. After data and sample collection, the samples were transported from Accra, Ghana, to Glasgow, UK, on gel ice packs (about 11

hours) to keep samples frozen. The samples arrived at the University of Glasgow Human Nutrition laboratory, still frozen.

3.4.8.1.2 Sample processing

The food samples were thawed, and a weighed amount was put into bijoux tubes that had holes created in the covers to allow for the successful evaporation of water during freeze-drying. These samples were kept in a -80^{0}C freezer for 24 hours, after which they were transferred quickly into the Scanvac Coolsafe freeze-dryer (LaboGene, Denmark) and dried following the manufacturer's protocol (Appendix 9). The drying process took between 24 hours to 72 hours, depending on the moisture content of the food. The dried samples were taken out of the freeze-dryer and weighed using an electronic weighing balance to note the weight change before and after drying. Samples were kept in racks and stored inside enclosed drawers to ensure they did not absorb moisture from the environment.

3.4.8.1.3 Calorie determination

About 1 g of dried food samples were weighed into crucibles to begin the bomb calorimetry process. Bomb calorimetry was done using a Parr 6100 calorimeter (Parr Instrument Company, Moline, Illinois, USA) and following the manufacturer's instructions (Appendix 10). The caloric value determined from the bomb calorimeter was for the dried samples. The number of calories for the wet samples (as consumed by participants) was calculated using the formula:

Energy in wet sample = [(weight of dry sample/ weight of wet sample) X energy of dried sample]

The results (in cal) were divided by 1000 to convert to kcal.

3.4.9 Data and statistical analysis

3.4.9.1 Data analysis

The energy compensation index score (COMPX) was calculated as the difference in calories consumed after the low-energy preload and the high-energy preload conditions, divided by the difference in calories consumed from both preload drinks, finally multiplied by 100%. A compensation deviation ($COMP_{dev}$) was then calculated by subtracting 100% from the compensation index score. These two calculations are summarised in the formulas below;

$$\text{COMPX (\%)} = \left[\frac{\text{Meal}_{(lep)} - \text{Meal}_{(hep)}}{\text{Preload}_{(hep)} - \text{Preload}_{(lep)}} \right] \text{X } 100$$

COMPX dev = COMPX – 100% (Johnson, 2000).

A COMPX of 100% represents perfect calorie-for-calorie compensation. A compensation deviation examines the dysregulation of energy intake by looking at overeating, which is associated with greater adiposity, as well as hyperresponsiveness associated with undernutrition (Johnson, 2000).

3.4.9.2 Statistical analysis

All data were entered into and analysed with the Statistical Package for Social Sciences version 22. Data cleaning was done by running descriptive and frequency analyses to check out for missing values and wrongly coded values. All missing values and wrongly coded values were filled out by going back to the filled questionnaires. A Shapiro-Wilk test was carried out to test for normality of the data. Basic descriptive and frequencies were run for the various child and caregiver variables to describe their background characteristics. The effect of the order of preload delivery on the main outcome (COMPX) was tested using a t-test or the non-parametric equivalent and adjusted for in the final analysis using multiple regression. Variations in the

wash-out period for the satiation study were also considered in the analysis to ascertain if there was any possible significant influence on energy compensation. This was done by comparing the mean and SD COMPX using a t-test or the non-parametric equivalent to compare those seen within two weeks to those seen at >2-4 weeks. In case of any substantial effect, the effect was tested, excluding those with longer intervals. A Chi square test and Mann Whitney-test were used to determine the association between compensation index and nutritional status of the children.

3.5 DATA HANDLING/MANAGEMENT

3.5.1 Coding

Participants were identified using identification codes with no personal identifying information such as names.

3.5.2 Data security and confidentiality

Data was entered and stored on a password-protected personal computer and made available only for the purpose of this study. Filled questionnaires have been kept under lock and key in cabinets at the Department of Dietetics, University of Ghana. All information collected is kept confidential and used solely for the purpose of this study.

3.5.3 Quality control

This is research work for a PhD Dietetics degree at the University of Ghana. The supervisors overseeing this work are researchers with several years of experience and a track record of publications. The questionnaires employed in this study were pre-tested among 10 caregiver /child (1-3 years) dyads at a selected CWC at the Korle Bu polyclinic, in the Greater Accra Region. The researcher was blinded to the type of preload for the child to avoid bias. There was a daily evaluation of the data collection process to ensure that the right things were being done. There were also weekly visual estimation exercises on dietary evaluations to minimize

any possible drift and to improve the measurement bias. Weighing scales were checked daily to ensure they were reading correctly by calibrating with an object of a known weight.

Two laboratory notebooks (one for the researcher and one for the laboratory) (Appendices 7 and 8) were kept for the genetics laboratory work as well as the bomb calorimetry work. This was meant to help with the daily evaluation of work done and to be referred to in case of inconsistencies in results. These notes were especially helpful in instances where some of the analysis had to be repeated. Samples of these notes are attached in the appendix.

Research assistants were trained in the data collection process. Two research assistants were involved in the cross-sectional study and one in the experimental study. These Research assistants were individuals with education up to tertiary level with degrees in Mathematics/ economics and computer science. Both assistants had been research assistants on another nutrition project in the past year.

3.6 ETHICAL CONSIDERATIONS

Ethical clearance was obtained from the Ethics Review Committee of the Ghana Health Service with ethics number GHS-ERC:022/11/21 (Appendix 5). Permission was sought from the Greater Accra Regional and the Health Directorates of the selected districts after ethical approval had been obtained (Appendix 6).

The known risks associated with this study included discomfort in answering certain questions and taking body measurements. Parents may have feared discussing a sensitive topic and invasion of privacy in some homes. Interviews were therefore carried out in a private area of the child welfare clinic, and mothers were assured of complete anonymity. There was also minimal physical risk associated with collecting genetic information. The genetic information collected in this study is very limited and will only be used to assess appetite-related

factors. All residual materials after testing for the 10 SNPs have been kept in a secure location and will not be used for other purposes without seeking additional consent.

The protocols of the study were explained to the caregivers by the researcher and the caregivers were assured of their ward's safety and confidentiality. All questions or concerns raised by caregivers on behalf of their wards were addressed. They were made to understand that their participation was solely voluntary and that they were free to withdraw at any point in the study without being disadvantaged in any way. In the event of a withdrawal, they were asked to decide if we could use the data we had collected up till then or not to use it. There was no direct payment to participants; however, children involved in the study received a souvenir in the form of a cup or a bowl. Caregivers who consented to the study had the expectations, benefits, and potential risk of the study as well as their rights communicated to them. They were made to sign the consent form on behalf of their wards before being recruited into the study.

It took about 40 minutes to complete the interview process, body measurement and saliva collection. All data were kept secure on a password-protected laptop and used only for the purpose of this study. Filled questionnaires were kept in locked cabinets at the Department of Dietetics, University of Ghana. All data-filled questionnaires and excess saliva samples will be kept for five years and discarded following standard procedures. The investigators declare no conflict of interest in the conduct of this study.

CHAPTER 4

4.0 RESULTS

4.1 BACKGROUND CHARACTERISTICS OF PARTICIPANTS IN THE CROSS-SECTIONAL STUDY

Table 4.1 (a and b) represents the background characteristics of children and their caregivers. A total of 262 child-caregiver pairs participated in the cross-sectional study (Phase 1). Children were an average age of 19.3 ± 6.7 months, with older children (20.9 ± 7.3 months) being significantly healthier than younger ones ($p < 0.001$). There were 75 wasted children, 53 stunted and 134 healthy children. The majority of the children were between ages 12-18 months (56.9%) and about half were females (55.7%). Compared to healthy children, a significantly greater number of younger children (12-18 months) (70.7%) were within the wasted group ($p<0.001$). Although not significant, wasting and stunting were more prevalent among females than males (54.7% vs. 45.3 % and 52.8% vs. 47.2%, respectively, $p=0.613$). Over a third of the children were firstborns (38.5%), with significantly more wasted than stunted children in birth order 1 ($p=0.019$), as revealed in chi square test.

The mean age of caregivers was 31.2 ± 8.8 years. There was a significant difference in the ages of caregivers for children in the three nutritional status groups ($p=0.025$). Specifically, a post hoc test showed that caregivers of wasted children were significantly older (32.3 ± 9.3 years) than caregivers of stunted children (28.2 ± 7.1 years) ($p=0.044$). Almost all the caregivers were the mothers of the children (91.6%). The majority of the caregivers had attained education up to junior high school level (42.2%), with a few having education up to tertiary level (13.0%). Generally, wasted and stunted children, respectively, had caregivers with lower education ($p= 0.032$ and $p= 0.029$) when compared to healthy children. More than a third of the caregivers were married or in a consensual union (89.7%) and were employed (78.2%). The most prevalent ethnic group was Ga (37.8%), followed by Akan (31.3%). More than a quarter of the

children (25.5%) did not have their fathers' residing with them. Across the nutritional status groups, almost all respondents reported not being food poor (89.3%) and this did not differ by nutritional status.

A principal component analysis was used to compute the wealth index score using various household asset ownership and housing characteristics as described in the methodology. The wealth index scores were then categorised into five groups to create wealth quintile groups, as shown in Table 4.1b. There was a significant difference in wealth quintile across the nutritional status groups ($p < 0.001$). Specifically, individual chi tests among the various nutritional status groups revealed that there were significantly more wasted or stunted children within lower wealth quintile groups than healthy children ($p=0.004$ and $p<0.001$). There were no significant differences in wealth quintile among wasted and stunted children. Most of the 75 wasted children were from the lowest middle (26.0%) and poor (23.3%) wealth quintiles. Over a third of stunted children were from the poor wealth quintile category (35.8%). Over a 5^{th} of the healthy children were from the middle (25.4%), upper middle (26.1%) and richest (21.6%) quintiles.

Table 4.1a: Background characteristics of children (N=262)

CHARACTERISTICS		TOTAL N (%)	Healthy (n=134) N (%)	Wasted (n=75) N (%)	Stunted (n= 53) N (%)	P Value
Age[aδ] (mean ± SD)		19.23 ± 6.7	20.9 ± 7.3[b]	16.8 ± 5.0[a]	18.7 ± 6.0[a]	**<0.001**
Age categories[a]						
	12-18	149 (56.9)	64 (47.8)	53 (70.7)	32 (60.4)	
	19-24	57 (21.8)	31 (23.1)	15 (20.0)	11 (20.8)	**<0.001**
	>24	56 (21.4)	39 (29.1)	7 (9.3)	10 (18.9)	
Gender						
	Male	116 (44.3)	57 (42.5)	34 (45.3)	25 (47.2)	0.613
	female	146 (55.7)	77 (57.5)	41 (54.7)	28 (52.8)	
Birth order						
	1	101 (38.5)	56 (41.8)	23 (30.7)	22 (41.5)	
	2	77 (29.4)	42 (31.3)	16 (21.3)	19 (35.8)	**0.029**
	3	50 (19.1)	21 (15.7)	22 (29.3)	7 (13.2)	
	4+	34 (13.0)	15 (11.2)	14 (18.7)	5 (9.4)	

[a] age in months, SD-Standard deviation, δ-ANOVA test, p<0.05-significant

Table 4.1b: Background characteristics of caregivers (N=262)

CHARACTERISTICS	TOTAL	Healthy (n=134)	Wasted (n=75)	Stunted (n= 53)	P value
Age[b§] (mean ± SD)	31.2 ± 8.8	31.6 ± 8.9	32.3 ± 9.3	28.2 ± 7.1	**0.025**
Relationship with child					
Mother	240 (91.6)	122 (91.0)	70 (93.3)	48 (90.6)	0.804
other	22 (8.4)	12 (9.0)	5(6.7)	5 (9.4)	
Education					
No school-primary	55 (21.0)	25 (18.7)	16 (21.3)	14 (26.4)	
Junior High school	111 (42.2)	46 (34.3)	40 (53.3)	25 (47.2)	**0.011**
Senior High school/Vocational/Technical	62 (23.7)	42 (31.3)	11 (14.7)	9 (17.0)	
Tertiary	34 (13.0)	21 (15.7)	8 (10.7)	5 (9.4)	
Marital status (N=261)					
Married/ Consensual union	234 (89.7)	118 (88.7)	67 (89.3)	49 (92.5)	
Separated / Divorced / Widowed	15 (5.7)	8 (6.0)	4 (5.3)	3 (5.7)	0.733
Never married	12 (4.6)	7 (5.3)	4 (5.3)	1 (1.9)	
Employment Status					
Employed	205 (78.2)	109 (81.3)	58 (77.3)	38 (71.7)	0.315
Unemployed	57 (21.8)	25 (18.7)	17 (22.7)	15 (28.3)	
Ethnicity					
Akan	82 (31.3)	47 (35.1)	23 (30.7)	12 (22.6)	
Ga	99 (37.8)	52 (38.8)	26 (34.7)	21 (39.6)	
Ewe	21 (8.0)	11 (8.2)	7 (9.3)	3 (5.7)	0.085
Nzema	4 (1.5)	2 (1.5)	1 (1.3)	1 (1.9)	
Hausa	17 (6.5)	6 (4.5)	6 (8.0)	5 (9.4)	
Other	39 (14.9)	16 (11.9)	12 (16.0)	11 (20.8)	
Availability of Child's father(n=255)					
Resident	190 (74.5)	100 (77.5)	51 (68.9)	39 (75.0)	
Non-resident but in contact	53 (20.8)	25 (19.4)	18 (24.3)	10 (19.2)	0.138
No contact	12 (4.7)	4 (3.1)	5 (6.8)	3 (5.8)	
Food poverty					
Food poor	28 (10.7)	17 (12.7)	5 (6.7)	6 (11.3)	0.212
Not food poor	234 (89.3)	117 (87.3)	70 (93.3)	47 (88.7)	
Wealth quintile (N=260)					
Poor	52 (20.0)	16 (11.9)	17 (23.3)	19 (35.8)	
Lowest middle	51 (19.6)	20 (14.9)	19 (26.0)	12 (22.6)	
middle	53 (20.4)	34 (25.4)	13 (17.8)	6 (11.3)	**<0.001**
Upper middle	59 (22.7)	35 (26.1)	14 (19.2)	10 (18.9)	
Richest	45 (17.3)	29 (21.6)	10 (13.7)	6 (11.3)	

b, age in years, SD-Standard deviation, §-Anova test, p<0.05-significant, other ethnicity-Kokomba, Dagomba, Kusasi, Mamprusi, Guang, Frafra,

4.2 SOCIO-DEMOGRAPHIC PREDICTORS OF NUTRITIONAL STATUS OF CHILDREN

All significant sociodemographic variables in table 4.1 were put in a linear regression model to determine significant independent predictors of nutritional status (Table 4.2). The results showed that the child's age and wealth quintile were significant predictors of nutritional status of children ($p<0.001$ and $p=0.012$, respectively). They both had a positive linear association with nutritional status. The ANOVA model results showed that the regression model predicts the dependent variable (nutritional status) significantly well ($p<0.001$).

Table 4.2: Sociodemographic predictors of nutritional status (Linear regression)

Predictors	Unstandardized coefficient		Standardised coefficient	t	P value
	B	Standard error	Beta		
Child's age	0.05	0.01	0.23	3.77	**<0.001**
Birth order	-0.13	0.08	-0.10	-1.64	0.102
Caregivers' age	0.00	0.01	-0.03	-0.44	0.658
Wealth quintile	0.16	0.06	0.16	2.53	**0.012**
Caregiver's education	0.09	0.09	0.06	0.96	0.339

4.3 ANTHROPOMETRY OF CHILDREN

There was no significant difference in anthropometry measurements between males and females (Table 4.3). However, there was a significant improvement in average WAZ ($p<0.001$), HAZ ($p<0.001$) and WHZ ($p=0.028$) with increasing age. A Scheffe posthoc analysis revealed that the difference in WAZ was between ages 12-18 months and 31-36 months ($p= 0.001$), while the difference in HAZ was between 12-18 months and 31-36 months ($p< 0.001$) as well as between 19-24 months and 31-36 months ($p=0.039$).

Table 4.3: Anthropometry among age and gender groups of children (n=257)

	Weight (kg) Mean (SD)	Height (cm) Mean (SD)	WAZ Mean (SD)	HAZ Mean (SD)	WHZ Mean (SD)	BMIZ Mean (SD)
Wasted	7.76 (1.1)	76.01 (6.2)	-2.57 (0.8)	-1.51 (1.5)	-2.50 (0.3)	-2.37 (0.4)
Stunted	8.91 (1.4)	74.20 (4.9)	-1.67 (0.7)	-2.60 (0.6)	-0.49 (0.8)	-0.09 (0.8)
Healthy	10.61 (2.0)	81.72 (8.1)	-0.51 (1.0)	-0.57 (1.0)	-0.33 (0.9)	-0.25 (0.9)
GENDER						
Male	9.59(2.1)	78.63 (7.6)	-1.40 (1.4)	-1.30 (1.5)	-1.04 (1.3)	-0.87 (1.3)
Female	9.34 (2.1)	78.51(7.9)	-1.28 (1.2)	-1.21 (1.2)	-0.95 (1.2)	-0.79 (1.3)
P value	0.333	0.897	0.410	0.546	0.572	0.649
AGE						
12-18 months	8.33 (1.4)	73.69 (4.0)	-1.58 (1.3)[a]	-1.49 (1.3)[a]	-1.17 (1.3)[a]	-0.96 (1.3)[a]
19-24 months	9.92 (1.4)	81.03 (3.9)	-1.20 (1.1)[b]	-1.16 (1.1)[b]	-0.88 (1.2)[b]	-0.73 (1.3)[b]
25-30 months	11.28 (2.1)	86.09 (6.0)	-1.01 (1.4)[c]	-1.04 (1.5)[c]	-0.66 (1.2)[c]	-0.54 (1.2)[c]
31-36 months	12.94 (1.5)	93.53 (5.5)	-0.48 (0.9)[a]	-0.20 (1.4)[a,b]	-0.55 (1.0)[d]	-0.60 (1.1)[d]
P value			**<0.001***	**<0.001***	**0.028***	0.201
TOTAL			-1.33 (1.3)	-1.25 (1.4)	-0.99(1.2)	-0.83 (1.3)

P<0.05= significant, WHZ-Weight for height z score, WAZ-weight for age z score, HAZ-Height for age z score, BMIZ-BMI z score, SD-standard deviation

4.4 FEEDING PRACTICES OF CHILDREN

The feeding characteristics of children, which involve breastfeeding, complementary feeding and dietary diversity, are presented in Table 4.4. More than half (58.4%) of the children had ceased breastfeeding at the time of data collection, with a significantly higher proportion of wasted children (56.0%) still breastfeeding compared to stunted and healthy children (p=0.001). The majority of the children were breastfed beyond 12 months, with 54.2% breastfed up to 12-18 months and 14.1% breastfed beyond 18 months. Among the children who were still being breastfed (n=109), the majority of them were fed >3x/day. The frequency of breastfeeding among children who were still being breastfed was significantly higher among wasted children (p=0.001) compared to healthy children and higher among stunted children compared to healthy children (p=0.050), as revealed in the individual chi square test. Most children (58.6%) met the recommendation to initiate complementary feeding at or near 6 months, with no difference by nutritional status.

Almost half (46.7%) of the children achieved minimum dietary diversity in the last 24 hours, but compared to healthy children, a significantly higher proportion of wasted (60.0%) and stunted (62.3%) children failed to meet the minimum dietary diversity (p= 0.035 and p= 0.039). Generally, more than a third of the children (82.2%) did not achieve a minimum meal frequency of at least 3x/day for breastfed children and 4x/day for non-breastfed children, and this did not differ by nutritional status (p=0.182). Almost all the children (94.6%) entirely or mostly self-fed their snacks but not meals (15.3%). Self-feeding snacks differed by nutritional status (p=0.034), but self-feeding meals did not differ.

The most frequently consumed food groups were starchy foods, meat/fish/poultry and foods cooked in oil, with about a third to half of the children eating them once daily or more than once daily (Figure 4.1). Legumes/nuts and leafy vegetables were the food groups least

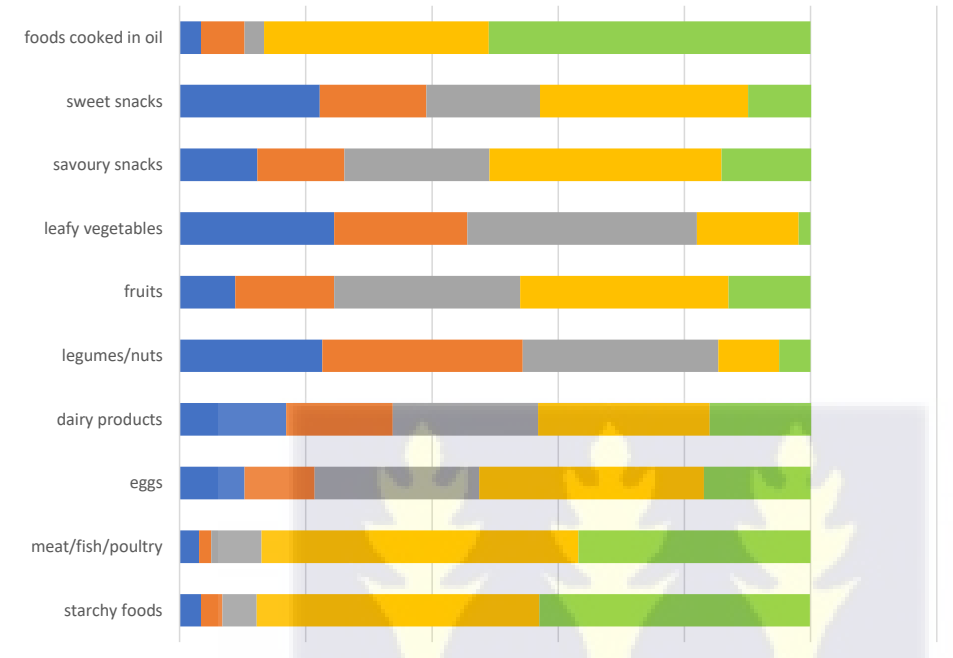
consumed among the studied children, with less than a quarter of the children eating them daily. Over a quarter of the children had never or rarely had legumes/nuts, leafy vegetables, and sweet snacks. About a third of the children ate eggs, dairy products, fruits, and sweet and savoury snacks once daily. The overall food frequency score – a combined measure of the number and frequency of consuming different food groups (Figure 1) differed by nutritional status, with wasted children having slightly lower consumption than stunted and healthy children (p=0.043) (Table 4.4).

The observed univariable associations of stunting and wasting with the various diet variables were no longer significant after adjustment for age – which differed significantly between the groups, however, frequency of drink intake (wasting: OR (CI) = 1.331 (1.024, 1.729), P= 0.032; stunting: OR (CI) = 1.344 (1.003, 1.801), P=0.047) and wealth quintile (stunting: 0R (CI) = 1.399 (1.078, 1.814), P=0.011) remained significant even after adjustment.

Table 4.4: Feeding practices of children (n=262)

FEEDING CHARACTERISTICS		TOTAL N (%)	Healthy N (%)	Wasted N (%)	Stunted N (%)	P value
Breastfeeding						
	Yes	109 (41.6)	43 (32.1)	42 (56.0)	24 (45.3)	**0.001**
	No	153 (58.4)	91 (67.9)	33 (44.0)	29 (54.3)	
Breastfeeding duration (months)						
	<6	8 (3.1)	4 (3.0)	3 (4.0)	1 (1.9)	0.200
	6-12	75(28.6)	33 (24.6)	25 (33.3)	17 (32.1)	
	12-18	142 (54.2)	76 (56.7)	36 (48.0)	30 (56.6)	
	>18	37 (14.1)	21 (15.7)	11 (14.7)	5 (9.4)	
Breastfeeding frequency (per day)						
	>3x	95 (36.3)	35 (26.1)	36 (48.0)	24 (45.3)	**0.001**
	2-3x	15 (5.7)	9 (6.7)	6 (8.0)	0 (0.0)	
	<1x	152 (58.0)	90 (67.2)	33 (44.0)	29 (54.7)	
Age of initiation of complementary feeding (months) (N=261)	<6	52 (19.9)	24 (18.0)	24 (32.0)	4 (7.5)	
	6	153 (58.6)	86 64.7)	36 (48.0)	31 (58.5)	0.770
	>6	56 (21.5)	23 (17.3)	15 (20.0)	18 (34.0)	
Minimum Dietary Diversity (N=261)						
	Yes	122 (46.7)	72 (54.1)	30 (40.0)	20 (37.7)	**0.020**
	No	139 (53.3)	61 (45.9)	45 (60.0)	33 (62.3)	
Minimum meal frequency (N= 258)						
	Yes	46 (17.8)	20 (15.0)	17 (22.7)	9 (18.0)	0.182
	No	212 (82.2)	113 (85.0)	58 (77.3)	41 (82.0)	
Self-feeding (snacks)						
	Yes	247 (94.6)	130 (97.0)	67 (89.3)	50 (96.2)	**0.034**
	No	14 (5.4)	4 (3.0)	8 (10.7)	2 (3.8)	
Self-feeding (meals)						
	Yes	40 (15.3)	23(17.2)	8 (10.7)	9 (17.3)	0.281
	No	221(84.7)	111(82.8)	67 (89.3)	43(82.7)	
		Median (25,75) for food frequency variables				**P Value$^{\beta}$**
Overall food frequency		3.400 (3.00, 3.80)	3.50 (3.20, 3.80)	3.20 (2.90, 3.70)	3.30 (2.90, 3.73)	**0.043**
Energy dense food frequency		2.00 (1.00, 3.00)	2.00 (1.00, 3.00)	2.00 (1.00, 2.00)	2.00 (1.00, 3.00)	0.696
Fruits and vegetables frequency		3.00 (2.50, 3.50)	3.00 (2.50, 3.50)	3.00 (2.00, 3.50)	3.00 (2.00, 3.50)	0.261
Snacks frequency		3.00 (2.50, 4.00)	3.00 (2.50, 5.00)	3.00 (2.00, 4.00)	3.00 (2.50, 4.50)	0.058
Drinks frequency		2.00 (1.00, 3.00)	2.00 (1.00, 4.00)	2.00 (1.00, 3.00)	1.00 (1.00, 3.00)	**0.010**
Spoonable food frequency		2.00 (1.00, 2.00)	2.00 (1.00, 2.00)	2.00 (1.00, 2.00)	2.00 (1.00, 2.00)	0.586

p<0.05=significant, β= Kruskal Wallis test



	starchy foods	meat/fish /poultry	eggs	dairy products	legumes/ nuts	fruits	leafy vegetables	savoury snacks	sweet snacks	foods cooked in oil
never/rarely	3.4	3.1	10.3	16.9	22.6	8.8	24.5	12.3	22.2	3.4
once a month	3.4	1.9	11.1	16.9	31.8	15.7	21.1	13.8	16.9	6.9
once a week	5.4	8	26.1	23	31	29.5	36.4	23	18	3.1
once daily	44.8	50.2	35.6	27.2	9.6	33	16.1	36.8	33	35.6
more than once daily	42.9	36.8	16.9	16.1	5	13	1.9	14.2	10	51

Figure 4.1: Frequency of food consumption among children

4.5 FEEDING BEHAVIOUR OF CHILDREN

The most prevalent feeding behaviour categories were low appetite (37%), low food refusal (38.5%), low force-feeding (60.3) and moderate caregiver feeding anxiety (34.7%) (Figure 4.2). Wasted children had a lower appetite (49.3%), high food refusal (41.3%), low force-feeding (54.7%) and high caregiver feeding anxiety (53.3%). Generally, poor feeding behaviours such as high to moderate food refusal, force-feeding and feeding anxiety were lower among stunted and healthy children than wasted children and good feeding behaviours such as high to moderate appetite, low food refusal, low force-feeding and low feeding anxiety were consistently lower among wasted children (Figure 4.2). There was no evidence of a U-shaped relationship between nutritional status and food refusal.

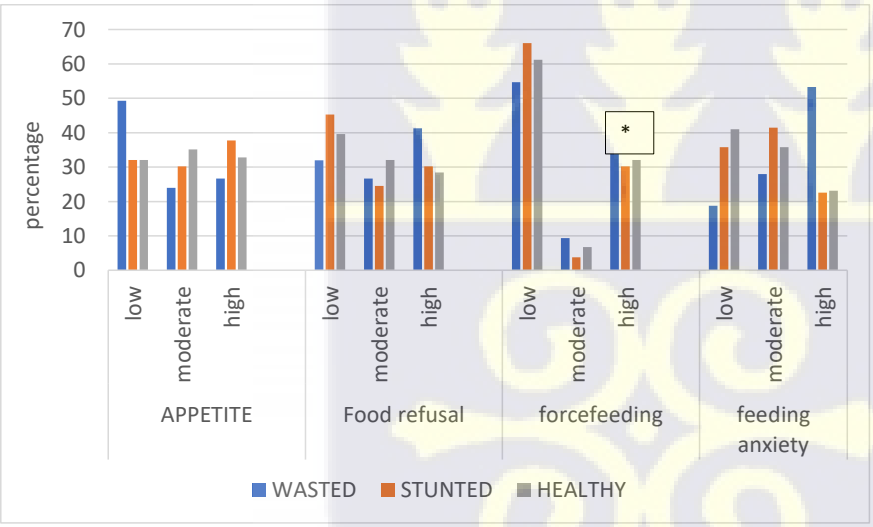


Figure 4.2: Feeding behaviour and nutritional status of study participants.

Wasted children had a lower z-score for appetite and higher z scores for food refusal, and caregiver feeding anxiety compared to both stunted and healthy children (Table 4.5 & Figure 4.3), but there were no significant differences in these feeding behaviours between the stunted group and the healthy group. There was no significant association between nutritional status and force-feeding. Overall, caregiver feeding anxiety was significantly higher in all malnourished children (stunted and wasted) than in healthy children ($p=0.002$) (Table 4.5). A multinomial regression with adjustment for significant sociodemographic characteristics (wealth index and child's age) revealed feeding anxiety as the only significant predictor of wasting ($p<0.001$) (Table 4.6). There was no significant association between feeding behaviour and stunting.

There was a significant correlation between a child's feeding behaviour and the caregiver's feeding behaviour ($p<0.001$) (Table 4.7). There was a significant negative correlation between appetite and food refusal ($r= -0.75$, $p<0.001$), force-feeding ($r = -0.38$, $p<0.001$) and caregiver feeding anxiety ($r = -0.70$, $p<0.001$). A positive correlation was seen between food refusal and force feeding ($r= 0.35$, $p<0.001$), food refusal and caregiver feeding anxiety ($r= 0.62$, $p <0.001$), as well as force-feeding and caregiver feeding anxiety ($r= 0.30$, $p<0.001$).

No significant association was found between feeding behaviour and other variables such as age, wealth quintile and child's current illness (Table 4.8). There was, however, a significant association between food poverty and force-feeding ($p= 0.016$), with force-feeding being higher among children with no food poverty.

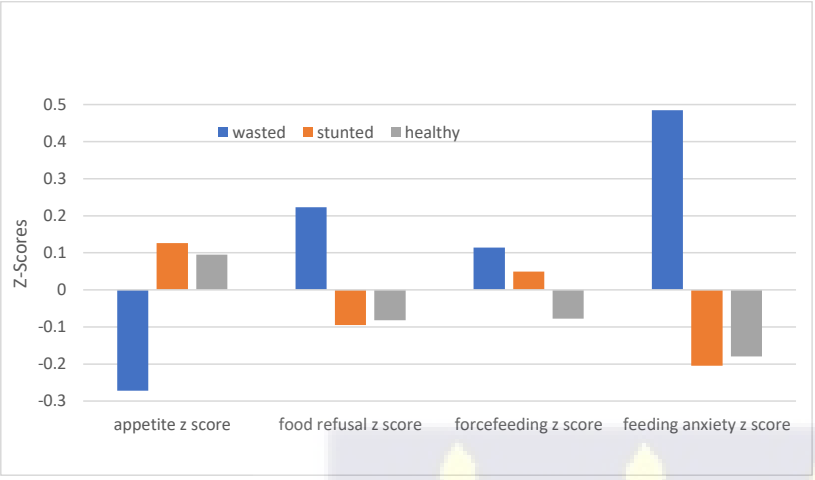


Figure 4.3: Feeding behaviour z scores across the different nutritional status groups.

Table 4. 5: Association between feeding behaviour and nutritional status of children (T-Test) (N=262)

Feeding behaviour Nutritional status	Appetite z-score Mean (SD)	p. Value	Food refusal z-score Mean (SD)	P. value	Force-Feeding z-score Mean (SD)	P. value	Feeding Anxiety z-Score Mean (SD)	P. Value
Wasted	-0.27 (1.1)	**0.010**	0.22 (1.1)	**0.032**	0.11 (1.1)	0.168	0.49 (1.0)	**<0.001**
Healthy	0.10 (0.9)		-0.08 (0.9)		-0.08 (0.9)		-0.18 (1.0)	
Mean difference (CI)	-0.37(-0.65, -0.09)		0.30 (0.03, 0.58)		0.19 (-0.08, 0.47)		0.67 (0.39, 0.94)	
Stunted	0.13 (1.0)	0.844	-0.10 (1.1)	0.930	0.05 (1.1)	0.418	-0.21 (1.0)	0.872
Healthy	0.10 (0.9)		-0.08 (0.9)		-0.08 (0.9)		-0.18 (1.0)	
Mean difference (CI)	0.03 (-0.28, 0.34)		-0.01 (-0.31, 0.29)		0.13 (-0.18, 0.44)		-0.02 (-0.33, 0.28)	
Wasted	-0.27 (1.1)	**0.036**	0.22 (1.1)	0.110	0.11 (1.1)	0.745	0.49 (1.0)	**<0.001**
Stunted	0.13 (1.0)		-0.10 (1.1)		0.05 (1.1)		-0.21 (1.0)	
Mean difference (CI)	-0.40 (-0.77, -0.03)		0.32 (-0.07, 0.71)		0.06 (-0.33, 0.46)		0.69 (0.35, 1.03)	
Overall nutrition								
Malnourished	-0.11 (1.1)	0.102	0.09 (1.1)	0.163	0.87 (1.1)	0.181	0.20 (1.0)	**0.002**
Healthy	0.10 (0.9)		-0.82 (0.93)		-0.08 (0.9)		-0.18 (1.0)	
Mean difference (CI)	-0.20 (-0.44, 0.04)		0.17 (-0.07, 0.42)		0.17 (-0.08, 0.41)		0.38 (0.14, 0.62)	

p<0.05-significant, malnourished-either stunted or wasted or both, CI-confidence interval.

Table 4.6: Association between feeding behaviour and nutritional status (Multinomial regression)

nutritional status	Feeding behaviour and significant sociodemographic parameters	B	Std. Error	Sig.	Exp(B)	95% Confidence Interval for Exp(B)	
						Lower Bound	Upper Bound
Wasted	Wealth quintile	-0.30	0.12	**0.013**	0.74	0.58	0.94
	Childs age	-0.09	0.03	**0.001**	0.91	0.86	0.96
	appetite	0.06	0.26	0.829	1.06	0.63	1.77
	food refusal	-0.31	0.25	0.211	0.73	0.45	1.19
	feeding anxiety	1.00	0.25	**0.000**	2.72	1.66	4.45
Stunted	Wealth quintile	-0.41	0.13	**0.001**	0.66	0.52	0.85
	Child's age	-0.04	0.03	0.168	0.96	0.91	1.02
	appetite	-0.06	0.30	0.836	0.94	0.53	1.68
	food refusal	-0.14	0.28	0.622	0.87	0.50	1.51
	feeding anxiety	0.00	0.26	0.997	1.00	0.60	1.65

Table 4.7: Correlation between child and caregiver feeding behaviours (Spearman Correlation)

	Food refusal	Force-Feeding	Caregiver feeding anxiety
	r (p value)	r (p value)	r (p value)
Appetite	-0.75 **(<0.001*)**	-0.38 **(<0.001*)**	-0.70 **(<0.001*)**
Food refusal		0.35 **(<0.001*)**	0.63 **(<0.001*)**
Force-feeding			0.30 **(<0.001*)**

Table 4.8: Association between other variables and feeding behaviour of children.

Feeding behaviour	Appetite z-score Mean (SD)	P value	Food refusal z-score Mean (SD)	P value	Force-Feeding z-score Mean (SD)	P value	Feeding Anxiety z -score Mean (SD)	P value
Poverty								
Food poor	-0.03 (1.0)	0.886	0.21 (0.9)	0.201	-0.28 (0.6)	**0.016**	0.06 (0.9)	0.749
Not food poor	0.00 (1.0)		-0.02 (1.0)		0.04 (1.0)		0.00 (1.0)	
Mean difference (CI)	0.03 (-0.38, 0.44)		-0.23 (-0.60, 0.13)		0.32 (0.06, 0.58)		-0.06 (-0.41, 0.30)	
Age (months)								
12-18	-0.09 (1.0)		0.12 (1.0)		0.07 (1.0)		0.10 (1.0)	
19-24	0.08 (0.9)	0.266	-0.14 (1.0)	0.134	0.05 (1.0)	0.316	-0.16 (1.1)	0.350
25-30	0.26 (1.0)		-0.27 (0.9)		-0.14 (1.0)		-0.09 (0.9)	
31-36	-0.03 (1.0)		0.03 (0.9)		-0.31 (0.8)		-0.04 (1.1)	
Wealth Quintile								
Poor	0.18 (0.9)		-0.14 (1.1)		0.16 (1.1)		-0.14 (0.9)	
Lowest middle	-0.13 (1.1)	0.547	0.16 (1.0)	0.479	-0.10 (0.8)	0.692	0.19 (0.9)	0.523
Middle	0.05 (1.0)		0.03 (1.1)		-0.08 (1.0)		0.01(1.0)	
Upper middle	-0.03 (0.9)		-0.19 (0.8)		0.05 (1.1)		0.03(1.1)	
Richest	-0.09 (1.1)		0.05 (1.0)		0.01 (1.0)		-0.08 (1.1)	
Child currently unwell								
Yes								
no	-0.28 (1.0)	0.121	1.96 (1.0)	0.249	-0.10 (0.8)	0.486	0.22 (1.1)	0.241
	0.03 (1.0)		-0.02 (1.0)		0.02 (1.0)		-0.02 (1.0)	
Mean difference (CI)	-0.31 (-0.71, 0.09)		0.22 (-0.16, 0.60)		-0.11 (-0.43, 0.21)		0.24 (-0.17, 0.65)	

p<0.05-significant, malnourished-either stunted or wasted or both.

4.6 GENETICS RESULTS

4.6.1 Genotype of children

The observed genotype frequencies were compared with the expected genotype frequencies (Appendix 13). All the genotyped SNPs were within Hardy-Weinberg equilibrium with p value>0.05. A comparison of the minor allele frequencies (MAF) for all SNPs with MAF in African populations with close ancestry with Ghana (i.e., Nigeria and Sierra Leone) also showed that the MAF were within ranges in African population.

The absolute number of participants that expressed each genotype category is presented in Table 4.9. The genotype frequencies were compared for all three nutritional status groups. There were no significant differences in genotype frequencies between healthy and malnourished groups except for SNP rs2274333, close to the gene CA6, which had significantly more wasted (87.1%) than healthy (76.0%) children being homozygous for the effect allele AA.

Table 4.9: Genotype of children across different nutritional status groups

SNP	Closest gene	No. of Samples	Genotype	TOTAL N (%)	HEALTHY N (%)	WASTED N (%)	STUNTED N (%)	P Value Healthy vs. wasted	P value Healthy vs. stunted	P value Wasted vs. stunted
rs13021737	TMEM18	181	AA	3 (1.7)	2 (2.1)	1 (2.3)	0 (0.0)	0.824	0.388	0.573
			AG	24 (13.3)	15 (15.6)	5 (11.6)	4 (9.5)			
			GG	154 (85.1)	79 (82.3)	37 (86.0)	38 (90.5)			
rs10246939	TAS2R38	224	CC	60 (26.8)	26 (22.2)	23 (36.5)	11 (25.0)	0.117	0.932	0.442
			CT	120 (53.6)	66 (56.4)	30 (47.6)	24 (54.5)			
			TT	44 (19.6)	25 (21.4)	10 (15.9)	9 (20.5)			
rs543874	SEC16B	219	AA	89 (40.6)	46 (40.7)	28 (44.40	15 (34.9)	0.887	0.773	0.587
			AG	109 (49.8)	56 (49.6)	29 (46.0)	24 (55.8)			
			GG	21 (9.6)	11 (9.7)	6 (9.5)	4 (9.3)			
rs1516725	ETV5	203	CC	137 (67.5)	70 (69.3)	43 (71.7)	24 (57.1)	0.780	0.374	0.252
			CT	61 (30.0)	29 (28.7)	15 (25.0)	17 (40.5)			
			TT	5 (2.5)	2 (2.0)	2 (3.3)	1 (2.4)			
rs2274333	CA6	208	AA	168 (80.8)	79 (76.0)	54 (87.1)	35 (83.3)	**0.018**	0.551	0.159
			AG	36 (17.3)	24 (23.1)	5 (8.1)	7 (16.7)			
			GG	4 (1.9)	1 (1.0)	3 (4.8)	0 (0.0)			
rs13078960	CADM2	225	GG	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.531	0.353	0.184
			GT	12 (5.3)	6 (5.1)	2 (3.1)	4 (9.1)			
			TT	213 (94.7)	111 (94.9)	62 (96.9)	40 (90.9)			
rs1558902	FTO	215	AA	1 (0.5)	1 (0.9)	0 (0.0)	0 (0.0)	0.655	0.289	0.081
			AT	18 (8.4)	10 (8.9)	7 (11.7)	1 (2.3)			
			TT	196 (91.2)	101 (90.2)	53 (88.3)	42 (97.7)			
rs6567160	MC4R	195	CC	10 (5.1)	6 (6.0)	2 (3.6)	2 (5.1)	0.804	0.939	0.903
			CT	66 (33.8)	33 (33.0)	19 (33.9)	14 (35.9)			
			TT	119 (61.0)	61 (61.0)	35 (62.5)	23 (59.0)			
rs10182181	ADCY3	216	AA	1 (0.5)	1 (0.9)	0 (0.0)	0 (0.0)	0.773	0.363	0.255
			AG	26 (12.0)	12 (10.5)	6 (10.3)	8 (18.2)			
			GG	189 (87.5)	101 (88.6)	52 (89.7)	36 (81.8)			

The frequency of effect allele (EA) homozygous is presented for the total studied population and compared for healthy versus each of the two malnourished groups (Table 4.10). From this, relative risk ratios were calculated with corresponding confidence intervals. There were no significant differences observed for all 9 SNPs.

Table 4.10: Effect allele (EA) frequencies in the studied population and relative risk of being homozygous for EA

SNP	Chr.	Position (bp)	Closest gene	EA	OA	Expected EA homozygous frequency	Observed EA homozygous frequency	Absolute Difference	Healthy vs. Wasted		Healthy vs. Stunted	
									Relative Risk (95%CI)	P value	Relative Risk (95% CI)	P value
rs13021737	2	632348	TMEM18	G	A	0.84	0.85	+0.01	0.96 (0.19, 4.85)	0.958	-	-
rs10246939	7	141972804	TAS2R38	C	T	0.29	0.27	-0.02	1.64 (0.90, 3.00)	0.089	1.12 (0.53, 2.37)	0.760
rs543874	1	177889480	SEC16B	G	A	0.12	0.10	-0.02	0.93 (0.46, 1.89)	0.845	1.08 (0.42, 2.80)	0.868
rs1516725	3	185824004	ETV5	C	T	0.68	0.68	-0.01	0.76 (0.28, 2.09)	0.629	0.77 (0.15, 3.94)	0.761
rs2274333	1	8957145	CA6	A	G	0.80	0.81	+0.01	0.54 (0.30, 0.99)	0.169	-	-
rs13078960	3	85807590	CADM2	G	T	0.00	0.00	0.00	-	-	-	-
rs1558902	16	53803574	FTO	A	T	0.00	0.01	0.00	0	0.469	0	0.520
rs6567160	18	57829135	MC4R	C	T	0.05	0.05	0.00	0.69 (0.20, 2.34)	0.515	0.91 (0.26, 3.19)	0.885
rs10182181	2	25150296	ADCY3	G	A	0.88	0.88	0.00	-	0.474	-	-

Effect allele (EA)=Thinness associated allele, OA= other allele, chr=Chromosome

4.6.2 Polygenic risk score

Polygenic risk scores (PGRS) were calculated for 122 participants as experimental error resulted in unknown genotypes for some SNPs, affecting about 98 participants. The mean PGRS generated for the total population was 9.52 (± 1.51) (out of a possible 18) (Table 4.11). Mean PGRS was slightly lower for stunted (9.35 ± 1.54)) and higher for wasted (9.84 ± 1.44) compared to healthy children (9.45 ± 1.52). Wasted children had a PGRS of about 0.4-0.5 scores, thus, higher than stunted and healthy. There were no significant differences in mean PGRS across different nutritional status groups ($p>0.05$). About 51% of participants had PGRS below the population mean PGRS (Table 4.13). The PGRS distribution was symmetric for both wasted and stunted children (Figure 4.4). The median PGRS score was the same for both stunted and healthy children, while wasted children had a higher median PGRS score (Figure 4.4, Table 4.11). The frequency distribution of PGRS was generally normal and similar for all three categories of nutrition status (Figure 4.5).

Table 4.11: PGRS across the different nutritional status groups

PGRS	Mean (SD)	Median (Min, Max)	95% CI for mean	P value
Nutritional status				
healthy	9.45 (1.5)	9.00 (7.00, 12.00)	9.04, 9.85	0.365
wasted	9.84 (1.4)	10.00 (7.00, 12.00)	9.32, 10.36	
stunted	9.35 (1.5)	9.00 (6.00, 13.00)	8.82, 9.89	
Total	9.52 (1.5)	9.00 (6.00, 13.00)	9.25, 9.79	

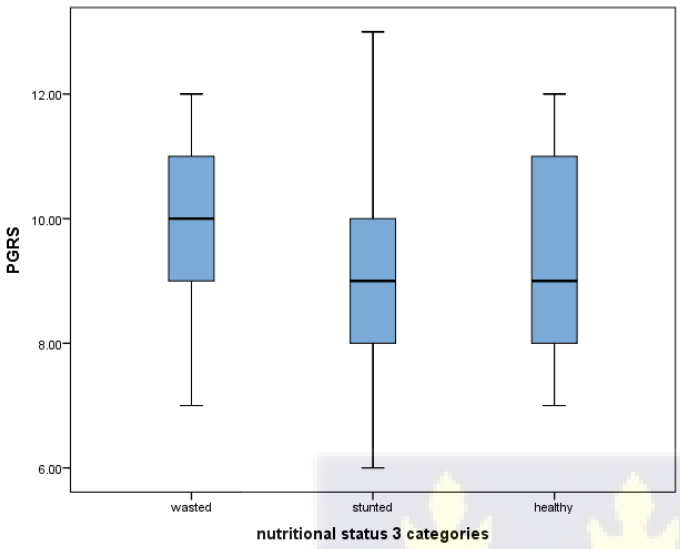


Figure 4.4: Box plot showing PGRS versus Nutritional status.

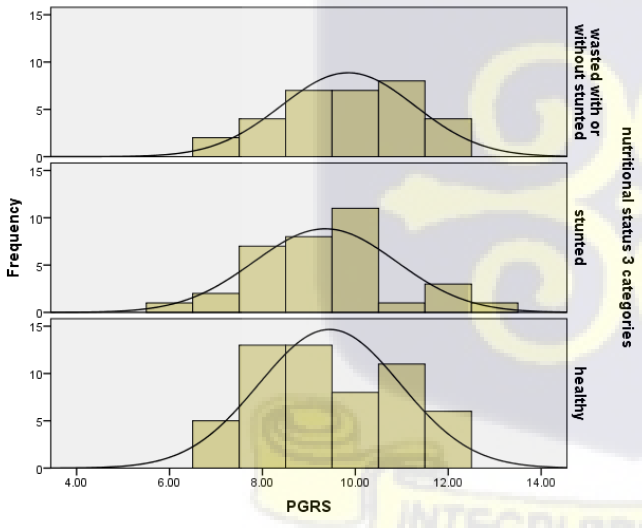


Figure 4.5: Frequency distribution of PGRS across nutritional status groups

There was no significant correlation between PGRS and nutritional status, as well as child feeding behaviour (appetite and food refusal) and mean food frequency (Table 4.12). A chi square test revealed a significant association between PGRS and appetite (p=0.046) (Table 4.13, Figure 4.6). The significant difference was particularly between low and moderate appetite (p=0.015). The median PGRS score was the same for children with low and high appetites and lower for children with moderate appetite scores (Figure 4.6).

Table 4.12: Correlation between PGRS and nutritional status and child feeding behaviours.

		Height for age z-score	Weight for height z-score	Appetite z-score	food refusal z-score	Mean food frequency
PGRS	Pearson Correlation	-0.06	-0.02	0.01	-0.06	0.04
	Sig. (2-tailed)	0.540	0.801	0.892	0.490	0.706
	N	122	122	122	122	121

Table 4.13: Association between PGRS and feeding behaviour (chi square)

		PGRS	
Child's feeding behaviour		Low (< 9.5) N (%)	High (>9.5) N (%)
Appetite			
	Low	13 (21.0)	21 (35.0)
	Moderate	23 (37.1)	11 (18.3)
	High	26 (41.9)	28 (46.7)
P value		**0.046**	
Food refusal			
	Low	28 (45.2)	33 (55.0)
	Moderate	20 (32.3)	18 (30.0)
	high	14 (22.6)	9 (15.0)
P value			0.456
TOTAL		62 (50.8)	60 (49.2)

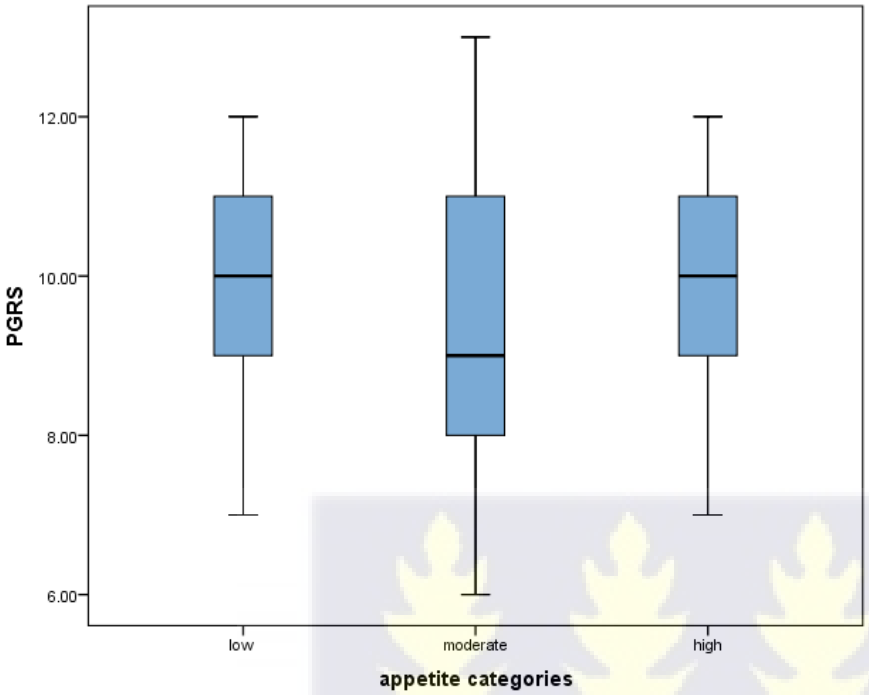


Figure 4.6: Box plot demonstrating the pattern of PGRS across appetite levels.

4.7 RESULTS FOR EXPERIMENTAL (SATIATION) STUDY

4.7.1 Background characteristics of children

Forty-one children were included in the experimental study, of which 17 (41.5%) were wasted, and 12 each (29.3%) were stunted or healthy. The children were of a median (range) age of 15 (12, 36) months, with wasted children being seemingly younger than stunted and healthy children. About half of the children were males (53.7%), but wasted children were slightly more likely to be male (64.7% vs. 35.3%). Their summary growth data are shown in Table 4.14.

Table 4.14: Background characteristics of children in experimental studies (N=41)

CHARACTERISTICS	Total N (%)	Healthy N (%)	Wasted N (%)	Stunted N (%)	P value
		12 (29.3)	17 (41.5)	12 (29.3)	
Age[a] (median (range))	15 (12, 36)	19.5 (12, 32)	15 (12, 22)	15.50 (12, 36)	0.289
Age[a] (mean ± SD)	18.02 ±6.8	20.25 ±7.7	15.35 ± 3.4	19.58 ± 8.5	0.098
Age categories (months)					
12-18	27 (65.9)	6 (50.0)	13 (76.5)	8 (66.7)	
19-24	6 (14.6)	1 (8.3)	4 (23.5)	1 (8.3)	**0.028**
>24	8 (19.5)	5 (41.7)	0 (0.0)	3 (25.0)	
Gender					
Male	22 (53.7)	6 (50.0)	11 (64.7)	5 (41.7)	0.494
female	19 (46.3)	6 (50.0)	6 (35.3)	7 (58.3)	
	Anthropometry	**Mean (SD)**			
Weight (kg)		10.91 (2.2)	7.80 (1.1)	9.28 (2.0)	<0.001
Height (cm)		82.99 (8.7)	75.91 (5.4)	75.08 (6.3)	0.011
WAZ		-0.35 (0.9)	-2.53 (0.8)	-1.44 (0.7)	<0.001
HAZ		-0.27 (0.9)	-1.47 (1.5)	-2.47 (0.5)	<0.001
WHZ		-0.33 (1.0)	-2.52 (0.3)	-0.28 (1.0)	<0.001

Wasted -WHZ <-2SD with or without stunting, stunted- HAZ <-2SD but WHZ >-2SD, Healthy-WHZ>-2 and HAZ >-2, WAZ-weight for age z score, HAZ-Height for age z score, WHZ-Weight for height z score

4.7.2 Food and drink energy intake and compensation characteristics

Over a third of the children had a washout period of 1 week (n=31, 75.6%), with a few having two weeks (n=6, 14.6%), three weeks (n=1, 2.4%) or four weeks (n=3, 7.3%). The average amount of energy consumed from the high energy (HEP) and low energy (HEP) preload drinks are shown in Table 4.15. Generally, children consumed more after a low energy preload than after the high energy preload (p= 0.033) (Table 4.15, Figure 4.7). While the total energy intake (preload drink plus test meal) under the high energy preload condition was slightly higher than under the low energy preload condition this difference was not significant (Figure 4.8). Overall, 21 children (51.2%) had minimal or no compensation (COMPX <30%), 11 children (26.8%) had moderate to accurate compensation (COMPX =30-100%) and nine children overcompensated (COMPX >100%). The average compensation index score (COMPX) for the children was 62.43% and males had a higher compensation than females (76.52 ± 172.25 % vs. 46.11 ± 64.97 %). This was, however, not significant (p =1.000).

Data collected on food consumption showed that commonly consumed foods were jollof and *banku* with okro (okra) stew (table 4.16, Appendix 14). The food sample with the highest number of calories was roasted peanut (721kcal/100g) followed by fried fish (403kcal/100g) (table 4.16). Okro stew (code-023) had the least number of calories (0.70 kcal/g) (Appendix 14).

Table 4.15: Energy intake and compensation characteristics of children (N=41)

Food and drink energy intake (kcal)	Mean	SD	Median (25%, 75%)	P Value
High energy preload (HEP)	101.79	17.5	109.50 (93.41, 109.50)	**<0.001**#
Low energy preload (LEP)	8.33	11.1	6.27 (4.75, 9.48)	
Test meal after HEP	147.71	130.8	113.56 (42.02, 209.22)	**0.033**#
Test meal after LEP	207.03	226.5	142.78 (80.87, 244.10)	
Preload difference (HEP-LEP)	93.45	15.4	100.00 (83.87, 104.08)	
Total energy intake (HEP condition)	249.50	134.2	223.06 (150.67, 313.95)	0.332#
Total energy intake (LEP condition)	215.37	225.8	172.80 (86.92, 253.60)	
Average total energy intake (both preload conditions)	232.43	172.8	202.96 (122.43, 282.03)	
COMPX (%)				
Males	76.52	172.3	35.30(-20.39, 116.98)	1.000∞
Females	46.11	65.0	24.56 (-3.22, 86.78)	
Total	62.43	133.1	24.56 (-10.47, 24.56)	
Compensation deviation				
Males	23.48	172.3	64.70 (-16.98, 120.39)	1.000∞
Females	53.88	65.0	75.44 (13.21, 103.23)	
Total	37.57	133.1	75.44 (5.28, 110.47)	

#Wilcoxon test, ∞ Mann Whitney test

Table 4.16: Calories in test meals (Bomb Calorimetry)

Food state	Food item	Description	Energy Kcal/ 100g (Kcal)
wet foods	Apapransa	One pot dish made with palmnut soup, powdered roasted corn and legumes	184
	Banku	Fermented cornmeal mixed with cassava that is cooked into a ball	105.5
	Boiled chicken	Fresh chicken boiled with spices	206
	Boiled egg	Hard-boiled egg	131
	Fish-fried	Fresh fish fried in oil	403
	Fish-Smoked	Smoked fish	224
	Grounded pepper	Grounded pepper, tomatoes, and onion with salt. This sauce is not heat treated	94
	Groundnut soup	Soup made from peanut butter, fish or meat and vegetables	103
	Jollof	*Rice cooked in a seasoned tomato-based sauce with local spices*	203.8
	Jollof and spaghetti	Jollof served with boiled spaghetti	170
	Ga Kenkey	Fermented white corn dough cooked into a ball in corn husk	158
	Kokonte	A swallow made from dried powdered cassava flour and cooked into a ball	118
	Mpotompoto	A flavoured yam porridge made from yam, tomatoes, fish, and palm oil.	190
	Okro stew	Thick sauce made from okro (okra) as main ingredient and fish, tomatoes, palm oil and local spices	89.5
	Roasted peanut		721
	Rice and garden egg stew	Boiled plain rice served with garden egg-based sauce made with garden eggs (a specific type of egg plant), fish, tomatoes, pepper, palm oil and local spices	207
	Rice and gravy	Boiled plain rice served with tomato sauce	175.5

	Rice and gravy and spaghetti	Boiled plain rice served with boiled spaghetti and tomato sauce	183
	Semolina	Semolina flour (a coarse, pale-yellow flour made from durum wheat) cooked into a ball	103
	Tombrown	Thick porridge made from roasted corn and legumes flour,	85
	Tumeric rice and egg stew	Rice boiled with turmeric and oil and served with egg sauce made from tomatoes, eggs, vegetable oil and spices	175
	Vegetable and fish stew	Sauce made from chopped cabbage, carrot, green bell pepper, tomatoes, fish, spices, and vegetable oil	122
Dry powdered foods	Yumvita-rice	The rice variant of yumvita- a commercially formulated infant cereal ideal for weaning	454.5
	Cerelac- millet	Millet variant of Cerelac®- a commercially formulated infant cereal ideal for weaning	465.4
	Cerelac -rice	Rice variant of Cerelac®- a commercially formulated infant cereal ideal for weaning	463.3
	Cerelac-fruitmix	Fruitmix variant of Cerelac®- a commercially formulated infant cereal ideal for weaning	479.6
	Gari and sugar and danu milk	Gari-dry crispy flakes made by roasting fermented cassava dough sieved into grits. Danu milk is a powdered milk brand.	405.9

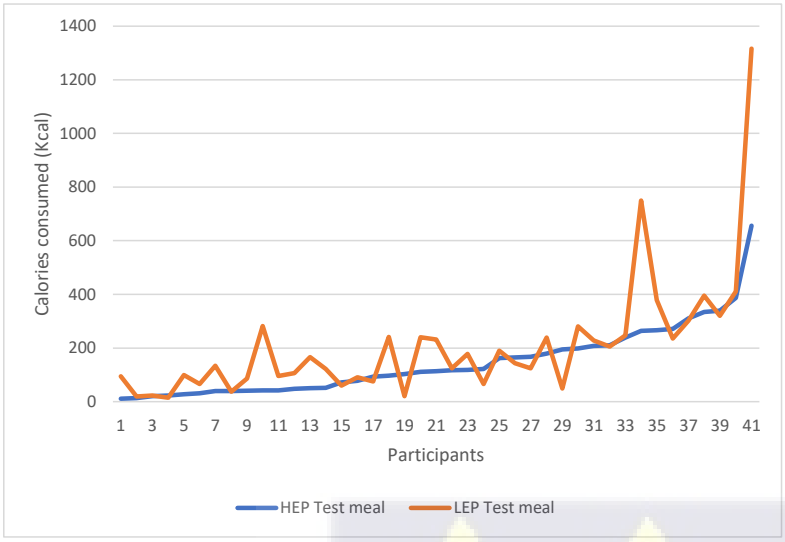


Figure 4.7: Energy intake from test meals after preload drinks

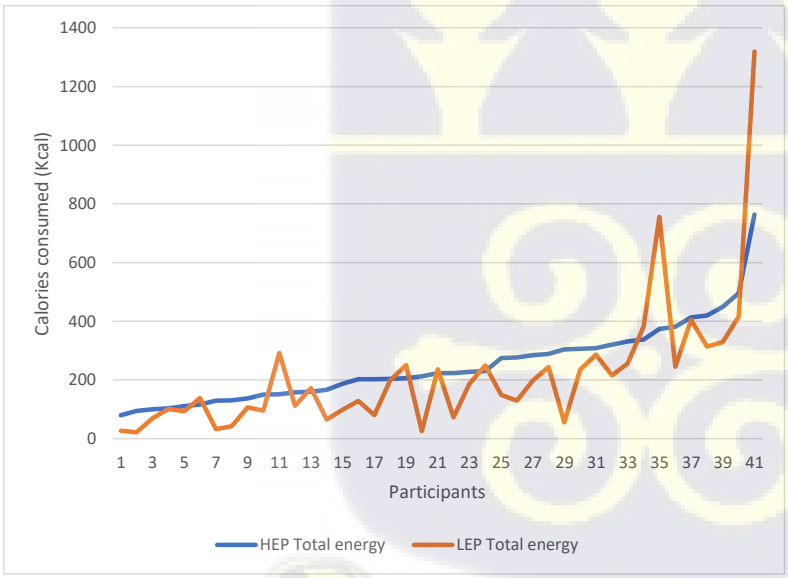


Figure 4.8: Total energy intake (preload drinks plus test meals) for both high and low energy preload conditions

4.7.3 Association between compensation index and nutritional status

It has been shown in previous studies that healthy toddlers have about 70% energy compensation (Kane *et al.*, 2011). The scatter plot in Figure 4.9 shows compx among the three categories of nutrition status. Compared to healthy children, stunted children tended to have a low a compx with a narrow range. In contrast, wasted children showed a much wider range, from little or no to over-compensation, but none of these differences reached statistical significance (Table 4.17).

However, when compared by category, between wasted and stunted children, wasted children showed either minimal or no compensation (41.2%) or over-compensation (35.3%) (figure 4.10, table 4.18), while a majority (66.7%) of the stunted children showed minimal or no compensation and none over-compensated and this difference was significant (p=0.023). There was no significant difference in energy compensation categories between wasted and healthy children or between stunted and healthy children.

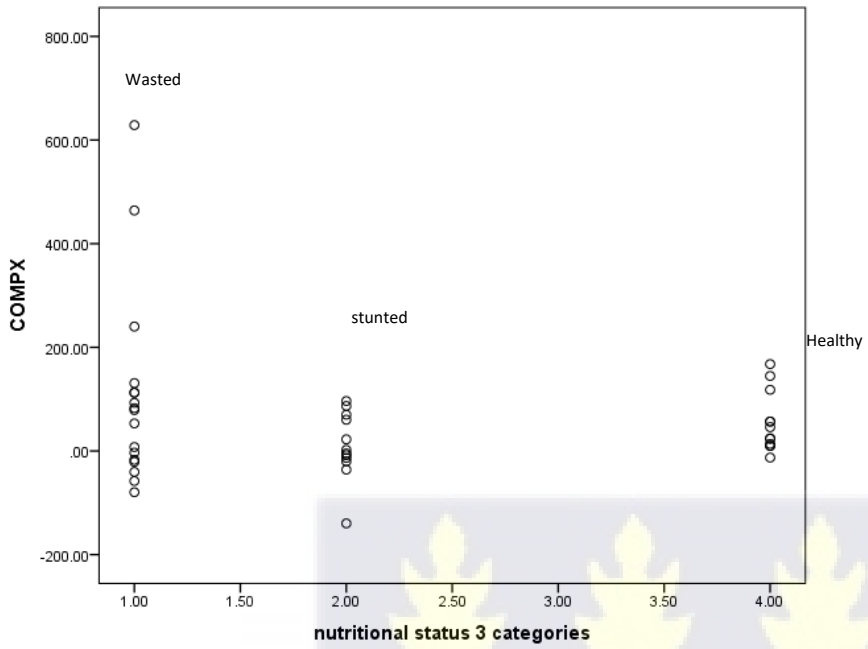


Figure 4.9: Compensation index across the three nutrition status groups

Table 4.17: Association between COMPX and nutritional status (Mann Whitney-test)

NUTRITIONAL STATUS	COMPX Mean (SD)	Median (25,75)	P value
Wasted	105.02 (187.1)	79.05 (-19.06, 121.84)	0.184
Stunted	9.64 (64.8)	-1.37 (-18.46, 67.51)	
Wasted	105.02 (187.1)	79.05 (-19.06, 121.84)	0.929
Healthy	54.88 (58.1)	35.28 (11.06, 102.73)	
Stunted	9.64 (64.8)	-1.37 (-18.46, 67.51)	0.094
Healthy	54.88 (58.1)	35.28 (11.06, 102.73)	
Malnourished (stunted +wasted)	65.55 (154.7)	22.43(-18.59, 94.71)	0.374
Healthy	54.88 (58.1)	35.28 (11.06, 102.73)	

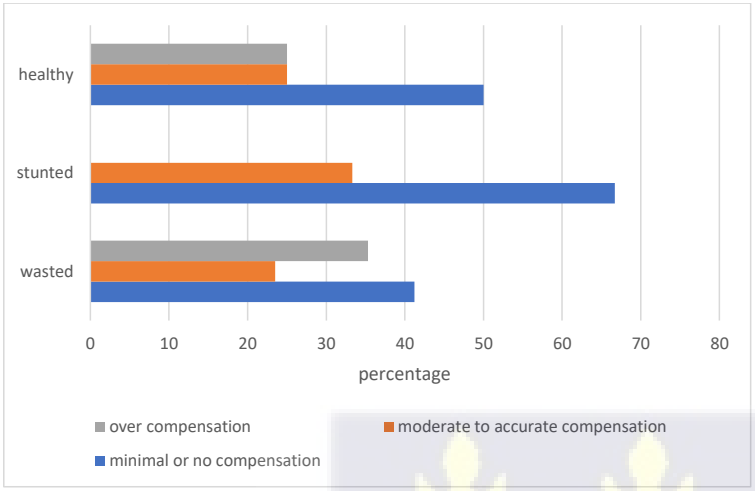


Figure 4.10: Compensation categories across nutritional status groups

Table 4.18: Association between nutritional status and compensation (Chi Square test)

NUTRITIONAL STATUS	Minimal or no compensation (COMPX <30%)	Moderate to accurate compensation (COMPX 30-100%)	Over-compensation (COMPX >100%)	P value
	N (%)	N (%)	N (%)	
Wasted	7 (41.2)	4 (23.5)	6 (35.3)	**0.023**
Stunted	8 (66.7)	4 (33.3)	0 (0.0)	
Wasted	7 (41.2)	4 (23.5)	6 (35.3)	0.830
Healthy	6 (50.0)	3 (25.0)	3 (25.0)	
Stunted	8 (66.7)	4 (33.3)	0 (0.0)	0.101
Healthy	6 (50.0)	3 (25.0)	3 (25.0)	
Wasted	7 (41.2)	4 (23.5)	6 (35.3)	0.121
Stunted + Healthy	14 (58.3)	7 (29.2)	3 (12.5)	

CHAPTER 5

5.0 DISCUSSION

There is evidence of the role of genetics and environmental exposure in the development of childhood malnutrition and that energy regulation plays a significant role in the cause, prevention, and treatment of malnutrition (Lansigan *et al.*, 2015); (Kane *et al.*, 2011); Carnell *et al.*, 2017; Almiron-Roig *et al.*, 2013). This research programme was conducted to describe the extent to which undernourished children (MAM and/or stunted) compared to healthy children living in urban Ghana regulate their energy intake using a standardised satiation study methodology and to determine whether genetic, anthropometry and environmental factors predict this.

5.1 FEEDING BEHAVIOUR AND NUTRITIONAL STATUS OF CHILDREN

This study showed a direct correlation between caregiver and child feeding behaviours. Wasted children showed significantly poor feeding behaviours, including low appetite, high food refusal and high caregiver anxiety, compared to healthy and stunted children. Feeding behaviour was, however, similar for healthy and stunted children. Generally, caregiver feeding anxiety was significantly higher among all malnourished groups and remained a significant predictor of wasting after adjustment for confounders.

Contrary to the findings in this study, Kakpovbia (2010) demonstrated that caregiver feeding behaviour was not a predictor of children's nutritional status in Ghana although it influenced how much food a child eats. Their study investigated how caregiver feeding styles affect child dietary intake and nutritional status in two regions of Ghana, specifically the Techiman and Kassena Nankana districts. In contrast to our study, their population is not diverse; they share similar demographics, backgrounds, or characteristics. It could also be argued that differences in operational variables for feeding behavior in their study contributed to this disparity. For

example, their study involved direct observation and video recording of feeding episodes by a researcher. The presence of the researcher at feeding events might have led to a Hawthorne effect (phenomenon where study participants alter their behavior simply because they know they are being observed), where observed practices do not truly reflect actual behavior

. Notwithstanding, experimental studies by Aboud *et al.* (2009) and Vazir *et al.* (2013) also found no significant impact of responsive feeding behaviour on nutritional status.

Divergent findings have been discovered from other studies. An earlier study found that children's nutritional status is affected by maternal feeding behaviour (Aboud *et al.*, 2008). It was also recently evidenced in a study by Papaioannou *et al.* (2023) that parental feeding behaviours did influence a child's BMI-z scores, with authoritative and indulgent feeding styles positively predicting BMI. This was similarly demonstrated earlier by Hughes *et al.* (2021). These researchers found that an indulgent feeding style at preschool age was associated with increased BMI at ages 7-9 years. This study was a longitudinal one and, hence, robust enough to establish a directional effect. The researchers, hence, found that parental feeding behaviour, specifically indulgent and authoritative feeding styles, positively predicted child BMI z-scores in later years. They also discovered that a child's BMI z-score positively predicted an indulgent feeding style and negatively predicted an authoritarian feeding style later.

The findings from this study clearly refute the null hypothesis that undernourished children will not have different feeding behaviour from healthy children. Indeed, children's nutritional status are largely influenced by their feeding behaviours and that of their caregivers. This was clearly demonstrated in China among preschool children that child's weight status is associated with caregiver's feeding behaviours as well as child's own feeding behaviours (Yuan *et al.*, 2021). The observation from this study that wasted children had poorer feeding behaviours is in tandem with previous findings among pre-schoolers (Jansen *et al.*, 2012; Parkinson *et al.*,

2010). Mutoro *et al*. (2020) also demonstrated that undernourished children living in low-income settings in Nairobi-Kenya had poorer feeding behaviours by refusing food more and having a poor appetite than healthy children. This is not surprising as it is largely expected that a lower appetite and higher food refusal will lead to a deficit in caloric intake and a resultant low weight. It has been previously demonstrated from a similar study that children with higher food refusal had higher odds of being undernourished compared with those with low food refusal (Mutoro *et al*., 2020). In rural areas of West Bengal, food avoidance or food refusal was also seen to be directly associated with thinness and protective against overweight (Roy *et al.*, 2020).

The direction of this association is, however, unknown from this study as it was not explored whether poor feeding behaviour is a cause or an effect of wasting. This requires in-depth exploration, and a longitudinal study will be more appropriate in ascertaining such causal directions. On the other hand, stunted children had similar feeding behaviour as healthy children. Stunting usually results from chronic undereating and poor hygiene (De Sanctis *et al.*, 2021; Woldesenbet *et al.*, 2023). It could, hence, be implied from this study that feeding behaviour is more associated with acute malnutrition rather than chronic malnutrition. This is contrary to discoveries in Kenya by Mutoro *et al.* (2022) where just like wasted children, stunted children were exposed to more risk factors, including poor feeding behaviour. Stunting was also seen to be associated with poor feeding behaviour in rural Ethiopia (Abebe *et al.*, 2017).

Interestingly, a multinomial regression analysis showed wealth quintile and caregiver feeding anxiety as the only remaining predictors of nutritional status after adjustment for all known sociodemographic confounders (Table 4.6). This shows that the association between feeding behaviour and nutritional status is more complex and possibly mediated by other external

factors such as wealth. This is not surprising as feeding also involves some financial demands. The availability of foods is largely dependent on the financial status of the household. This was explored as food poverty in this study.

Food poverty was only significantly associated with force feeding which was paradoxically higher among children with no food poverty. This could be due to the lack of dichotomy in the food poverty and force-feeding data i.e the data does not clearly separate or distinguish between issues of food poverty and force-feeding. No food poverty and low force-feeding were ubiquitous. It could also be attributed to the fact that children from food insecure homes are not forced to eat once they refused meals because foods are already not available. The low prevalence of force-feeding in this study, could be due to the fact that the children were recruited from child welfare clinics where caregivers are educated against force-feeding. Force-feeding may also be a normal practice in this population, such that a child does not need to refuse food before being pressured to eat or that a child's ability to take in food when being pressured is interpreted as good appetite. This pattern was also seen in Kenya where force-feeding was seen to be widely practised irrespective of the child's nutritional status (Mutoro *et al.*, 2020) .

Nonetheless, there was no significant association between force-feeding and nutritional status. This is notable as there was a significant correlation between force -feeding and food refusal which in turn was strongly associated with wasting. It could mean that the relationship between force-feeding and nutritional status is not direct but mediated by factors such as food refusal, as established earlier in this discussion. In contrast, other studies have found a significant negative association between a child's BMI and parental pressure to eat (Nowicka *et al.*, 2014; Jansen *et al.*, 2012). Mutoro *et al.* (2020) also found from their study that force-feeding was significantly more prevalent among children with severe acute malnutrition than among healthy

and MAM children. This style of feeding has also been reported in other studies carried out mostly in LMICs (Abebe *et al.*, 2017; Ha *et al*., 2002; Moore *et al.*, 2006; Nti & Lartey, 2008).

Force-feeding was generally uncommon in this study and, hence, could not be ascertained to be either protective against or a risk factor for malnutrition. Although force-feeding is positively correlated with food refusal and, hence, not an ideal way of feeding, it has been seen to be associated with food acceptance in some children (Ha *et al.*, 2002) and thus could be protective against malnutrition. However, force-feeding is not a sustainable or protective practice against malnutrition. Research consistently shows that force-feeding can have negative psychological and physical impacts on children, leading to aversions to food, increased anxiety around eating, and potential nutritional deficiencies due to disrupted eating patterns (Kerzner *et al*., 2015). While force-feeding may temporarily increase food intake, it can be harmful in the immediate term and affect longer term eating behaviour. It is also not a viable long-term solution for preventing malnutrition and should not be encouraged.. Instead, fostering a supportive and responsive feeding environment is key to ensuring sustainable and healthy nutrition for children. All the same, this exposes the complexities in measuring behavioural correlates that have multiple directions of causation and presents with collinearity or multicollinearity (Tu *et al.*, 2005; Mutoro *et al.*, 2020). Prospective research or intervention studies are more useful to disentangle this concept.

Although the direction of the association between caregiver's feeding behaviours and child's feeding behaviours was not ascertained in this study, it could be extrapolated that the association is bidirectional. This is explained by the fact that negative child's feeding behaviours were directly correlated with negative caregiver feeding behaviours and positive child behaviours were directly correlated with positive caregiver feeding behaviours. One could safely argue that feeding episodes are marked by an action and a corresponding reaction between caregiver and child. For example, a child may refuse food more when force-fed, and

a caregiver may force-feed when a child refuses food more. This may be augmented by an increased caregiver anxiety. This association has been evidenced by Ha *et al.* (2002).

It was also discovered in an earlier study among preschool children that parental pressure to eat was strongly associated with children's food avoidance (Ek *et al.*, 2016) . Consequently, such negative feeding behaviours lead to less caloric intake and invariably result in malnutrition (Kakpovbia, 2010). In a systematic review and meta-analysis, the researchers concluded that the bidirectional association between parental non-responsive feeding and child's feeding behaviour are inconsistent and, hence, requires in-depth exploration (Wang *et al.*, 2022).

On the other hand, mothers also become worried and interact negatively in feeding when they perceive their children to be underweight (Gueron-Sela *et al.*, 2011). However, Hidalgo-Mendez *et al.* (2019) demonstrated that child's actual weight rather than maternal perceived child's weight was a stronger predictor of maternal feeding practices and styles. It was discovered from this study that wasted children had caregivers with higher feeding anxiety scores and caregiver feeding anxiety remained a consistent predictor of wasting after adjustment for all known confounders. Nti and Lartey (2008) also gave credence to this relationship when they found that positive deviant children in Manya-Krobo in the Eastern Region of Ghana were children who were responsively fed, while negative deviant children were non-responsively fed. In the same vain, undernourished children in low income setting in Nairobi-Kenya were also observed to have caregivers with higher feeding anxiety (Mutoro *et al.*, 2020). They also discovered that caregiver anxiety was a consistent predictor of nutritional status and severity. It is imperative to include psychological measures in behavioural interventions aimed to prevent or manage malnutrition in order to deal with caregivers feeding anxiety.

5.2 POLYGENIC PREDICTION OF NUTRITION STATUS AND FEEDING BEHAVIOUR OF CHILDREN

This genetic pilot work involving a heterogeneous sample of children with different feeding behaviours used a new PGRS and found a borderline significant association between PGRS and appetite. However, no significant associations were detected between the PGRS and measures of nutritional status or feeding behaviour. Apart from the SNP rs2274333 close to the gene CA6, which had significantly more wasted than healthy children being homozygous for the effect allele AA, genotype frequencies of the SNPS were not statistically different across the nutrition status groups. These findings agree with the hypothesis for this genetic work that undernourished children will not carry more gene polymorphisms associated with thinness or shortness than healthy children. This hypothesis was made based on the recent evidence that thinness is heritable and polygenically driven (Riveros-McKay *et al.*, 2019). The findings from this study indicate that, although genetics may play a role in food energy regulation, its impact on nutritional status is not huge compared to other modifiable factors such as feeding behaviour and feeding practices. This has implication for clinical practice; a focus on the other factors such as feeding behaviour and feeding practices will help achieve malnutrition treatment goals much easier and faster than genetic factors. Indeed, the genetic findings from this study slightly deviates from recent evidence on the heritability of thinness due to several possible reasons such as small sample size of the study, problems with selection of the SNPs used due to unknown allelic frequencies in the Ghanaian population. These factors are discussed in details in the paragraphs below.

This was a novel study investigating a relatively understudied area of nutrition. Genetics of obesity has greatly advanced from studies into severe monogenic forms, genome-wide linkage analyses, and GWAS; identifying over 250 susceptibility loci (Singh *et al.*, 2017) and over 700 polymorphisms (Orthofer *et al.*, 2020). On the other hand, there has been little interest into the

genetics of undernutrition. It was just recently discovered that thinness is a heritable trait and also polygenically driven, just as obesity (Riveros-McKay *et al.*, 2019). It is, therefore, difficult to compare these findings to other published literature, as a PGRS for undernutrition has not been previously created; however, a BMI genetic risk score, composed of 15 SNPs, has been tested in 3,179 preschool aged children involved in the Generation R study, a prospective population-based cohort in Rotterdam- Netherlands (Monnereau *et al.*, 2017). The score did not significantly associate with any of the 5 CEBQ subscales, mirroring the lack of association found in this study. The authors, however, discovered a potential role for BMI SNPs in satiety responsiveness during childhood. On the other hand, adult BMI genetic risk score composed of 97 SNPs was seen to be nominally associated with some feeding behaviour subscales. The authors postulated that some SNPs have an age-dependent effect, with smaller or no effects seen at a young age.

Since the prevalence of homozygosity was significantly different for only 1 (SNP rs2274333 close to the gene CA6) out of the 9 SNPs, it may be that the SNPs chosen for the PGRS do not actually associate with undernutrition. Though there is evidence of the role of the selected SNPs in regulating appetite or satiety and in body weight regulation (Cecil *et al.*, 2012; Carnell *et al.*, 2008; Riveros-McKay *et al.*, 2019; Garcia-Bailo *et al.*, 2009), this study did not find any significant association between the SNPs and nutritional status. For example, the specific *TAS2R38* SNP used in this study, *rs10246939*, was a candidate SNP whose prevalence did not differ between healthy and malnourished children. This SNP was seen to have no significant association with BMI in Italians (Tepper *et al.*, 2008), nor in a larger sample of Germans (Sausenthaler *et al.*, 2009). This is despite evidence suggesting that *TAS2R38* polymorphisms influence eating behaviour via bitter and sweet taste preferences (Diószegi *et al.*, 2019).

Another SNP investigated in this study was a *CA6* variant, *rs2274333*, which contributes to an increased taste response to bitter thiourea compounds, affecting food perception and

preference. Genotype at this SNP has previously been demonstrated to relate inversely to BMI (Padiglia *et al.*, 2010), just as seen in this study where significantly more wasted children were homozygous for the effect allele AA. Among 11,586 Japanese individuals, there was a significant association between BMI and polymorphisms in or around the candidate genes FTO and TMEM18 (Iwase *et al.*, 2021). The association with FTO variants was significant across the life course and after adjustment for dietary intake, whereas TMEM18 was only significant for BMI at 20 years. This suggests that the effect of some genetic polymorphisms on BMI differs across different life course. This age -dependence effect was also suggested by Monnereau *et al.* (2017), as mentioned earlier. Similarly, Winkler *et al.* (2015) confirmed from a systematic review and meta-analysis of 114 studies that the effect of some 15 genetic loci on body size are age dependent. Amongst these 15 loci were ones near some of the candidate genes SEC16B, TMEM18, FTO and MC4R selected for this current study. Age or life course was, however, not considered in the selection of SNPs for this current study.

Most important is the cumulative effect of possessing multiple BMI-associated SNPs. The majority of the 9 SNPs are located in non-coding regions, with their exact mechanistic influence on body composition unknown. Many of the closest genes are highly expressed in adipose tissue and the brain, specifically the hypothalamus - an area known as the master of whole-body energy homeostasis (Timper & Brüning, 2017). Llewellyn *et al.* (2012) explained that genetic differences in appetitive traits accounted for differences in weight among infants. They discovered from the Gemini twin study that shared genetic effects explained 41 - 45% of appetitive traits, particularly satiety responsiveness, slowness in eating and appetite size, with a significant correlation found between these traits and the weight of infants. It is likely that the SNPs act through pathways controlling appetite and energy regulation. This can explain why this current study found a close to significant association between PGRS and appetite. The association is clearly not huge but gives a hint of the possibility of genetic polymorphisms and

appetite regulation. The small sample size in this study makes it underpowered to detect a huge effect.

Again, there was no significant association observed between PGRS and other feeding behaviour variables as well as nutritional status. Apart from the small sample size affecting the ability to detect significant effects, the small number of SNPs used to compute the PGRS could also be a factor. The predictive power of PGRS is enhanced by not just using SNPs with genome-wide significance in GWAS but by using a large number of SNPs (Agerbo *et al.*, 2015; Mavaddat *et al.*, 2019; Purcell *et al.*, 2009).

It is imperative to consider the effect size of individual SNPs in similar populations when generating a PGRS (Choi *et al.*, 2020; Torkamani *et al.*, 2018). This study, however, did not weight each SNP due to a lack of existing data on the effect sizes of these SNPs in the Ghanaian population. Another genetic study in Ghana that required a PGRS could not also weight the individual effect sizes of the SNPs owing to a similar reason of lack of data in the Ghanaian population (Alsulami *et al.*, 2020). This pilot study, therefore, serves as a preliminary study, forming a baseline for future studies in Ghana.

The underrepresentation of the African population in GWAS, which has been mostly Eurocentric, makes genetics studies in Africa quite challenging. For instance, due to the paucity of data in Ghana, the minimum allele frequency (MAF) in this current study had to be compared with MAF from another African country with close ancestry with Ghana, leaving room for genetic biases and possible errors. It is largely believed that humans originated from Africa and, hence, this continent has a very rich genetic diversity. This diversity places Africa on an advantageous pedestal for novel discoveries in genetic studies, enhances the generalisability of genetic risk models and provides a better understanding of the genetic architecture of complex

diseases and phenotypes (Martin, Teferra, Möller, *et al.*, 2018). Unfortunately, genetic studies in Africa are very negligible.

The SNPs selected for this study were selected based on their discoveries across Europe and other non-African populations with little consideration for their penetrance in Ghana especially. This could possibly explain why most of the associations were not statistically significant. The closest was to consider their allelic frequencies in other African populations with close ancestry with Ghana. A simulated PGRS for the Ghanaian population would have been very helpful for detailed comparison with the studied population; however, this was impossible as the genotype frequencies of the selected SNPs are unknown in Ghana and the use of the healthy groups as controls for comparisons.

Overall, while there is probably some association between reported appetite and genetic score, which is expected, this difference has no observable impact on nutritional status. Meanwhile, an association between appetite and wasting has been found in this current study, but this implies that that is not genetically driven. A confirmed result of PGRS could be useful in the clinical setting. It would be beneficial for parents to know if their child has a genetic propensity to be thin or to exhibit poor feeding behaviour in the future, when the contributions of the genes to thinness are fully characterised and understood, it could translate into therapeutic recommendations. Understanding genetic aetiology in children with poor feeding behaviour and nutritional status could deliver valuable information that would improve nutritional status and overall health outcomes.

5.3 ENERGY REGULATION AND NUTRITIONAL STATUS OF CHILDREN

This experimental study, though small with about 41 children, is one of the largest conducted using such mechanistic and slow methodological approach among a hard-to-reach group. A significant search shows that this is one of the first if not the only to be conducted among a

heterogenous group of children living in a developing country. It was hypothesized in this experimental study that children with MAM will have different satiation patterns compared to healthy children and satiation patterns of stunted children will not be different from healthy children. Generally, children ate more following the low energy preload than high energy preload drink; however, the overall total energy intake, although not statistically different between the two preload conditions, was higher under the high energy preload condition than the low energy preload condition. The overall average compensation was 62.43% with males compensating slightly more than females. Most of the children had minimal to no compensation, and this was most profound among stunted children. This refutes the second hypothesis. Compared to MAM children, stunted children seemed not to compensate at all and will keep eating beyond hunger if given the chance. MAM children showed a much more varied response, and this is in line with the first hypothesis.

The average COMPX value for toddlers in affluent countries is estimated to be 70% (Kasese-Hara *et al.*, 2002). The confidence interval for the healthy children in this current study extends above 70%, suggesting that the true value for healthy children (in the population) could actually be around 70% and the same applies to the wasted children. However, for the stunted children in this current study, the upper limit for the confidence interval is considerably below the 70% estimate, making it likely that this group of children who are stunted, actually have a lower compensation than healthy children.

Indeed, the data are not truly normal, so this has only limited validity, but it does tend to support the idea that the stunted children at least have learnt to not compensate and thus tend to eat beyond hunger when given the chance. This could be an adaptive mechanism for individuals chronically exposed to undereating. It has been previously observed that children from food insecure settings eat more in the absence of hunger (Kral *et al.*, 2017). Although there is lack of uniformity in the operationalization of eating in the absence of hunger (EAH), it has been

consistently evidenced that EAH is positively associated with increased weight status, with EAH being significantly observed among overweight and obese children from both cross sectional and prospective studies (Lansigan *et al.*, 2015).

If the findings from this study are linked to treatment approaches using ready-to-use supplemental foods (RUSF), it suggests that MAM children are more likely than stunted to have their appetite ruined by a preload (or RUSF) while stunted children seem to eat the same whatever. Ironically that might suggest that the non-wasted stunted children might even get fat if given RUSF, while around a third of the MAM children would eat less in total. On the other hand, the big difference between the MAM and both other groups (stunted and healthy) is in the size of the standard deviation and variance, so clearly the wasted children are a very mixed group.

Although the children were supplied with their favourite foods as determined from their caregivers, the overall average energy intake (both preload conditions) was 232kcal, an intake much lower than required for such an age group (330 - 460kcal) (Faizan & Rouster, 2023). This may also reflect the fact that most of the children involved in the experimental study were younger and small with lower energy requirement. Similar findings were reported by Kane *et al.* (2011).

Indeed, there is equivocal evidence on energy regulation in humans due to interindividual differences in metabolism as well as ecological factors such as the setting in which food is eaten (Almiron-Roig *et al.*, 2013). Nonetheless, the evidence is consistent that normally, humans will consume more after a low energy preload than after a high energy preload drink or meal (Rolls, 2009; Tey *et al.*, 2018) . This was also true for this study. The study by Kane *et al.* (2011) also agrees with this current study when they demonstrated that males compensate slightly higher than females with healthy toddlers having about 70% energy compensation.

They also used similar methodological approach as used in this study to assess energy compensation or satiation. Again, the trend for boys to compensate fairly accurately than girls have also been evidenced in previous studies (Davy *et al.*, 2007). It has also been previously revealed that children with failure to thrive lack the normal responses to internal hunger/satiation cues and may either overcompensate or undercompensate following a preload drink or meal (Kasese-Hara *et al.*, 2002). This could explain why the MAM children in this current study seemed to have varied responses to the preload conditions.

These results suggests that children have food energy compensatory mechanisms that are dependent on their nutritional status and influences their ability to eat to meet their nutrient and energy needs. This obviously has implications for clinical practice, especially on the use of high energy supplemental foods or drinks to manage MAM and/or stunting as discussed earlier. The use of RUSF to treat MAM is largely common in LMICs; however, trial evidence has found that high energy supplements (e.g. RUSF) have only modest effects on MAM and almost no effect on stunting (Lazzerini *et al.*, 2013). Ready-to-use supplemental foods (RUSF) have been seen to not prevent progression from MAM to severe acute malnutrition (SAM) or mortality (Lenters *et al.*, 2013; Lazzerini *et al.*, 2013). It only produces a meagre effect on weight gain (0.2 SDS gain in weight after 2-4 months) and no effect on stunting (Lazzerini *et al.*, 2013). A systematic review and meta-analysis showed that the differences in weight gain seen from RUSF compared to children fed corn and soy blend are only statistically significant but not clinically relevant (Lenters *et al.*, 2013). As a result, WHO does not recommend using RUSF to treat MAM. While this lack of efficacy is often attributed to non-compliance (Kenneth Maleta *et al.*, 2004), an already inherent poor energy regulation mechanism may also affect treatment efficacy. The findings from this study and other studies among children are suggestive that offering children high energy preloads, especially as snacks before actual

meals, would, on average, slightly increase their net energy intake but affect the actual amount of food eaten from the main meal and possibly undermine the family food system.

It is worth noting that there were some practical difficulties associated with this experiment. Recruitment and commitment of caregivers were quite challenging due to scheduling difficulties. About 18 participants could not go on to do the second trial due to the unavailability of caregivers 1 - 4 weeks after the first visit. Though most children, especially malnourished ones, tend to have neophobic tendencies, this was quite minimal in this study as children were offered familiar foods that they mostly liked; however, one child would not take any drink apart from Vitamilk; a commersial soyabean based sweetened drink. This made blinding not achievable for such a child and caused a lack of preload uniformity and energy density inconsistencies. This inconsistency did not, however, affect the outcome of the experiment as a sensitivity analysis conducted by taking out the one child and including him did not make any difference.

5.4 CHALLENGES AND SUCCESSES IN RECRUITMENT OF STUDY PARTICIPANTS

Overall, 261 and 41 caregiver-child dyads completed the cross-sectional study and the experimental study, respectively. These participants included both children who were healthy and malnourished in the required sample size. The numbers for the cross-sectional study were beyond the required sample size by about 11 counts and the numbers for the experimental study was just as needed. Healthy children were easier to recruit, and their caregivers were more responsive to the study than malnourished children. There were health staff and peer influence encouraging or discouraging participation. Malnourished children were difficult to recruit, possibly because of their low numbers, as there were clinic days where no malnourished child was identified. It could also be due to caregivers' fear of stigmatisation. There were also instances in some clinics where the health staff were very interested in the study and

encouraged caregivers to participate. However, there were other clinics where individual health staff who did not receive prior notice of the study (notices were given to the clinic leaders) were just indifferent about the study and that affected participant enrolment.

Another interesting observation during the recruitment was the influence of family and friends in communities where people who enrolled in the study encouraged their peers and families to enrol as well. But more intriguing is a particular community where some participants decided not to allow their wards to participate in the genetic trials due to fear of what their saliva samples could be used. They managed to convince other participants to continue with the questionnaire but not to provide saliva samples for genetics analysis.

Participants' recruitment and retention are essential in any successful research. An interview was conducted among low-income urban parents who participated in a parent training study to ascertain what motivated their participation or subsequent withdrawal (Gross *et al.*, 2001). Most parents participated because they wanted to become better parents, while those who dropped out cited schedule and time constraints as their reasons. Most of them cited Program location and qualities of the recruiter as very important factors and financial compensation as least important. Although not explored, these factors may also be true for this study. It is possible those who participated in this current study did so to show they are good parents to their children, knowing the overall benefit of the study to children in general. About 18 participants dropped out of the experimental study after the first visit as they could not be available for the second visit due to time and schedule constraints. Again, the fact that the study was being conducted at the child welfare clinics where they were already attending as well as the experimental study being conducted in their homes, probably did influence their participation as proximity and convenience would have been a factor. However, for those who refused to participate in the experimental study, it could be for reasons of not wanting their private space intruded.

An earlier work by Gorelick *et al.* (1998) explored reasons why minority groups, specifically African Americans, participated or refused to participate or voluntarily withdrew from a study. They reported that some barriers to entry into clinical trials were mistrust of the medical system, economic disadvantages, lack of awareness of study programs and communication barriers. Participants consistently gave reasons for being afraid to be used as ''guinea pigs", hence, their refusal to enrol or to withdraw from a study. The outcome of their investigation led to the emergence of the recruitment triangle, which has become a useful tool to consider when recruiting participants for research work. The three walls of the triangle represent the patient, key family members and friends, and the patient's primary medical doctor and other medical personnel. They explained that the walls are held together by social support, education about the nature of the research, and trust in study personnel and the overall program. The triangle will crush, resulting in withdrawal from or refusal to participate in the study if any of the walls pull away.

Figure 5.1: The recruitment triangle (Gorelick *et al.*, 1998)

The key components of this recruitment triangle were enlisted and adopted for a successful recruitment for this current study. Specifically, the strategies employed included first contacting the clinicians directly involved in running the child welfare clinics, explaining the study to them, and seeking their support well before the start of recruitment. On the days of recruitment, a general nutrition education was given to all participants to build their interest in nutrition research. Then an education on the nature of the research being conducted, the researcher and research assistants involved, and their identities were also made known to the attendants. This information was detailed to them one on one when an eligible participant was approached before their consent was sought to partake in the study. Souvenirs were given to health staff and participants as well. For participants who enrolled in the experimental studies, the cost of procuring food for the children was catered for and the research team went to the homes of the participants to carry out the study so that economic and transport challenges were not barriers to their participation.

Nicholson *et al.*, (2011) described their recruitment strategy that was successful in enrolling and retaining participants for the MOMS' Study (Making Our Mealtimes Special), a longitudinal study that involved low-income, ethnically diverse group of mothers. Their recruitment approach included commitment of research leadership, piloting procedures, frequent team reporting, emphasis on participant convenience, incentives, frequent contact with participants, expanded budget, clinical staff buy-in, a dedicated phone line, and the use of research project branding and logos. They did not encounter any barrier to recruitment, and they had a very high retention rate at 6 months and 12 months after recruitment. Other researchers have documented successful recruitment approaches such as cultural responsiveness, cultural sensitivity and training peers as recruiters (Chang *et al.*, 2009; Flaskerud & Nyamathi, 2000).

5.5 CHARACTERISTICS OF PARTICIPANTS IN THE CROSS-SECTIONAL STUDY

5.5.1 Variations with age

The MAM children tended to be younger which could reflect the oversampling of MAM children who were younger. In general, older children may have better nutritional status than younger children due to several factors. The gut microbiome which is very important for adequate nutrient utilisation and absorption (Bakker *et al.*, 2015; Blaut, 2015; Duca & Lam, 2014) develops with age (Wernroth *et al.*, 2022). Indeed, there are several evidence on the association between gut microbiota and malnutrition (Iddrisu *et al.*, 2021). There have been observed changes in infant microbiome after age 6-months when there is transition to complementary foods with diets getting complex and richer in proteins and fibre (Iddrisu *et al.*, 2021; Laursen *et al.*, 2017; Laursen *et al.*, 2016). The diversity and composition of infant gut microbiome assume adult likeness by ages 2-3 years (Bäckhed *et al.*, 2015; Koenig *et al.*, 2011; Yatsunenko *et al.*, 2012) although some studies suggest that the gut microbiota matures to full adult composition not until age 5 (Cheng *et al.*, 2016; Hollister *et al.*, 2015; Ringel-Kulka *et al.*, 2013; Zhong *et al.*, 2019).

Older children are also exposed to more diverse diet than younger ones (Na *et al.*, 2018). Ariff *et al.* (2020) found child's age to be significantly associated with minimum dietary diversity (MDD) with the likelihood of meeting MDD increasing as the children aged. These could explain why older children in this study had better nutritional status than younger children.

5.5.2 Variations in socio economic status

In this current study, children from poorer households had poor nutritional status than those from wealthy homes. This was especially true for stunted children although they were not significantly different from wasted children in terms of wealth quintile. Wealth index which involves using principal component analysis to analyse household asset is currently considered

a more robust proxy to measure household socio economic status especially in low- middle income countries (LMICs) where data on household income and consumption are unreliable (Poirier *et al.*, 2020).

The economic inequalities in malnutrition have been largely studied with evidence suggesting that undernutrition is more prevalent in poor households than in wealthy ones (Alao *et al.*, 2021). Generally, in Ghana, the 2022 demographic health survey observed an inverse relationship between household wealth (wealth quintile) and stunting (Ghana Statistical Service (GSS) and ICF International., 2023). Children from the poorest households had about three times the likelihood of becoming stunted than children from the wealthiest households (30% versus 9%). There was, however, no clear correlation between wealth quintile and wasting. Frempong and Annim (2017) found a positive correlation between household wealth quintile and nutritional status. They further discovered that the effect of wealth on stunting was between those in the second and middle quintiles but increased between the fourth and richest quintiles; however, wasting was more prevalent among children in the second and middle quintiles. Hence, they suggested that the relationship between wealth and nutritional status may not be linear.

The association between poverty and childhood malnutrition is commonly mediated by poor dietary plan or pattern (dietary diversity), reduced purchasing power, poor sanitation and hygiene facilities, poor living conditions, lack of access to proper health care and many others (Panda *et al.*, 2020; Rahman *et al.*, 2021). Although people from poor households may have low purchasing power and, hence, may not be able to afford some foods, this study did not find any significant relationship between food poverty and nutritional status. This could be because food poverty was measured in terms of accessibility or availability but not quality. Besides,

almost all participants responded to not being food poor; hence, there was no clear dichotomy to draw a comparison among the different nutritional status categories.

5.6 DIETARY DIFFERENCES BETWEEN HEALTHY AND MALNOURISHED CHILDREN

5.6.1 Breastfeeding

A low prevalence of continuous breastfeeding was recorded in this study and was not significantly different from the wasted and stunted cohorts. While the majority of the children in this study had ceased breastfeeding, more wasted children were still being and most frequently breastfed. It may seem paradoxical that breastfeeding was more common among wasted children in this study but it may not be surprising considering their lower average age; however, age was not an explanatory variable for this association.

The WHO recommends continued breastfeeding up to the age of 2 years in addition to complementary feeding (WHO, 2021a). There is convincing evidence of the benefit of continuous breastfeeding to the health and development of a child (Mortensen & Tawia, 2013; Victora *et al.*, 2016). Apart from breastmilk offering the infant certain essential nutrients, the bioactive immunological factors that help protect the child from diseases and infection are sustained even after two years of lactation, hence making continuous breastfeeding beneficial in lowering the risk of morbidity and mortality in children (Andreas *et al.*, 2015; Czosnykowska-Łukacka *et al.*, 2020).

Prolonged breastfeeding positively shapes mother-child feeding practices. Mothers who breastfeed for long have a better bonding effect with their children and are better at recognising and interpreting the hunger and satiety cues of their babies and exert less control during episodes of infant feeding (Fisher *et al.*, 2000; Taveras *et al.*, 2004). This may well influence

how they respond to their children's nutritional needs, allowing children to have better feeding patterns.

Current data shows that globally, 45.1% of children are breastfed for up to two years (Development Initiatives, 2018). In Ghana, data from 2017 shows that about 42% of children were breastfed for up to two years (UNICEF, 2019). Though approximately 80% of the children in this study were between one and two years old, only 14.1% were breastfed beyond 18 months. The prevalence of continuous breastfeeding was lower than global statistics and the general Ghanaian population. A population based study that looked at breastfeeding and weaning practices of caregivers in the Volta Region of Ghana found a higher prevalence (30.6%) of continuous breastfeeding up to 2 years (Appiah *et al.*, 2021). Similarly, in North western Romania, the rate of continued breastfeeding up to 2 years was 30.3% (Cozma-Petruţ *et al.*, 2021).

Some determinants of early cessation of full breastfeeding are lower maternal education level, lower socioeconomic status, full-time employment and short maternity leave (Chimoriya *et al.*, 2020; Scott *et al.*, 2019). Most of these factors are true for the caregivers in this study as the majority of them had education up to Junior high school level, were mostly employed and were from the poor to middle wealth quintile. Additionally, the study setting is the central business area in Greater Accra, with most people, including mothers, being very busy engaging in one economic activity or the other. Promotion of continuous breastfeeding up to 2 years is among the cost effective interventions to reduce malnutrition (Nisar *et al.*, 2016). It is, hence, necessary to give wider attention to this population by increasing education on this subject to the caregivers.

5.6.2 Feeding characteristics of children

5.6.2.1 Complementary feeding

Findings from this study showed that approximately half of the study population had timely introduction to complementary feeding, a rate similar to that recorded among children 6 - 24 months of age in the Ga South Municipality of the Greater Accra Region but lower than that recorded in the northern region of Ghana (86.3%) (Saaka *et al.*, 2022; Kusi, 2016)). The differences in study location could account for the observed differences in these studies. The two studies in the Greater Accra region showed similar rates, much lower than the Northern Region, possibly because of the relatively high level of economic activities in the Greater Accra Region. Unlike mothers in the Northern Region, most mothers in the Greater Accra Region may be busy engaging in one economic activity or the other and possibly allocating less time for feeding the infant. More education on the need for timely introduction of complementary feeding is warranted in Greater Accra Region.

More than half of the wasted children in this study did not meet the recommendation for complementary feeding and were introduced to complementary foods either before or after six months. Generally, early introduction to complementary feeding was more common among wasted children, whereas late introduction was more common among stunted children. This finding is similar to the findings of a recent study conducted in Tanzania to determine the effect of inappropriate complementary feeding practices on the nutritional status of children aged 6–24 months in urban Moshi (Masuke *et al.*, 2021). In this large study with over 3355 participants, early introduction to complementary foods, especially between 0-1 months, was associated with a higher risk of wasting and underweight.

Indeed, untimely introduction to complementary foods poses a threat to a child's growth and development (Tessema *et al.*, 2013; Teshome *et al.*, 2010). Early as well as late introduction to

complementary foods have repercussions on a child's health. Early introduction could lead to complementary foods displacing breastmilk and increasing the risk of diarrheal diseases and infections, which invariably makes a child susceptible to malnutrition (Abiyu & Belachew, 2020). Late introduction may also lead to an insufficient supply of energy and nutrients needed for a child's growth, as breastmilk alone is not enough to support a child's growth and development beyond six months of age (Abiyu & Belachew, 2020).

A major approach to preventing,, treating, or managing malnutrition is to ensure adequate and timely introduction of complementary food. About 6% of deaths among children under five years old in developing countries can be prevented by ensuring optimal complementary feeding (Black *et al.*, 2003). Among the WHO indicators for complementary feeding are minimum dietary diversity (MDD) (5 or more food groups out of 8 groups), minimum meal frequency (MMF) (solid, semi solid of soft foods - 3X for breastfed children 9 - 23 months/ 4x for non-breastfed children 6-23months) and minimum acceptable diet (MAD) (a proportion that achieves both MDD and MMF) all of which are obtained from a 24-hour recall of foods eaten (Donkor *et al.*, 2021).

5.6.2.2 Dietary diversity

A relationship between dietary diversity and nutritional status was found in this current study, where children who met the minimum dietary diversity were of a better nutritional status than those who did not meet. More than half of wasted and stunted children did not achieve minimum dietary diversity; however, about half (46.7%) of the children in this study achieved minimum dietary diversity, a rate slightly higher than that determined by Donkor *et al.* (2021) among Ghanaian children (42%) and quite higher than current national prevalence (41%) (Ghana Statistical Service (GSS) and ICF International., 2023). The recorded rate was also higher than that recorded from pooled data of Demographic Health Surveys in 32 sub-Saharan

African countries (25.1%) (Aboagye *et al.*, 2021). Again, using the 2014 GDHS, Anane *et al.* (2021) discovered that generally, the mean dietary diversity score among children 6 - 23 months was low, and the prevalence of achieving dietary diversity was 22%; however, they found regional differences in the prevalence of MDD with Greater Accra having about 43% prevalence, a rate just a bit lower than what was recorded in this study. The generally high prevalence of MDD observed in this study could be explained by the fact that the participants were recruited from child welfare clinics where they are constantly educated on infant and young child feeding practices.

Improved dietary diversity potentially enhances nutritional status because a diverse diet provides a child with all the necessary nutrients required for their growth (Verger *et al.*, 2021; Khamis *et al.*, 2019). The observed association between MDD and nutritional status in this study was corroborated by a study in Tanzania, where the consumption of a diverse diet was significantly associated with a reduction in undernutrition (Khamis *et al.*, 2019b). It is also in line with studies by researchers Aboagye *et al.* (2021), who examined the association between dietary diversity and undernutrition among 48,968 children 6 - 23 months living in 32 countries in sub-Saharan Africa and found that children who achieved MDD had a 12% less risk of being stunted, 17% less risk of being underweight and 13% less risk of wasting.

Although not explored in this study, other studies have documented some factors that determine a child's ability to achieve MDD. For instance, Anane *et al.* (2021) found that dietary diversity and food consumption among children 6 - 23 months in Ghana were influenced by geographic location and socioeconomic status. Using the 2014 GDHS, these investigators discovered that the probability of achieving adequate dietary diversity was higher in Greater Accra Region (43%) than Ashanti Region (8%). They further demonstrated that maternal education was a significant factor in achieving MDD. Most children (65%) with mothers having higher

educational backgrounds did achieve MDD compared to 19% of those with mothers with no education. They also found some differentials related to household wealth, where more children from the wealthiest homes achieved MDD compared to those from the poorest homes (38% vs. 13%). Bandoh and Kenu (2017) also demonstrated that a child's age did influence the possibility of achieving MDD. They found from their study in the Central Region of Ghana that the younger children aged 6-11 months had lower dietary diversity scores than older children 36 - 47 months old. Similar factors have been reported in other studies carried out in and outside Ghana (Amugsi *et al.*, 2015; Kandala *et al.*, 2011; Meng *et al.*, 2018; Nguyen *et al.*, 2013; Nti, 2011).

Generally, in Ghana, there seems to have been a significant improvement in the prevalence of MDD, with the 2014 GDHS recording 28% and the current 2022 GDHS recording 41% (Ghana Statistical Service (GSS) and ICF International., 2023; Ghana Statistical Service (GSS), Ghana Health Service (GHS), 2015). Although the prevalence of MDD is relatively high in this study compared to other studies, it is actually low, with more than half of children not achieving MDD at all. An increase in dietary diversity shows a corresponding increase in micronutrient and energy intake (Acham *et al.*, 2013; Bandoh & Kenu, 2017); hence, it is likely that the majority of the children in this study did not achieve their daily nutrient and energy requirements. Therefore, it is imperative to give appreciable attention to this area of need to improve dietary diversity when designing nutrition interventions for such a group of children.

5.6.2.3 Food consumption and meal frequency

Generally, the majority of the children, irrespective of nutritional status, did not achieve the recommended minimum meal frequency (MMF) of 3x/day for breastfed children and 4x/day for non-breastfed children. Wasted children seemed to have had a slightly lower overall food frequency than stunted and healthy children. The prevalence of MMF in this study was much

lower than that recorded in the Northern Region of Ghana, where close to half (49%) of children 6-23 months achieved MMF (Agbadi *et al.*, 2017). It was also lower than that recorded in Ethiopia (69.2%) (Wagris *et al.*, 2019). Globally, only half (52%) of children 6-23 months are meeting MMF with low prevalence even among the richest regions and much lower in the poorest regions (UNICEF, 2016).

It is not surprising to observe that the commonly consumed food groups were starchy foods, meat/fish/poultry and foods cooked in oil. Starchy foods are major staples and in high abundance in Ghana. Similar trends have been recorded in other studies carried out in Africa, including Uganda (Acham *et al.*, 2013). Specifically in Ghana, over three-quarters of children in Ekumfi-Central Region were recorded to have consumed starchy foods daily (Bandoh & Kenu, 2017). However, the commonly consumed food group was mainly fish as it is a fishing community. Nti (2008) also reported that the commonly consumed food groups among women in the Manya Krobo District in the Eastern Region of Ghana were starchy roots and plantains as well as grains and cereals, as these were the most abundant foods in the study area. This is reflective of the fact that dietary patterns are dictated by foods that are in abundance and readily available and accessible. The findings seen in this study could be because the study setting is bounded by the major markets such as *Makola, Agbogbloshie, and Kwasiadwaso* where most food items are wholesaled or retailed and procured to other parts of the country. Hence, staples and fleshy foods may be relatively cheaper around this district.

Adequate food quantities, frequencies and quality are essential needs for a child's growth and development, especially in the first 1000 days of life. It is well documented that the nutritional needs of children 6-23 months are much higher per kg body weight than any other age (UNICEF, 2016). However, younger children have small stomach sizes and are unable to eat larger food quantities at a single mealtime. Hence, they need increased meal frequencies to be

able to meet their growing nutritional needs (UNICEF, 2016). A lower meal frequency will put a child at risk of malnutrition. Although not found in this study, possibly because almost four-fifths of the children, irrespective of nutritional status, did not achieve MMF, other studies have documented a significant association between meal frequency and nutritional status. For example, a larger study among children 6 - 23 months old in India revealed 63% higher odds of stunting among children who did not achieve MMF (Aguayo *et al.*, 2016). In this current study, wasted children seem to have had a slightly lower overall food frequency compared to healthy and stunted children. Similarly, Yazew (2022) found that child meal frequency was an associated risk factor for wasting.

The lower prevalence of MMF in this study underscores the urgent need to improve the adequacy of infant diet in the Ashiedu Keteke sub–Metropolitan District. In a systematic review and meta-analysis that aimed to determine the pooled prevalence of MMF practice and associated factors among children aged 6 to 23 months in Ethiopia, the researchers discovered that factors such as post-natal care visit, wealth index and age of the child were significantly associated with MMF (Wake, 2021). In Ghana, factors that contribute to women empowerment, such as decisions on large household purchases, family visits, home ownership, mothers' education and place of residence, were positively associated with the attainment of MMF (Dadzie *et al.*, 2021). These factors, including enhancing women's empowerment could, hence, be considered in designing interventions to improve MMF among children.

5.6.2.4 Self-feeding

Almost all the children self-fed snacks, with a rate higher among healthy children than stunted and wasted. However, the majority of them were caregiver-fed meals and this practice did not differ between healthy and malnourished children. Similar to the findings from this study, this practice has also been previously recorded in resource constraint settings such as Bangladesh

and Vietnam (Moore *et al.*, 2006; Ha *et al.*, 2002). This is not surprising as it seems infant self-feeding of meals is a less common practice in food insecure settings, possibly to avoid food waste (Arlinghaus & Laska, 2021).

Irrespective of their psychomotor abilities or nutritional status, most children from food insecure households are more likely to be caregiver fed meals but allowed to self-feed snacks as these snacks are mostly finger foods that can easily be picked up when dropped. It has been well postulated that scarcity of resources and food insecurity influences parental feeding practices, food provision and delivery as well as infant feeding behaviour (Arlinghaus & Laska, 2021; Berge *et al.*, 2020; Kral *et al.*, 2017) . Nonetheless, it has been suggested that by age 12 months, children should begin to self-feed finger foods and should be able to self-feed a wide range of meals by age 12-24 months (Black & Aboud, 2011).

Although responsive feeding involves encouraging self-feeding, the benefit of self-feeding to a child's weight gain has been quite conflicting from various studies. For instance, Aboud *et al.* (2009) reported from their feeding intervention program that promoted responsive feeding and self-feeding in Rural Bangladesh that self-feeding increased the number of mouthfuls of food eaten by children but did not significantly increase the weight of children 8 - 20 months old. Similarly, Mutoro *et al.* (2020) found no significant association between self-feeding and nutritional status. However, an earlier intervention study, also in Bangladesh, found that self-feeding was positively correlated with the number of mouthfuls eaten and associated with increased weight of children 12-24 months old (Aboud *et al.*, 2008). These two studies in Bangladesh were both randomised controlled trials and, hence, provided stronger evidence of the association between self-feeding and the nutritional status of children. Flesher *et al.* (2020) also discovered from their experimental study that children who were made to self-feed

consumed more food and significantly accepted novel foods compared to those who were never made to self-feed.

Indeed, self-feeding alone may not lead to the attainment of a desired nutritional status. The frequency and quality of meals and snacks are important factors to consider in addition to self-feeding. However, self-feeding is associated with improved food acceptance (Flesher *et al.*, 2020) and a child's control over energy intake. This could account for the higher rate self-feeding, especially of snacks, among healthy children than malnourished ones in this study. Nonetheless, increasing snacks alone may not suffice for a high deficit in calories from the main meal.

5.7 STRENGTHS OF THE STUDY

This is a novel study that used robust methodologies and tools to assess energy regulation among a heterogeneous group of children. To the best of my knowledge, this is the first study in a developing country to comprehensively assess energy regulation among children touching on behavioural, genetic and environmental factors.

Most existing studies assessing feeding behaviour used child eating behaviour questionnaire (CEBQ) and child feeding questionnaire (CFQ), which is more suitable for children susceptible to obesity or obese children (de Lauzon-Guillain *et al.*, 2012; Vaughn *et al*., 2013; Birch *et al.*, 2001) with little consideration for undernourished children. The ICFET used in this study is validated and more tailored to assess feeding behaviour among undernourished children, hence providing a more robust tool to answer the research questions.

Most genetic studies in nutrition have focused on overweight and obese children living in affluent countries, with less attention given to genetics of undernutrition. To the best of my knowledge, this is the first genetics study in Ghana focusing on undernutrition. The use of

PGRS also helped to strengthen the outcome as no single genetic variant is enough to explain a particular trait.

The experimental study conducted is by far the largest to be conducted in a developing country using such a robust and mechanistic approach. Most satiation studies conducted even in developed nations have done so in schools and laboratories, which are not the natural habitat of the participants and using foods strictly prescribed with little or no modification. This experiment was conducted in participants' own homes, and children were fed with their preferred foods in a manner already familiar to them to ensure they were comfortable, thereby eliminating ecological effects on the outcome of the study. Blinding was highly achieved in this study, with caregivers and researchers blinded to the preload sequence. The cross over design used for this experiment ensured that the children were their own controls thereby enhancing the internal validity of the experiment. The caloric content of foods eaten was objectively analysed using a bomb calorimeter, which is the gold standard. Hence, this study did not rely on food tables and software, which are usually flawed and full of approximations and assumptions.

5.8 LIMITATIONS OF THE STUDY

Despite these strengths, there were some major limitations in this research study. Firstly, the feeding behaviour of children and caregivers was subjectively assessed as they relied solely on the caregivers' report; hence it was open to recall biases and some level of under or over reporting. For instance, it was observed that some caregivers involved in both cross-sectional and experimental studies reported feeding behaviours differed from what they actually did. Some of them who reported never force-feeding their child were seen pressurizing their child to eat during the experiment when they were asked to feed the child in their usual feeding manner. A better approach to assessing feeding behaviour is through observation of feeding

episodes of different mealtimes using a longitudinal design. The cross-sectional design used does not allow for making any causal inferences. Again, a longitudinal study will be more appropriate to ascertain causal directions.

Secondly, the genetic study was underpowered to ascertain any meaningful associations. This number is, however, good enough for such a hard-to-reach, vulnerable group of children. The under-representation of Ghana in GWAS also made selection of SNPs for this study difficult and more reliant on non-African populations. This also led to the use of unweighted SNPs for the generation of the PGRS. The number of SNPs used for the PGRS was also small, as more numbers would increase the predictive power of a PGRS.

Certainly, satiation studies only assess short term energy compensation that may not reflect long term energy regulation (Kane *et al.*, 2011). For instance, this methodology only assessed two meals of a child, which may not be typical of the child's eating pattern. The presence of the researcher during feeding could have also introduced a Hawthorne effect in this study. Again, the use of maxijul could not fully mask the taste and texture of the drink. This was, however, overcome by vigorous stirring and leaving the drink to sit for a while until it became clear before serving it to the child. The drinks were also served in transparent but coloured sippy cups so that the slight changes in colour caused by the maxijul, remained undetected. It is, however, possible that some of the children could notice a change in taste as maxijul slightly increased the sweetness of the drinks, which could have affected the amount of preload consumed.

CHAPTER 6

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

This study aimed to describe the extent to which undernourished children (MAM and/or stunted) living in urban Ghana regulate their energy intake using a standardised satiation study methodology and to determine whether genetic, anthropometry and environmental factors predict this. It was demonstrated that the nutritional status of children is influenced by their own feeding behaviour and that of their caregivers, with a direct correlation between the caregiver's behaviour and the child's feeding behaviour. Wasted children exhibited poorer feeding behaviours, specifically food refusal and low appetite, than stunted and healthy children who share similar patterns of feeding behaviour: low food refusal and high appetite. Caregivers' feeding anxiety remained a consistent predictor of the nutritional status of children. Although the genetic study was underpowered to ascertain a clear association between PGRS and nutritional status as well as feeding behaviour, the borderline significant association seen between PGRS, and appetite provided an insight into a possible association. Significantly more wasted children were homozygous for the effect allele AA of the SNP rs2274333 close to the gene CA6. Although not clearly proven in this study, the PGRS provided an awareness of the cumulative genetic susceptibility to undernutrition. Stunted children did not compensate at all and would keep eating beyond hunger if given the chance. On the other hand, MAM children showed a much more varied response to food energy compensation. These findings give insight into the complex interaction between genetics, environmental factors, and nutrition among children and that these factors seem to work together to influence a child's food energy regulation ability.

This study also revealed a low prevalence of continued breastfeeding for up to 24 months and suboptimal complementary feeding practices that reflected low dietary diversity, low MMF

and reduced child self-feeding. Sub-optimal feeding was more profound among MAM and stunted children. A statistically significant association between dietary diversity and nutritional status was found in this study, where children who met the minimum dietary diversity had better nutritional status indicators than those who did not meet it. Starchy foods, meat/fish/poultry and foods cooked in oil were the most consumed food groups among the study participants.

Although a longitudinal study will be more suitable to ascertain causal directions, efforts to prevent or treat undernutrition in children might benefit from a focus on modifiable factors such as feeding practices and feeding behaviours of both children and their caregivers. Precision nutrition can then be considered when the genetics of undernutrition are fully confirmed from larger studies.

6.2 RECOMMENDATIONS

The cost of infant malnutrition is enormous, and the economic losses it poses to individuals and the national economy are huge. It is. Therefore, it is vital to encourage intentional investment in infant nutrition. The findings from this study have implications for research, practice, and policy. These recommendations are, therefore, being made based on this study;

1. This study showed an association between feeding behaviour and nutritional status, with undernourished children having poorer feeding behaviour than healthy children. Unfortunately, assessing feeding behaviour is not readily done as part of the therapy for malnutrition. It is, hence, recommended that the assessment of feeding behaviour should be part of the general assessment during the management of malnutrition.
2. The study also revealed that caregiver anxiety was a significant predictor of nutritional status. The involvement of a psychologist as part of the management team for infant malnutrition is recommended in order to fully attend to caregiver stress and anxiety

associated with feeding children. Caregivers should also be trained on responsive feeding.

3. Africa and Ghana, specifically, is largely underrepresented in GWAS, making genetics studies very challenging; however, Africa has a rich genetic diversity. It is recommended that investment be made into genetic studies in Ghana and in Africa to enhance our representation in GWAS, to increase evidence on genetic studies and to provide a larger scope for comparative studies.
4. Studies, including the present one, have indicated that high-energy supplemental foods or drinks have a limited impact on the treatment outcomes for moderate acute malnutrition (MAM) and stunting. While high-energy supplemental foods, such as Ready-to-Use Therapeutic Foods (RUTF), are not intended for long-term use, they have proven highly effective in managing severe acute malnutrition. These supplements help quickly stabilize severely malnourished children, especially when combined with the treatment of any concurrent infections during critical periods of wasting. For treatment of MAM, Ready to use supplemental foods (RUSF) is utilized. However, these supplements are prohibitively expensive. Therefore, a cost-effective approach that maintains the integrity of family food systems should be adopted. This approach could include training on responsive feeding practices and increased education on infant nutrition to better manage MAM and stunting.
5. This study also revealed a low prevalence of continued breastfeeding up to 24 months as well as suboptimal complementary feeding practices among the studied population. Caregiver education on these feeding practices is usually more common during postnatal clinics. An intentional approach to educating caregivers and providing practical guidelines for continuous breastfeeding and complementary feeding should start right from antenatal clinics.

6.2.1 Practical implementation of recommendations in the context of the study

1. *Integrating feeding behaviour assessment in malnutrition management*

Healthcare providers: Paediatricians, dietitians, and nutritionists should incorporate structured feeding behaviour assessments into routine evaluations for children undergoing treatment for malnutrition. This could involve standardized questionnaires or observation protocols during clinic visits.

Training and resources: Training workshops for healthcare workers on how to assess and interpret feeding behaviours, along with the development of easy-to-use assessment tools, can facilitate this integration.

Community health workers: Community health workers can be trained to conduct feeding behaviour assessments during child welfare clinics and home visits, ensuring that even those in remote areas receive comprehensive evaluations.

2. *Addressing caregiver anxiety with psychological support*

Psychologists and Counsellors: A psychologist should be integrated into the malnutrition management team to provide counselling and support to caregivers, addressing anxiety and stress related to feeding practices.

Training programs: Workshops for caregivers on stress management and responsive feeding techniques can be organized regularly at healthcare facilities.

Support groups: Establishing support groups for caregivers can provide a platform for sharing experiences and coping strategies, facilitated by a mental health professional.

3. *Enhancing genetic research representation in Africa*

Funding and collaboration: Governments and international health organizations should allocate funds specifically for genetic research in Africa, encouraging collaboration between local and international research institutions.

Capacity building: Training programs for African scientists in genomics and bioinformatics can build local expertise. Establishing state-of-the-art genetic research labs in universities and research centers across Africa is crucial.

Community engagement: Engaging local communities in genetic research through awareness campaigns can enhance participation and understanding of the importance of genetic diversity studies.

4. *Adopting cost-effective nutritional interventions*

Responsive feeding training: Health educators and nutritionists can conduct workshops on responsive feeding techniques, demonstrating how to incorporate these practices into daily routines using locally available foods.

Education campaigns: Public health campaigns through radio, television, and community meetings can raise awareness about the benefits of responsive feeding and proper infant nutrition.

Community-based programs: Community health programs can include cooking demonstrations and nutrition classes to show how family foods can be prepared to meet the nutritional needs of malnourished children.

5. *Promoting continued breastfeeding and optimal complementary feeding*

Antenatal and postnatal education: Healthcare providers should deliver consistent messages about the importance of continued breastfeeding and complementary feeding during both antenatal and postnatal visits.

Practical guidelines: Developing and distributing practical, culturally relevant guidelines on breastfeeding and complementary feeding can help caregivers implement these practices effectively.

Peer support programs: Establishing peer support groups for new mothers can provide continuous education and encouragement for maintaining optimal feeding practices.

By involving various stakeholders, including healthcare providers, community health workers, psychologists, researchers, and caregivers, these recommendations can be effectively implemented to improve the nutritional status of children and support their development in Ghana and beyond.

6.3 FUTURE STUDIES

Some possibilities for future research are suggested based on the findings from this study;

1. The interview approach as well as the cross-sectional design used to assess feeding behaviour, provided an outcome based on the caregivers' report and did not allow for drawing an inferential conclusion. Future studies should consider using a longitudinal design with the researcher observing actual meal episodes (making sure to avoid Hawthorne effect) in order to reduce subjectivity and provide a clear inference on the association between behaviour and nutritional status.
2. The PGRS generated for this study could not give a clear prediction of nutritional status and feeding behaviour because of the low sample size and the small number of SNPs used in generating the score. Future studies involving larger sample sizes and more SNPs are warranted.
3. The satiation methodology used in this study to assess food energy compensation only assessed short term energy regulation among a small sample of children. Future studies

Commented [11]: But how? How do you avoid hawthorne effect? Do not assume everyone knows

employing a more robust methodological approach should consider assessing longer term energy regulation among a larger sample of children.

6.4 CONTRIBUTION TO KNOWLEDGE

1. This study has reinforced the existing knowledge on dietary causes of malnutrition by looking at dietary diversity and feeding practices of young children.
2. It has contributed to the understanding of non-dietary determinants of malnutrition among young children living in urban Ghana by carefully observing and measuring the feeding behaviour of children and their caregivers.
3. This study is probably the first to provide evidence on the genetics of undernutrition in developing countries, particularly Ghana.
4. It has also provided insight into how malnourished and healthy children compensate for their energy intake following a preload drink.

6.5 CONTRIBUTIONS OF RESEARCH CANDIDATE

The research candidate was significantly involved with all aspects of the study. She particularly contributed to the following areas;

- Conceptualisation of the thesis.
- Data collection with the help of research assistants.
- Molecular (genetic) laboratory analysis with the help of a research assistant.
- Bomb calorimetry.
- Data entry and cleaning.
- Data analysis and interpretation
- Writing up the entire thesis.

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APPENDICES

APPENDIX 1- PARENTAL INFORMATION AND CONSENT FOR CROSS-SECTIONAL STUDY

PARENTAL INFORMATION SHEET AND CONSENT 1

UNIVERSITY OF GHANA

COLLEGE OF HEALTH SCIENCES, SCHOOL OF BIOMEDICAL AND ALLIED HEALTH SCIENCES

PROJECT TITLE: ENERGY REGULATION AND NUTRITIONAL STATUS OF CHILDREN: A SATIATION STUDY METHODOLOGY

Principal Investigator: Eunice Nortey, University of Ghana, College of Health Sciences, School of Biomedical and Allied Health Sciences, Korle Bu-Accra. Tel 0246046253. Email: eunicearthur@rocketmail.com / earthur056@st.ug.edu.gh

General Information about Research

Children are expected to be able to control their food intake, with healthy children increasing or reducing their food intake, when they have under- or over-eaten previously. Children need to be able to do this to keep them healthy. If they are unable, they may become thin or short and prone to illnesses. We are interested in finding out whether children with feeding problems have underlying genetic differences and /or control their food intake differently from other children. This study is part of a research degree towards the attainment of PhD in Dietetics at the University of Ghana.

You have been invited to participate in this study because you have a child aged 1-3years who is attending child welfare clinic within selected communities in Greater Accra Region. Accepting to participate in this study will involve answering some questions about yourself and your child's diet. Your child's weight and height will be measured. A swab will also be used to take saliva from your child's mouth to test for genes that control eating. This test will be done at the West Africa Genetic Medicine Centre in Accra. It will take about 40 minutes to complete the process of interview, body measurement and saliva collection.

Possible Risks and Discomforts

There is no known risk associated with this study except for a little discomfort in answering certain questions and taking the body measurements of your child. All Covid-19 safety protocols of hand hygiene, wearing of face mask, sanitising all measuring equipment and safe distancing will be observed.

Possible Benefits

You will not receive direct feedback on your information; however, the information collected from you will benefit you and other people because it will help stakeholders in developing appropriate interventions in managing nutrition among children. The results of this study will also be used to influence and guide the design of subsequent research.

Confidentiality

We will protect information about you and your child to the best of our ability. You will be identified by an ID number, and any information about you will have your name and address removed so that you cannot be recognised from it. Confidentiality will be strictly adhered to except in the unlikely event of finding evidence of serious harm, or risk of serious harm, when the University would be obliged to contact relevant statutory bodies/agencies.

Compensation

You will not receive any direct payment for your participation. However, your child will receive a souvenir in the form of a cup or a bowl.

Voluntary Participation and Right to Leave the Research

It is up to you to decide whether to allow your child take part and if you choose not to, this will not have any influence on the services he/she receives at the child welfare clinic. If you decide to take part, you are still free to withdraw at any time and without giving a reason. If so, you can tell us if we can use the data we have collected up till then.

Contacts for Additional Information

If you wish to ask more questions or complain or are unhappy about any aspect of this survey, please contact Mrs Eunice Nortey, University of Ghana, College of Health Sciences, School of

Biomedical and Allied Health Sciences, Korle Bu-Accra. Tel 0246046253. Email : eunicearthur@rocketmail.com / earthur056@st.ug.edu.gh. You may also contact the Administrator of the Ethics Review Committee, Ghana Health Service, Nana Abena Apatu, 0503539896, ethics.research@ghsmail.org.

Your child/ward's rights as a Participant

If you decide to let your child take part in the study, you have the right to information collected from you (before, during and after the study). You also have the right to refuse to participate or discontinue at any stage/level. This research has been reviewed and approved by the Ethics Review Committee, Ghana Health Service. If you have any questions about your rights as a research participant you can contact the Administrator of the Ethics Review Committee, Ghana Health Service, Nana Abena Apatu, 0503539896, ethics.research@ghsmail.org.

CONSENT FORM

STUDY TITLE: ENERGY REGULATION AND NUTRITIONAL STATUS OF CHILDREN: A SATIATION STUDY METHODOLOGY

PARTICIPANTS' STATEMENT

I acknowledge that I have read or have had the purpose and contents of the Participants' Information Sheet read and all questions satisfactorily explained to me in a language I understand (English/ Twi/ Ga). I fully understand the contents and any potential implications as well as my right to change my mind (i.e. withdraw from the research) even after I have signed this form.

I voluntarily agree to be part of this research.

Name of Participant……..

Participants' Signature ……...OR Thumb Print……

Date:…….

INTERPRETERS' STATEMENT

I interpreted the purpose and contents of the Participants' Information Sheet to the afore named participant to the best of my ability in the (English / Twi / Ga) language to his proper understanding.

All questions, appropriate clarifications sort by the participant and answers were also duly interpreted to his/her satisfaction.

Name of Interpreter……

Signature of Interpreter…….. OR Thumb Print …….....

Date:…… Contact Details

STATEMENT OF WITNESS

I was present when the purpose and contents of the Participant Information Sheet was read and explained satisfactorily to the participant in the language he/she understood (English / Twi /Ga)

I confirm that he/she was given the opportunity to ask questions/seek clarifications and same were duly answered to his/her satisfaction before voluntarily agreeing to be part of the research.

Name:……

Signature…….. OR Thumb Print …….....

Date:……

INVESTIGATOR STATEMENT AND SIGNATURE

I certify that the participant has been given ample time to read and learn about the study. All questions and clarifications raised by the participant have been addressed.

Researcher's name…….

Signature …… Date…….

APPENDIX 2- PARENTAL INFORMATION SHEET AND CONSENT FOR EXPERIMENTAL STUDY

PARENTAL INFORMATION SHEET AND CONSENT 2

UNIVERSITY OF GHANA

COLLEGE OF HEALTH SCIENCES, SCHOOL OF BIOMEDICAL AND ALLIED HEALTH SCIENCES

PROJECT TITLE: ENERGY REGULATION AND NUTRITIONAL STATUS OF CHILDREN: A SATIATION STUDY METHODOLOGY

Principal Investigator: Eunice Nortey, University of Ghana, College of Health Sciences, School of Biomedical and Allied Health Sciences, Korle Bu-Accra. Tel 0246046253. Email: eunicearthur@rocketmail.com / earthur056@st.ug.edu.gh

General Information about Research

Children are expected to be able to control their food intake, with healthy children increasing or reducing their food intake, when they have under- or over-eaten previously. Children need to be able to do this to keep them healthy. If they don't, they may become thin and prone to illnesses. We are interested in finding out whether children with feeding problems have underlying genetic differences and /or control their food intake differently from other children. This study is part of a research degree towards the attainment of PhD in Dietetics at the University of Ghana.

You have been invited to participate in this study because you have a child aged 1-3years who is attending child welfare clinic within selected communities in Greater Accra Region and has already completed the first phase of this study. Accepting to participate in this study will involve answering some questions about yourself and your child's diet. It will take about 10 minutes to complete the process of interview. In addition, we will pay two visits (at least one week apart and at most 4 weeks apart) to your home. All visits will take place around mid-morning, arriving two hours after your child has had their breakfast. Your child should have at least two hours fast before we arrive. On each visit, your child will first be given a drink and after 30 minutes he/she will be served lunch – we will discuss with you beforehand what s/he

likes to eat and how to obtain the food either you or us providing the food. We will pay for all meals even if you decide to provide. We will weigh the drink and food before and after intake and the meal will be observed by the researcher.

Possible Risks and Discomforts

There is no known risk associated with this study except for a little discomfort in answering certain questions and some inconveniences with us visiting your home. All Covid-19 safety protocols of hand hygiene, wearing of face mask, sanitising all measuring equipment and safe distancing will be observed.

Possible Benefits

You will not receive direct feedback on your information; however, the information collected from you will benefit you and other people because it will help stakeholders in developing appropriate interventions in managing nutrition among children. Findings from this study will be included in the researcher's final thesis write-up to be submitted to the University of Ghana. The results of this study will also be used to influence and guide the design of subsequent research.

Confidentiality

We will protect information about you and your child to the best of our ability. You will be identified by an ID number, and any information about you will have your name and address removed so that you cannot be recognised from it. Confidentiality will be strictly adhered to except in the unlikely event of finding evidence of serious harm, or risk of serious harm, when the University would be obliged to contact relevant statutory bodies/agencies.

Compensation

You will not receive any direct payment for your participation; however, your child will receive a souvenir in the form of a cup or a bowl.

Voluntary Participation and Right to Leave the Research

It is up to you to decide whether to allow your child take part and if you choose not to, this will not have any influence on the services he/she receives at the child welfare clinic. If you decide

to take part, you are still free to withdraw at any time and without giving a reason. If so, you can tell us if we can use the data we have collected up till then.

Contacts for Additional Information

If you wish to ask more questions or complain or are unhappy about any aspect of this survey, please contact Mrs Eunice Nortey, University of Ghana, College of Health Sciences, School of Biomedical and Allied Health Sciences, Korle Bu-Accra. Tel 0246046253. Email : eunicearthur@rocketmail.com / earthur056@st.ug.edu.gh. You may also contact the Administrator of the Ethics Review Committee, Ghana Health Service, Nana Abena Apatu, 0503539896, ethics.research@ghsmail.org.

Your child/ward's rights as a Participant

If you decide to let your child take part in the study, you have the right to information collected from you (before, during and after the study). You also have the right to refuse to participate or discontinue at any stage/level. This research has been reviewed and approved by the Ethics Review Committee, Ghana Health Service. If you have any questions about your rights as a research participant you can contact the Administrator of the Ethics Review Committee, Ghana Health Service, Nana Abena Apatu, 0503539896, ethics.research@ghsmail.org.

CONSENT FORM

STUDY TITLE: ENERGY REGULATION AND NUTRITIONAL STATUS OF CHILDREN: A SATIATION STUDY METHODOLOGY

PARTICIPANTS' STATEMENT

I acknowledge that I have read or have had the purpose and contents of the Participants' Information Sheet read and all questions satisfactorily explained to me in a language I understand (English/ Twi / Ga). I fully understand the contents and any potential implications as well as my right to change my mind (i.e. withdraw from the research) even after I have signed this form.

I voluntarily agree to be part of this research.

Name of Participant……..

Participants' Signature ……...OR Thumb Print……

Date:…….

INTERPRETERS' STATEMENT

I interpreted the purpose and contents of the Participants' Information Sheet to the afore named participant to the best of my ability in the (English/ Twi /Ga) language to his proper understanding.

All questions, appropriate clarifications sort by the participant and answers were also duly interpreted to his/her satisfaction.

Name of Interpreter……

Signature of Interpreter…….. OR Thumb Print …….....

Date:…… Contact Details

STATEMENT OF WITNESS

I was present when the purpose and contents of the Participant Information Sheet was read and explained satisfactorily to the participant in the language he/she understood (English/ Twi /Ga)

I confirm that he/she was given the opportunity to ask questions/seek clarifications and same were duly answered to his/her satisfaction before voluntarily agreeing to be part of the research.

Name:……

Signature…….. OR Thumb Print …….....

Date:……

INVESTIGATOR STATEMENT AND SIGNATURE

I certify that the participant has been given ample time to read and learn about the study. All questions and clarifications raised by the participant have been addressed.

Researcher's name…….

Signature …… Date…….

APPENDIX 3 - INTERNATIONAL COMPLEMENTARY FEEDING

EXAMINATION TOOL

EATING AND FEEDING BEHAVIORS OF HEALTHY AND MALNOURISHED CHILDREN

CHILD CHARACTERISTICS (CIRCLE APPROPRIATE ANSWER)

CHILD		
Age (years)	Age (months):	Date of Birth:
Gender: Male [ ] female [ ]		
Birth order: 1 [ ] 2[ ] 3 [ ] 4 [ ] 5 [ ] 6 [ ] other ----------		
CAREGIVER		
Relationship with child: ……		
Age:……..		
Education: No school [ ] primary [ ] JHS [ ] SHS [ ] Vocational/Technical [ ] Tertiary [ ]		
Employment: Employed [ ] unemployed [ ] self-employed [ ]		
Marital status: Married [ ] consensual union [ ] separated [ ] divorced [ ]widowed [ ]never married [ ]		
Ethnicity: Akan [ ] Ga [ ] Ewe [ ] Nzema [ ] Hausa [ ] Others----------		
Address (place of residence):		
ANTHROPOMETRY		
Weight 1 (kg):	Weight 2(kg)	WAZ:
Height 1 (cm)	Height 2 (cm)	HAZ :
MUAC 1 (cm)	MUAC 2 (cm)	WHZ:
		BMIZ:
Who measured child? Researcher [ ] Health staff [ ] Research assistant [ ] Other (specify) _____		
GENETICS		
Saliva samples taken: Yes [ ] No [ ]		
ID on sample:		

1. **Child illness**
   - **a.** Is your child currently unwell? Yes/ No **If yes ask:**
     - i. What is his/her current illness? _____
   - b. Has your baby had any major health problems since birth? Yes [ ] No [ ] (If yes please describe them)
   - c. Has your child ever been admitted to hospital? Yes [ ] No [ ] (if yes)

   When was the most recent admission? Duration: _____
   Reason for admission? _____
   Are you giving any vitamin supplements? Yes [ ] No [ ]
   Routine vitamin A Yes [ ] No [ ]
   Micro nutrient powders Yes [ ] No [ ]
   Other: specify_____

2. **Child Development**

Can your baby do any of the following?	**Tick if present**	How old was your baby when they first did this? Age in months*
Reach out for objects and take them to their mouth		
Sit for at least 10 seconds without using hand or arms to support them		
Stand holding on to a stable object (e.g. furniture, not a person) with hands only for at least 10 seconds		
Stand alone with no contact with a person or object for at least 10 seconds		
Walk at least 5 steps with back straight and no contact with a person or object		

*'not yet' if not yet attained

3. **Child care and feeding**
   - **d.** Did you ever breastfeed your child? Yes/ No **If yes ask:**

   Is your baby still breastfeeding? >3 feeds per day / 2-3 feeds per day/ 1 feed per day / <1 feed per day
   (If response to (i) is <1 feed per day ask) At what age did you last give breast milk?
   _____ months

   At what age did you first feed your child solid foods, including purees, cereals, porridge?
   _____ months

4. What sort of food did you give your child during the past 24 hours? (tick and write down the food given if child was not offered anything write nothing offered)

	Plated foods requiring feeding by hand or spoon	**Dry Finger foods** (e.g. biscuits, crisps, bread)	**Moist finger foods** (e.g. Fruit)	**Milk drinks** (formula milk, cow milk, tea, yoghurt)	**Other drinks*** (e.g. juice, porridge)	**Ready to use foods**
Morning						
Mid-morning						
Afternoon						
Evening						
Night						

*does not include water

How often does the child eat following? (*please tick one answer for each*):	**Never/ rarely**	**once a month but not weekly**	**once a week but not daily**	**Once daily**	**More than once daily**
Starchy foods (Bread, potatoes, yam, plantain, banku, rice, fufu, kokonte, tuo zaafi, kenkey etc)	[ ]	[ ]	[ ]	[ ]	[ ]
Meat/fish/poultry	[ ]	[ ]	[ ]	[ ]	[ ]
Eggs	[ ]	[ ]	[ ]	[ ]	[ ]
Dairy products (milk, yoghurt, fermented milk)	[ ]	[ ]	[ ]	[ ]	[ ]
Legumes/nuts, beans, peas, peanuts)	[ ]	[ ]	[ ]	[ ]	[ ]
Fruits (bananas, oranges, melons, mango)	[ ]	[ ]	[ ]	[ ]	[ ]

Leafy Vegetables (spinach, cabbage, kontomire, aleefu, boma, ademe)	[ ]	[ ]	[ ]	[ ]	[ ]
Savoury snack foods (crisps, chips, nuts, popcorn, plantain chips, savoury biscuits)	[ ]	[ ]	[ ]	[ ]	[ ]
sweet snack foods (chocolates, sweets, sweet biscuits)	[ ]	[ ]	[ ]	[ ]	[ ]
Food cooked in oil (vegetable oil, cooking fat, palm oil, blue band)	[ ]	[ ]	[ ]	[ ]	[ ]

5. Feeding practices (Who feeds the child)

a. *Does mother usually feed the child?* Always / *Mostly* / *Sometimes* / *Never*

b. *When the mother is not there, who usually feeds the baby? Specify* _____

Please tick one answer for each	**entirely self feeds**	**mostly self feeds**	**Half and half**	**Carer mostly feeds**	**Carer always feeds**	**Not given**
Who feeds the child meals (foods that are served on a plate and eaten with a spoon/hand)?	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Who feeds the child snacks (finger foods pieces of fruit, biscuits)?	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

6. How does your child eat?

Would you say your child? *(tick one answer for each)*	**All the time**	**Most of the time**	**Sometimes**	**Rarely**	**Not at all**
Likes food a lot	[ ]	[ ]	[ ]	[ ]	[ ]
Is interested in food	[ ]	[ ]	[ ]	[ ]	[ ]
Enjoys eating	[ ]	[ ]	[ ]	[ ]	[ ]
Enjoys a wide variety of food	[ ]	[ ]	[ ]	[ ]	[ ]

Eats quickly	[ ]	[ ]	[ ]	[ ]	[ ]
Finishes his/her meal	[ ]	[ ]	[ ]	[ ]	[ ]

Food refusal

7. How often does your child do the following when offered food?

	All the time	**Most of the time**	**Sometimes**	**Rarely**	**Not at all**
Turns head away when offered food	[ ]	[ ]	[ ]	[ ]	[ ]
Pushes food away	[ ]	[ ]	[ ]	[ ]	[ ]
Cries/ screams	[ ]	[ ]	[ ]	[ ]	[ ]
Spits out food	[ ]	[ ]	[ ]	[ ]	[ ]
Meals last More than 1 hour	[ ]	[ ]	[ ]	[ ]	[ ]

8. What sort of things do you do if your child refuses to eat?

	All the time	**Most of the time**	**Sometimes**	**Rarely**	**Not at all**
Restrain him by holding his/her hands	[ ]	[ ]	[ ]	[ ]	[ ]
Pour food in to his/her mouth	[ ]	[ ]	[ ]	[ ]	[ ]
Try to forcefully open his/her mouth	[ ]	[ ]	[ ]	[ ]	[ ]

Other (What else do you do if your child refuses food)	

9. How do you feel when feeding your child? (*please tick one answer for each that best applies*):

	All the time	**Most of the time**	**Sometimes**	**Rarely**	**Not at all**
Do you worry that your child is not eating enough	[ ]	[ ]	[ ]	[ ]	[ ]
Does your child's feeding cause you significant anxiety	[ ]	[ ]	[ ]	[ ]	[ ]
<u>Are you concerned about</u>					
The variety of foods your child eats	[ ]	[ ]	[ ]	[ ]	[ ]
Your child's behaviour at mealtimes	[ ]	[ ]	[ ]	[ ]	[ ]
Your child's lack of interest and/or enjoyment of food	[ ]	[ ]	[ ]	[ ]	[ ]
Your child's eating speed	[ ]	[ ]	[ ]	[ ]	[ ]

10. Household hunger score

Can we ask you a few questions on whether you have ever been in short of food at home	0=Never	1 = Rarely (once or twice in the past 30 days)	2 = Sometimes (3–10 times in the past 30 days)	3 = Often (more than 10 times in the past 30 days
Was there ever no food to eat of any kind in your house because of lack of resources to get food? *If so, how often did this happen?*				
Did you or any household member go to sleep at night hungry because there was not enough food? *If so, how often did this happen?*				
Did you or any household member go a whole day and night without eating anything because there was not enough food? *If so, how often did this happen?*				

11. Is there anything else you would like to say about feeding your baby?

12. Housing characteristics

The house is: *Owned by family/Rented /Shared / Other*_____

Number of rooms in house_____

House construction - *Permanent /semi-permanent / Temporary / Other specify*_____

Household possessions *Car/ Motorcycle / Bicycle /Refrigerator / Television / Radio / Mobile phone*

13. Water and sanitation facilities

	Piped into house	**Public tap (purchase**	**Well/rain water**	**Vendor (truck**	**bottled water**	**Other**
Main source of water for household use:						
Main source of drinking water						

Type of toilet: *Pit latrine (without flash system) /latrine (Flush system) /Flush toilet /bucket/pail open place / other* ___

i) Is toilet shared by other households? *Yes / No*

ii) Do you pay to use the toilet? *Yes/No*

Garbage disposal: *Collected by "Private firm" /Disposal within compound/ Unauthorized heap outside the compound / other (specify)* _____

14. Family characteristics

Mother's age: _____

Education level: *None/ primary / JHS /Secondary education/ Tertiary/ Other* _____

Father's age: _____ *Resident / non resident and contributing to household / no contact*

Education level: *None/ primary / JHS /Secondary education/ Tertiary/ Other* _____

Number of children born to this mother (including this child) _____

Age of eldest child: _____ Age of youngest child: _____

How many children are under the age of 5 years_____?

APPENDIX 4- SATIATION STUDY DATA COLLECTION FORM

BASIC INFORMATION

ID		
CHILD'S AGE (months)		
GENDER	MALE [ ]	FEMALE [ ]
CARER'S NAME		
CARER'S PHONE NUMBER		
CARER'S EMAIL		
RELATIONSHIP WITH CHILD		
ADDRESS		

CHILD'S ANTHROPOMETRY

	1	2	AVERAGE
WEIGHT (KG)			
HEIGHT/LENGTH (CM)			
WAZ			
HAZ			
WHZ			

WEEK 1	Type	High [ ]	Low [ ]
	Flavour		
	Volume before (V1) /ml		
PRELOAD DRINK	Volume after (V2) / ml		
	Volume intake (V1-V2) / ml		
	Kcal / 100 ml		

WEEK 2	Type	High [ ]	Low [ ]
	Flavour		
	Volume before (V1) /ml		
PRELOAD DRINK	Volume after (V2) / ml		
	Volume intake (V1-V2) / ml		
	Kcal /100 ml		

1ST VISIT DATE:…….....

Food 1	Description	
	Weight before (M1) /g	
	Weight after (M2) /g	
	Weight intake (M1-M2) / g	
	Kcal / 100g	
Food 2	Description	
	Weight before (M1) /g	
	Weight after (M2) /g	
	Weight intake (M1-M2) / g	
	Kcal / 100g	
Food 3	Description	
	Weight before (M1) /g	
	Weight after (M2) /g	
	Weight intake (M1-M2) / g	
	Kcal / 100g	

BREAKFAST TIME:……..

FOOD	AMOUNT	CALORIES

FOOD RECIPE

RESEARCHERS COMMENT:

2ND VISIT DATE:…….....

Food 1	Description	
	Weight before (M1) /g	
	Weight after (M2) /g	
	Weight intake (M1-M2) / g	
	Calories / kcal	
Food 2	Description	
	Weight before (M1) /g	
	Weight after (M2) /g	
	Weight intake (M1-M2) / g	
	Calories / kcal	
Food 3	Description	
	Weight before (M1) /g	
	Weight after (M2) /g	
	Weight intake (M1-M2) / g	
	Calories / kcal	

BREAKFAST TIME:……..

FOOD	AMOUNT	CALORIES

FOOD RECIPE

RESEARCHERS COMMENT:

APPENDIX 5 - ETHICAL CLEARANCE

GHANA HEALTH SERVICE ETHICS REVIEW COMMITTEE

In case of reply the number and date of this Letter should be quoted.

My Ref. GHS/RDD/ERC/Admin/App | 022/005
Your Ref. No.

Research & Development Division
Ghana Health Service
P. O. Box MB 190
Accra
Digital Address: GA-050-3303
Mob: +233-50-3539896
Tel: +233-302-681109
Email: ethics.research@ghsmail.org
17th January, 2022

Eunice Nortey
Department of Dietetics
School of Biomedical and Allied Health Sciences
University of Ghana
Korle-Bu

The Ghana Health Service Ethics Review Committee has reviewed and given approval for the implementation of your Study Protocol.

GHS-ERC Number	**GHS-ERC: 022/11/21**
Study Title	Energy Regulation and Nutritional Status of Children: A Satiation Study Methodology
Approval Date	17th January, 2022
Expiry Date	16th January, 2023
GHS-ERC Decision	**Approved**

This approval requires the following from the Principal Investigator

- Submission of a yearly progress report of the study to the Ethics Review Committee (ERC)
- Renewal of ethical approval if the study lasts for more than 12 months,
- Reporting of all serious adverse events related to this study to the ERC within three days verbally and seven days in writing.
- Submission of a final report after completion of the study
- Informing ERC if study cannot be implemented or is discontinued and reasons why
- Informing the ERC and your sponsor (where applicable) before any publication of the research findings.

You are kindly advised to adhere to the national guidelines or protocols on the prevention of COVID -19

Please note that any modification of the study without ERC approval of the amendment is invalid.

The ERC may observe or cause to be observed procedures and records of the study during and after implementation.

Kindly quote the protocol identification number in all future correspondence in relation to this approved protocol

SIGNED.....
Dr. Cynthia Bannerman
(GHS-ERC Chairperson)

Cc: The Director, Research & Development Division, Ghana Health Service, Accra

APPENDIX 6 - PERMISSION LETTER

In case of reply the number and date of this letter should be quoted.

My Ref. No. ***GHS/GARHD/001/22***

Your Ref. No.

GHANA HEALTH SERVICE
REGIONAL HEALTH DIRECTORATE
GREATER ACCRA
P. O. BOX 184
ACCRA

Tel: +233-0302-248997

29th March, 2022

THE METRO DIRECTORS OF HEALTH SERVICES
- ACCRA
- TEMA

THE MUNICIPAL DIRECTORS OF HEALTH SERVICES
- KORLE KLOTTEY
- LEDZOKUKU
- AYAWASO EAST
- ASHAIMAN
- ABLEKUMA WEST
- ABLEKUMA CENTRAL
- KPONE KATAMANSO
- GA CENTRAL

THE DISTRICT DIRECTOR OF HEALTH SERVICES
ADA EAST

RE: PERMISSION TO CARRY OUT RESEARCH IN GREATER ACCRA REGION – MS. EUNICE NORTEY

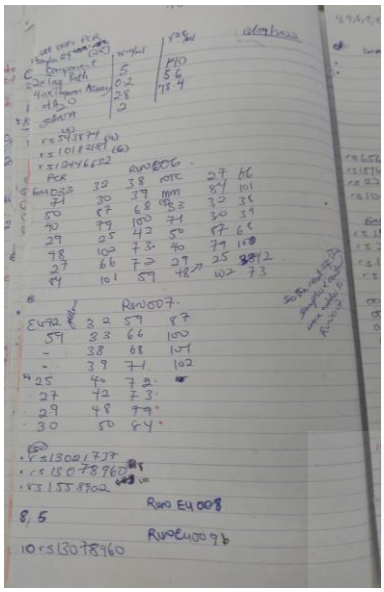
Kindly find attached a letter dated 22nd March, 2022 from the Head, Department of Dietetics, School of Biomedical and Allied Health Sciences of the University of Ghana on the above subject matter for your information and necessary action.

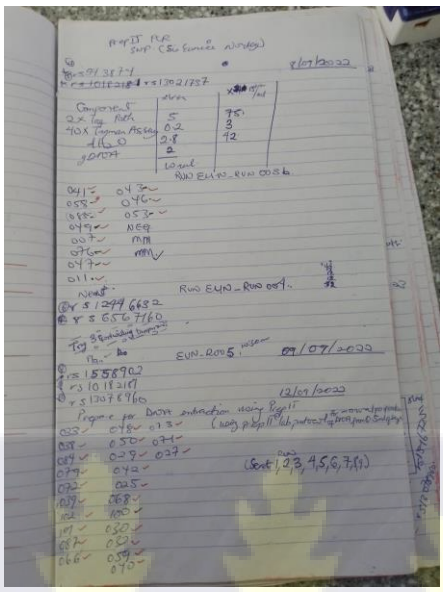
Thank you.

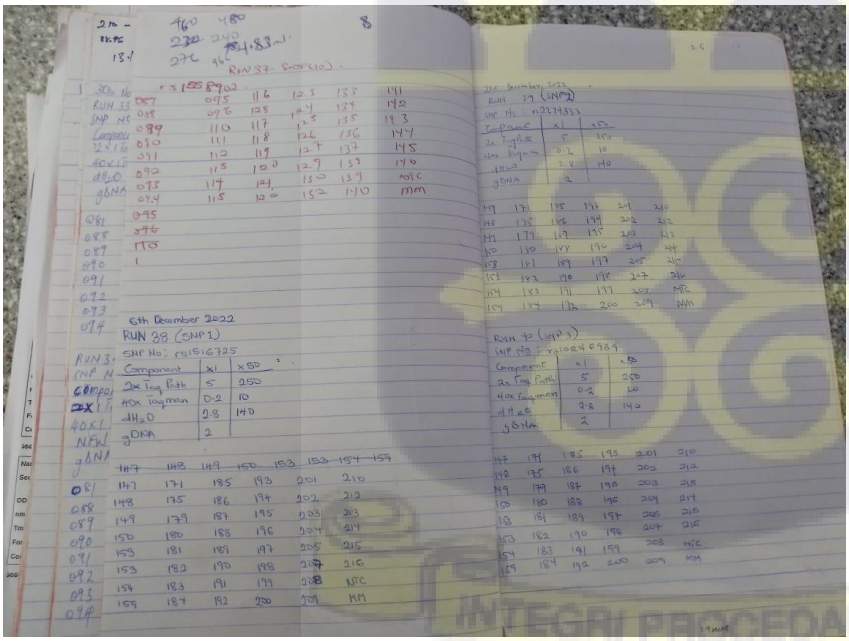
DR. (MRS.) CHARITY SARPONG
REGIONAL DIRECTOR OF HEALTH SERVICE
GREATER ACCRA

Cc: Deputy Director Clinical Care

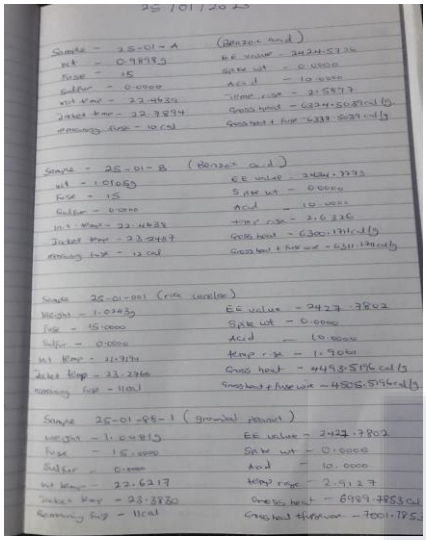
APPENDIX 7 - LABORATORY NOTE: GENETICS TEST

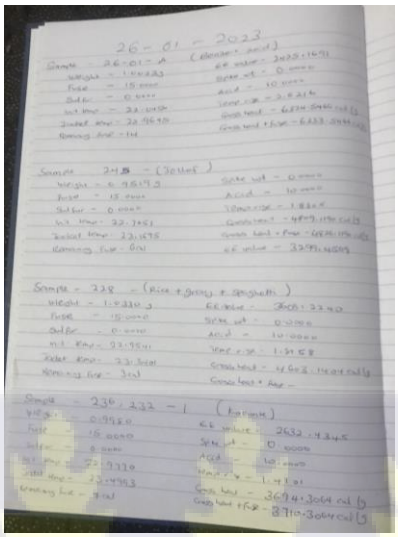


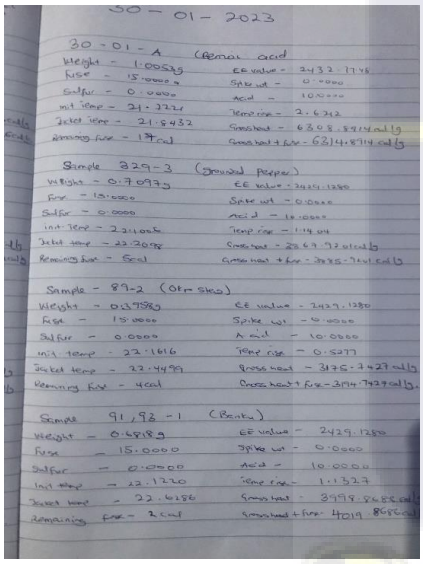


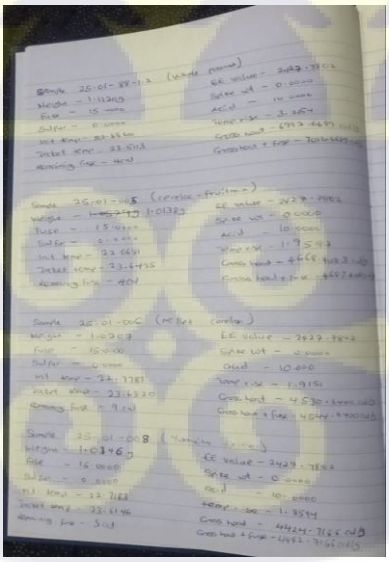


APPENDIX 8 - LABORATORY NOTE: BOMB CALORIMETRY









APPENDIX 9 – FREEZE DRYING PROTOCOL

Scanvac Coolsafe Freeze-dryer

Before you begin:

- The samples and Sample Stand should be stored in a -80^{o}C freezer for 24 hrs.
- Ensure the inside of the unit is clean and dry.
- Place the Manifold on the chamber. The 3 white plugs should face down inside the chamber and the red ring should face upwards.
- Place the clear Perspex cover onto the Manifold so that it forms a seal with the red ring.
- Make sure the beaker is under the Drain valve.
- **Make sure the Drain valve is turned fully closed.** Do not overtighten.
- The **Power switch** and **Vacuum valve** on the Freeze dryer should both be switched off.
- The connected Vacuum pump should also be switched off.

Using the Freeze-dryer:

1. Turn the Freeze dryer on using the **Power switch**.
2. Turn the Vacuum pump on at the wall switch. (DO NOT switch on the **Vacuum valve** of the Freeze dryer until you enter Freeze drying mode in step 7)

   User interface reads **STATUS: WAIT**

   Leave the unit running for 30 mins to achieve lowest vacuum performance.

   Temperature will drop to around -50^{o}C or lower.

   User interface will change to **STATUS: OK**
3. Remove your samples and Sample Stand from the -80 freezer.
4. Remove the Perspex cover from the Manifold.

   NOTE: temperatures can be lower than -50^{o}C.

Be very careful not to touch any surfaces with bare skin.

5. Place the Sample Stand on the Manifold and carefully load your samples onto it.

   You can use racks or wrap samples with elastic bands to prevent them from falling over.

6. Replace the Perspex cover onto the Manifold taking care not to knock the samples or the Sample Stand.
7. Start Freeze drying mode by switching the **Vacuum valve** to OPEN. Leave for desired time.
8. Once freeze drying is complete, switch the **Vacuum valve** to CLOSED.
9. Release the pressure by SLOWLY turning open the **Drain valve** in order to break the vacuum.

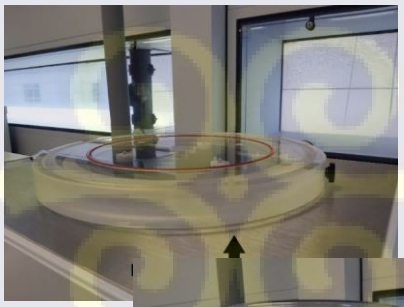
   Do this extremely slowly – it may take up to 30 seconds to complete.

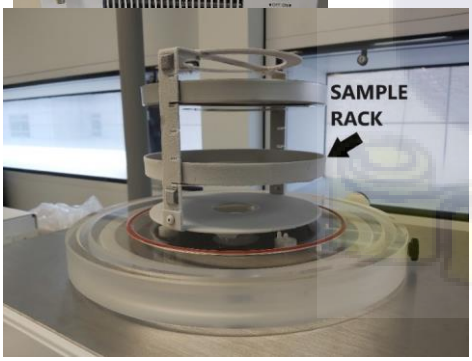
10. You can now lift the Perspex cover and carefully remove the Sample Stand with your samples.

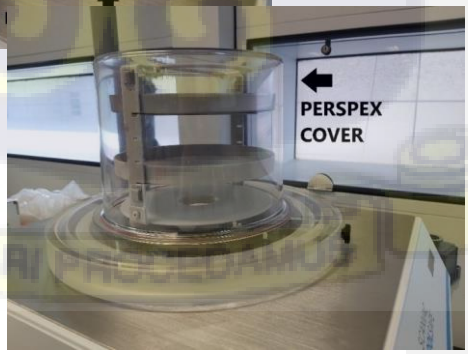
    If someone else is using it after you, turn the **Drain valve** closed, replace the Sample Stand and Perspex cover on the Manifold and keep the freeze dryer running.

<u>At the end of the freeze drying process:</u>

11. Remove Manifold, Sample Stand and Perspex cover from Freeze dryer and leave aside to dry.
12. Turn off Freeze-dryer using the **Power switch** and ensure the **Drain valve** is turned open.
13. Leave the Vacuum pump running for another 45 mins to remove condensation from the pump oil. Switch off at the wall.







APPENDIX 10 – BOMB CALORIMETRY PROTOCOL

Parr 6100 calorimeter

Extreme caution must be used when dealing with the Parr Calorimeter and the Oxygen.

Turn on the Calorimeter (switch is located on the back at the Left hand side)

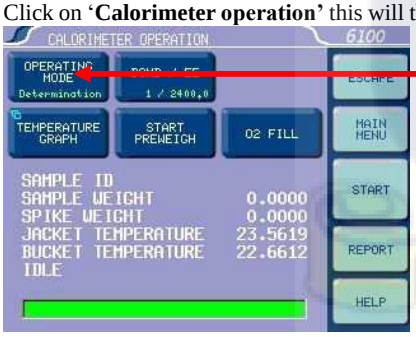
The Calorimeter will 'boot up' and the stirrer shaft will rotate until the boot up is complete.

It will then show the **main menu** on the screen.

Turn on the oxygen supply till it reaches 400psi.

Make sure the oxygen levels are on 3000 and 440-450psi flow rate. If the level drops below 450psi then change the oxygen cylinder.

Click on '**Calorimeter operation'** this will then take you to the operation menu



To choose the operating mode click on the '**operating mode'** button. This will switch from '**Determination'** to '**Standardisation'**.

Determination ~ to analyse samples

Standardisation ~ for use with the Benzoic acid tablets to standardise your machine *(this should be done on a weekly basis if the machine is in regular use.*

Go to the fine balance and tare it with a clean, dry, empty Parr sample crucible.

For Standardization: Prepare the crucible (*ensure that it is clean and dry before use –do not use paper to dry it –it must be air dried*.) Tare (zero the weight of) the crucible sample cup. Add 1 tablet of Benzoic acid and weigh the sample to the nearest .0001 mg. (***Keep a written note of this exact result as it needs to be entered into the Parr menu****).*

Calorimeter **must be calibrated** with Benzoic acid prior to use with any determination samples. The energy value of benzoic acid is a known quantity and we use pellets @1g.

For Determination: Prepare the crucible (*ensure that it is clean and dry before use –do not use paper to dry it –it must be air dried*.) Tare (zero the weight of) the crucible sample cup. Add about 1 gram of the unknown sample and weigh to the nearest .0001 mg. (*Keep a note of this result as it needs to be entered into the Parr menu).*

Important note: The amount of material used is normally around 1 g, however, be aware that the calorific level of the samples can vary greatly. If a sample is thought to have a high calorific value,

then less of the sample should be used.

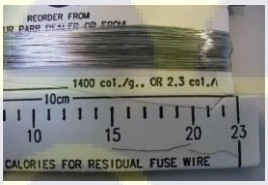
Samples should not be freeze dried or oven dried. Any samples that have been dried overnight or for 48hrs samples are not usable –they must be reconstituted before analysis as the powder form is a huge surface area and results in flash burning (very hot, very intense reaction –explosive results!)

Additional note: Samples that are too wet can result in a misfire (analysis failure).

Please note: Samples that have a suspected high fat/oil content or very high calorific level must be used with extreme care.

Set up the bomb head mechanism. Place the mechanism on the special holder for ease of use.

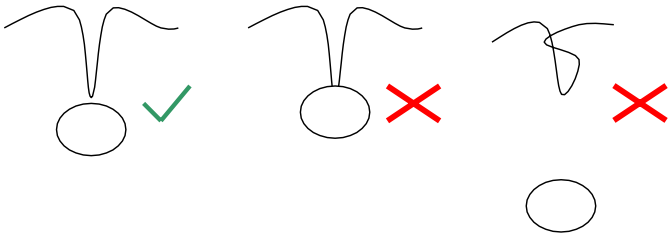
Place the crucible with the sample or benzoic acid into the bomb head (*you must have this sitting in the head holder to ensure the bomb head is not damaged!)*



Cut one strip of the fuse wire, **ensure that it is only one side length (fold to fold) from 0 to 23**-as this is vitally important, as it will affect your results. One fold is exactly 15kcal

Attach the fuse wire to the bomb head: Move the wire grips gently upwards to access the holes to put the wire through. Attach the wire through these holes and leave about 5mm space at the other side. Gently push the grips down but not far as it may cause the wire to snap.

Carefully bend the fuse wire above the sample.

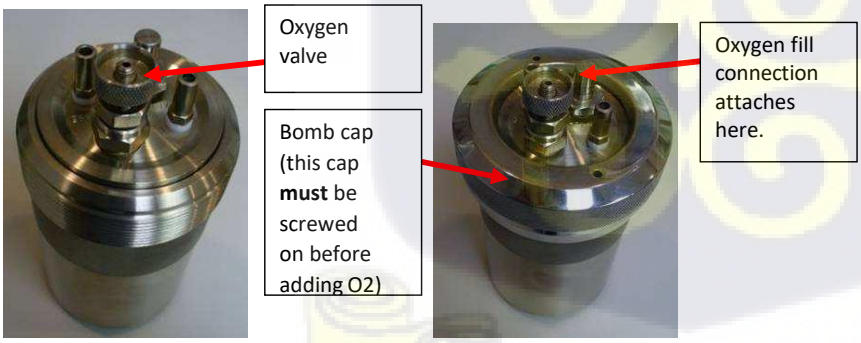


Please note the fuse wire **should NOT** touch the sample in any way –it should be placed just **above** the surface of the sample. Also the wire must not touch at any point as this will cause a short.

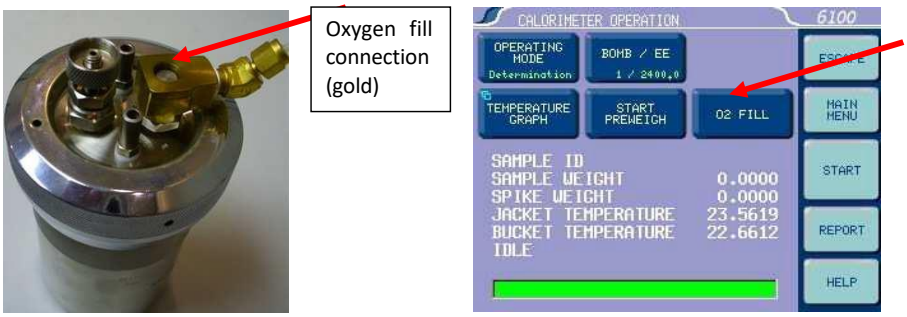
You **must** add a little distilled water (@2ml) to the base of the bomb cylinder before loading the bomb head into the cylinder. **Ensure the oxygen valve is open**. *Do not use oil or grease to lubricate the seals, if required only use a dH2O damp finger to rub gently around the seal –Never anything else!!*

Place the head into the cylinder and press down firmly, there should still be a little lip as it will not completely push down so don't force it too much! Tighten the **Oxygen valve** until it is finger tight only.

Screw on the cap as far as it will go. Do not over-tighten.



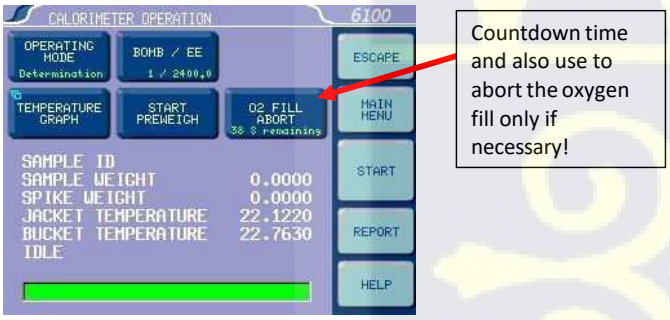
Place the gold **Oxygen Fill Connection** on to the bomb. Press down firmly to ensure it is locked in place.



Press the '**O2 Fill button'** on the '**Calorimeter Operation'** screen.

Oxygen will then flow into the bomb (*there will also be a countdown on the screen~ it generally takes 60 seconds to fill*). When it has filled with O2 there will be a loud "*psst*" sound as the gas is sealed off.

Note: Pressing the **O2 Fill key** while the timer is counting down will **abort** the fill process.



Fill the calorimeter bucket with 2 liters (2000 ± .5 g) of water. *(**Important:** Using the same amount of water each time is critical!!!)*

Place the bucket inside the calorimeter. **Do not tip the bomb!** -as the sample will fall out of the crucible! **Note:** There are three plastic pieces at the bottom of the Parr aircan that line up with the depressions in the bottom of the bucket.

Make sure to weigh out 2litres exactly. Use the balance scales.

Line up the bucket with the grooves in the bottom of the Parr aircan. Ensure the cables are clear of the water

Using the bomb lifter position the bomb part way into the bucket. It is usually better to place the bomb in with the lifter positioned north to south. (*The Bucket is shaped so that you can open the lifter*)

IMPORTANT!!!! You must note where the bubbles come from on the bomb!

If they come from the **oxygen valve** then remove the bomb and start from scratch again (*otherwise there will be a really nasty explosion!)*

Do NOT continue if there are bubbles coming from the bomb!

Bubbles from this point – **very bad!**

Bubbles from this points - okay

Ignition wire insertion

Attach the ignition wires securely to the terminals on the 1108 bomb head but try to avoid getting your fingers wet. Lower the bomb the rest of the way into the bucket. There is an embossed circle in the bottom of the bucket –**the bomb must sit in this**!

Close the lid making sure that neither the **stirrer shaft** nor the **bucket thermistor** are touching the 1108 bomb or bucket.

On the touch screen Press **Start**.

The screen will change and it will ask you to add in the follow

Input the Sample ID. Eg Sample 2

Input the Bomb ID. It is not necessary to alter this setting. **Input the Sample weight.** Input the exact result that you had from the weighing of your sample.

Input the Spike weight (if spiking is turned on). Not necessary (*unless using Urine/ paraffin)*

The test will then automatically proceed through the following steps:

Preperiod Cycle

Fire the sample It will bleep 5 times before firing

Post Period Cycle It will bleep once to tell you it has finished its run

Do not open machine before Post Period has completed its run. It usually takes between 6-15mins to complete a run *(longer time may be due to water temperature)*

Results

You can watch the graph for the cycle on the touch screen by choosing **Temperature Graph**

Red line is the bucket temperature, blue is sample

If after 40secs there has been no temperature rise then the machine will stop. This could be due to:

- ◊ Fuse wire problem

- ◊ Sample too wet
- ◊ Low O2 pressure
- ◊ Connecting wires problem

The expected temperature rise is 2 ½ -3oC- If the ΔT goes over 4oC then there is a warning from the machine.

Once the calorimeter is finished with the **post period cycle** the results will be displayed on the touch screen. When the **post period cycle** is over and the results are displayed the machine can be used again for another sample *(–once you have prepared the sample/bucket /bomb etc again*!)

Finishing up

Open the lid and carefully remove the bucket with the bomb using the **bomb lifter** and place into the sink.

If another pre-prepared bomb and bucket combination is ready it may be put in at this time.

Once you have removed the 1108 bomb from the bucket and carefully placed in the sink, release the pressure by gradually loosening the oxygen valve knob.(***Do not stand over the bomb as the release of gas can include nitrates, also the O2 release is like a pressure cooker!*)**

If you are analysing the bomb rinse water then release the pressure slowly (*over not less than one minute*) to avoid entrainment losses.

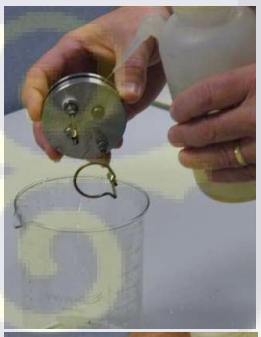
If the screw cap does not release turn the oxygen valve a little more to release any residual O2.

Fuse correction determination *

Remove the unburned remains of the fuse wire from the holder

Measure the unburned length and subtract from 10 cm. Multiply this by 2.3 cal/cm to get the result. The 45C10 Fuse Wire card may be used to directly measure how many calories the fuse wire contributed. **This step can be skipped if you are using a fixed fuse correction.*

Rinse the bomb head and cylinder with distilled water thoroughly.



Do not use soap or detergent or any other substance to clean any part of the Parr bomb apparatus!

Allow the bomb head, cylinder and screw cap to air dry.

Do not paper dry it!

Rinse the crucible clean and air dry in the drier oven –use a fresh clean, dry crucible each time you need to test a sample. The crucible needs to be completely dry before using the

benzoic acid. (*The crucibles will allow you to do analysis on 6 samples per hour*)

The bomb is now ready to prepare for the next test.

ADDITIONAL INFO

To analyse urine you will need to run the sample with a 'spike' this is usually paraffin. Run with the paraffin first to detect this spike first *then* add to urine -this needs to be accurately carried out.

If the Parr Calorimeter is serviced, then you need to 'clear the history' and start up the standardization from scratch again. The more calibrating that is done the more accurate the Parr calorimeter becomes.

APPENDIX 11 - SALIVA SAMPLE COLLECTION INSTRUCTIONS

ORAcollect
for pediatrics
REF OC-175

Collection precautions:

Ensure the sponge tip does NOT come into contact with any surface prior to collection.

Donor should NOT eat, drink, smoke or chew gum for 30 minutes before collecting oral sample.

Intended for the collection of human DNA from pediatric oral samples.

Contents: Contains 1 collection kit.

Warning and precautions: Sponge tip is a choking hazard. Caution should be used when inserting sponge into the mouth. For assisted collections do NOT leave infant or child unattended.

Wash with water if liquid comes in contact with eyes or skin. Do NOT ingest.
See MSDS at www.dnagenotek.com

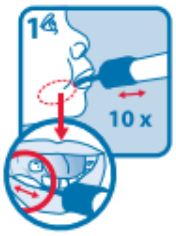
Storage: 15 – 25°C

Summary and explanation of the kit:
ORAcollect for pediatrics is an assisted collection kit that provides the materials and instructions for collecting and stabilizing human DNA from pediatric oral samples.

Label legend:

- Manufacturer
- REF Catalog number
- Non-sterile
- LOT Lot number
- Collect sample by (Use by)
- 15°C – 25°C Storage instructions

Instructions for sample collection:



Open package and remove collector without touching sponge tip. Place sponge as far back in the mouth as comfortable and rub along the lower gums (see close up image) in a back and forth motion. Gently rub the gums 10 times. If possible, avoid rubbing the teeth.

Gently repeat rubbing motion on the opposite side of the mouth along the lower gums for an additional 10 times.

Hold the tube upright to prevent the liquid inside the tube from spilling. Unscrew the blue cap from the collection tube without touching the sponge.

Turn the cap upside down, insert the sponge into the tube and close cap tightly.

Invert the capped tube and shake vigorously 10 times.

For Research Use Only
Not for use in diagnostic procedures

APPENDIX 12 - DNA EXTRACTION AND GENOTYPING PROTOCOL

GDNA EXTRACTION FROM SALIVA USING DNA GENOTEK ORAGENE KIT

Reagents and Consumables

- prepIT®•L2P (catalog #: PT-L2P)
- 1.5 mL microtubes (e.g., Axygen #MCT-150-C)
- Ethanol (95% to 100%) at room temperature
- Ethanol (70%) at room temperature
- DNA storage buffer: TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) or similar solution
- Filter pipette tips

Equipment

- Microcentrifuge capable of running at 15,000 × g
- Air or water incubator at 50 °C
- Micropipettes

Protocol

1. Set the oven to 50 °C.
2. Mix the sample in the DNA Genotek kit by inversion and gentle shaking for a few seconds.
3. Incubate the sample at 50°C in a water incubator for a minimum of 1 hour or in an air incubator for a minimum of 2 hours. ***Note:*** The use of an air incubator may be preferable since the sample tubes may float in a water bath. If a water bath must be used, ensure the sample containing portion of the tube remains immersed in water.
4. Transfer 500 µL of the mixed sample to a 1.5 mL microcentrifuge tube.

5. For 500 µL of sample, add 20 µL (1/25th volume) of PT-L2P to the microcentrifuge tube and mix by vortexing for a few seconds.
6. Incubate on ice for 10 minutes.
7. Centrifuge at room temperature for 5 minutes at 15,000 × g.
8. Carefully transfer the clear supernatant with a pipette tip into a fresh microcentrifuge tube. Discard the pellet containing impurities.
9. To 500 µL of supernatant, add 600 µL of room temperature 95% to 100% ethanol. Mix gently by inversion 10 times.
10. Allow the sample to stand at room temperature for 10 minutes to allow the DNA to fully precipitate.
11. Place the tube in the microcentrifuge ***in a known orientation***. Centrifuge at room temperature for 2 minutes at 15,000 × g.
12. Carefully remove the supernatant with a pipette tip and discard it. Take care to avoid disturbing the DNA pellet.
13. Ethanol wash: Carefully add 250 µL of 70% ethanol. Let stand at room temperature for 1 minute. Completely remove the ethanol without disturbing the pellet.
14. Add 100 µL of TE solution (see Page 1) to dissolve the DNA pellet. Vortex for at least 5 seconds.
15. To ensure complete rehydration of the DNA (pellet and smear) incubate at room temperature overnight followed by vortexing or at 50°C for 1 hour with occasional vortexing
16. Options for storage of the fully rehydrated DNA: a) Recommended in TE, in aliquots at -20°C for long-term storage, or b) In TE at 4°C for up to 2 months.

CHECKING QUANTITY AND QUALITY OF GDNA

The buffer is simply used to zero the spectrophotometer and should be the same as the buffer used for eluting the sample.

Consumables

Filter pipette tips

Optical lens wipes

1X TE / dH_2O

Equipment

Pipettes

DeNovix Spectrophotometer (https://www.denovix.com/ds-11/)

Protocol

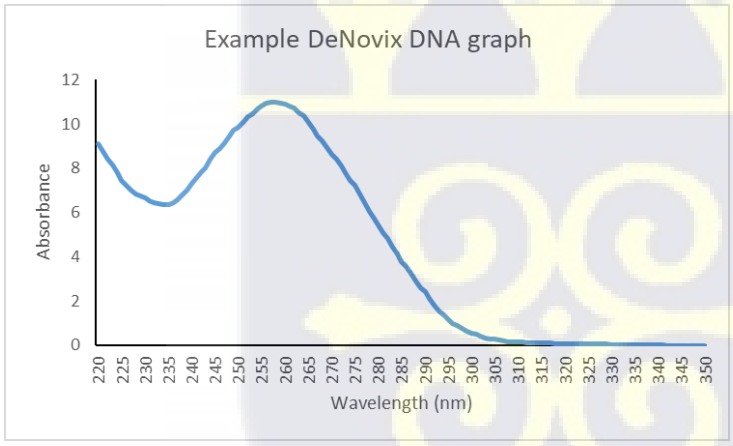
1. Open the DeNovix Spectrophotmeter and wipe down both the base and arm with an optical lens wipe – *don't use ordinary tissue as fibres will clog up the machine.*
2. Select the dsDNA icon (highlighted above).
3. Type in 'Blank' (in the space highlighted to the right).
4. Pipette 1.5 µl of dH_2O onto the platform.
5. Carefully close the arm.
6. Select the 'Blank' button.
7. Wipe down both the base and arm with an optical lens wipe.
8. Pipette 1.5 µl of sample onto the platform.
9. Carefully close the arm.
10. Type in your sample name (in the space highlighted to the right).

11. Select the 'Measure' button to read the sample.
12. Note down the A_{260}, A_{260}:A_{230} and the A_{260}:A_{280} ratio as well as the DNA concentration.

Note

1. High quality gDNA should have an A_{260}:A_{280} ratio of approximately 1.8. Higher values indicate RNA contamination, lower values indicate protein contamination.
2. The A_{260}:A_{230} ratio is used as a secondary measure of nucleic acid purity. High quality gDNA should have an A_{260}:A_{280} ratio of approximately 2.0. Strong absorbance around 230 nm can indicate that organic compounds or chaotropic salts are present in the purified DNA. Lower values indicate protein contamination.

The graph should look like this.



13. Check that all the values (for A_{260}:A_{280} ratio and total extracted value) are acceptable, if the result is strange,
    a. Re-run the samples by clicking on read samples again.
    b. If still not correct then go back to step 6 and repeat process

14. Dilute the DNA to ~10 ng/µl ready for the qPCR reaction.
    a. Take 10-50 µ l of your DNA into a fresh 1.5ml tube.
    b. Add dH_2O to required volume – *note you may need to do two serial dilutions*.
    c. For example, if your DNA is at 500 ng/ µl, take 10 µ l of DNA and add 490µ l of dH_2O to give 10 µ g/ µl.

QPCR FOR GENOTYPING

Consumables

Filter pipette tips

384-well qPCR plates

Thermal optically clear seals

1.5 ml tubes

2X TaqPath™ ProAmp™ Master Mix

40X Taqman probe assay

dH2O

Equipment

Pipettes

Roche LC480 with 384-well blockTaqman probes can be used for genotyping or gene expression. Although they are slightly different versions in each case. For genotyping, each assay contains one pair of primers and **TWO** probes of different colours: each probe recognises and binds to a different version of the SNP. Set up a mastermix using the ingredients below. You should always make a little extra to make sure you don't run out.

qPCR Mix for Taqman genotyping. Note that the gDNA is not added to the mastermix.

Mastermix	Mix for 1 (µl)	Mix for 3 (µl)
2X TaqPath™ ProAmp™ Master Mix	5	15
40X Taqman assay	0.2	0.6
dH_2O	2.8	8.4
gDNA (5-10 ng/µl)	2	-
Total	10	24

NOTE. This reaction must be protected from light to avoid bleaching of the fluorescence. Once ready, place enough for one reaction, along with the gDNA sample, in one of the wells of the qPCR plate (which the members of staff have) and note down which well you placed it in. This can be sealed and stored in the fridge until all samples are loaded and ready to run it.

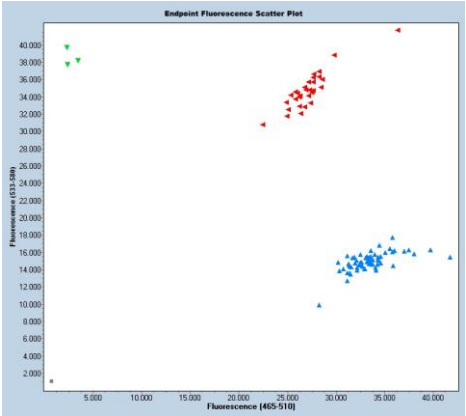
Place the 384-well plate with all the samples in the qPCR machine (Roche LC480) and set the reaction running using the cycling conditions below.

qPCR cycle settings for Taqman genotyping.

95ºC	15 min	
95ºC	15 s	┐
60ºC	1 min	├ 40 cycles
37ºC	1 s	

Note – This is known as two-step PCR. The polymerase does not only work at 72 ºC, it also works (less efficiently, but well enough) at nearby temperatures, which allow for a shorter experimental time. The qPCR genotyping reaction will take approximately 75 minutes.

A typical result from Taqman genotyping:



This shows the fluorescence from each probe plotted on one axis. The three genotypes cluster into different groups.

APPENDIX 13 - STATISTICAL QUALITY CONTROL- HARDY WEINBERG EQUILIBRIUM (HWE)

SNP	Closest gene	No. of Samples	Allele frequency p	q	Genotype	Observed (o)	Expected (E)	O-E	$(O-E)^2/E$	Chi sq	P. value		African MAF
rs13021737	TMEM18	187	0.92	0.08	GG	159	157.28	1.72	0.02		0.099	Range	5-15%
					AG	25	28.43	-3.43	0.41	2.72		Nigeria	7%
					AA	3	1.28	1.72	2.29			Sierra Leone	15%
rs10246939	TAS2R38	239	0.53	0.47	CC	62	66.43	-4.43	0.30		0.251	Range	41-58%
					CT	128	119.15	8.85	0.66	1.32		Nigeria	53/54%
					TT	49	53.43	-4.43	0.37			Sierra Leone	51%
rs543874	SEC16B	227	0.66	0.34	AA	94	98.88	-4.88	0.24		0.187	Range	23-37%
					AG	111	101.88	9.12	0.82	1.74		Nigeria	25/27%
					GG	22	26.24	-4.24	0.69			Sierra Leone	28%
rs1516725	ETV5	205	0.83	0.17	CC	141	141.81	-0.81	0.01		0.688	Range	12-21%
					CT	59	57.39	1.61	0.00	0.16		Nigeria	17/19%
					TT	5	5.81	-0.81	0.11			Sierra Leone	13%
rs2274333	CA6	214	0.89	0.11	AA	173	171.37	1.63	0.02		0.235	Range	6-15%
					AG	37	40.27	-3.27	0.27	1.41		Nigeria	6/11%
					GG	4	2.37	1.63	1.13			Sierra Leone	8%
rs13078960	CADM2	229	0.97	0.03	GG	0	0.18	-0.18	0.18		0.658	Range	1-7%
					GT	13	12.63	0.37	0.01	0.20		Nigeria	3/4%

					TT	216	216.18	-0.18	0.00			Sierra Leone	1%
rs1558902	FTO	218	0.95	0.05	TT	199	198.46	0.54	0.00		0.402	Range	2-10%
					AT	18	19.08	-1.08	0.06	0.70		Nigeria	6%
					AA	1	0.46	0.54	0.64			Sierra Leone	3%
rs6567160	MC4R	197	0.78	0.22	TT	119	118.83	0.17	0.00		0.943	Range	16-25%
					CT	68	68.35	-0.35	0.00	0.00		Nigeria	16/23%
					CC	10	9.83	0.17	0.00			Sierra Leone	21%
rs10182181	ADCY3	220	0.93	0.07	GG	192	191.96	0.04	1.02		0.961	Range	4-19%
					AG	27	27.09	-0.09	0.00	0.00		Nigeria	6/7%
					AA	1	0.96	0.04	0.00			Sierra Leone	4%

Appendix 14- Calories in test meals (Bomb Calorimetry)

code	Food item	Wet sample weight (g)	Dry sample weight (g)	Moisture content (%)	R1 calories (cal)	R2 calories (cal)	Average sample calories (cal)	Wet sample calories (cal)	Wet sample Kcal/ g (Kcal)
001	Apapransa	2.82	0.90	68.08	5197.94	6275.83	5736.89	1835.31	1.84
002	Banku	2.67	0.75	71.91	3969.77	4041.04	4005.40	1128.10	1.13
003	Banku	2.85	0.68	76.14	3879.18	3964.81	3921.99	942.66	0.94
004	Banku	2.15	0.66	69.30	4019.87	3982.78	4001.32	1225.89	1.23
005	Banku	4.66	1.07	77.04	4083.82	3941.46	4012.64	919.92	0.92
006	Boiled chicken	3.30	1.12	66.06	6091.50	6071.50	6081.50	2059.58	2.06
007	Boiled egg	5.72	1.20	79.02	6640.63	5864.08	6252.35	1309.38	1.31
008	Fish-fried	3.77	2.41	36.07	6366.96	6275.83	6321.40	4028.29	4.03
009	Fish-Smoked	3.00	1.18	60.67	5534.14	5916.20	5725.17	2243.44	2.24
010	Grounded pepper	3.25	0.72	77.85	3885.92	4623.78	4254.85	942.29	0.94
011	Groundnut soup	4.56	0.76	83.33	7115.30	5140.17	6127.73	1026.21	1.03
012	Jollof	2.35	1.25	46.81	4864.21	4352.06	4608.13	2449.02	2.45
013	Jollof	2.37	0.87	63.29	5326.40	4794.71	5060.56	1855.89	1.86
014	Jollof	3.44	1.51	56.10	4723.34	4486.26	4604.80	2018.25	2.02
015	Jollof	2.73	1.08	60.44	4593.59	4590.36	4591.98	1818.49	1.82
016	Jollof and spaghetti	4.15	1.64	60.48	4349.82	4287.50	4318.66	1703.30	1.70
017	Ga kenkey	3.18	1.21	61.95	4156.34	4130.37	4143.35	1580.99	1.58
018	Kokonte	3.34	1.04	68.86	3710.31	3835.09	3772.70	1175.68	1.18
019	Mpotompoto	2.34	1.06	54.70	5406.15	5313.88	5360.01	2416.60	2.42
020	Mpotompoto	5.34	1.27	76.22	5307.78	6238.64	5773.21	1375.92	1.38
021	Okro stew	5.01	0.43	91.42	3194.74	3306.78	3250.76	276.08	0.28
022	Okro stew	4.76	1.08	77.31	7327.65	7526.43	7427.04	1678.22	1.68
023	Okro stew	4.77	0.57	88.05	6255.56	5594.26	5924.91	703.66	0.70
024	Okro stew	5.32	0.76	85.71	6331.88	6480.84	6406.36	919.81	0.92
025	Roasted peanut				7016.67	7409.45	7213.06		7.21
026	Rice and garden egg stew	3.85	1.54	60.63	5028.98	5361.17	5195.07	2074.68	2.07
027	Rice and gravy	2.89	1.18	59.17	4769.00	4240.55	4504.78	1841.45	1.84
028	Rice and gravy	3.86	1.46	62.18	4717.99	4147.09	4432.54	1671.17	1.67
029	Rice and gravy and spaghetti	2.66	1.10	58.65	4623.14	4253.26	4438.20	1830.44	1.83
030	Semolina	3.60	0.93	74.17	4020.81	4020.81	4020.81	1033.17	1.03
031	Tombrown	5.12	1.03	79.88	4305.84	4209.35	4257.60	853.70	0.85
032	Tumeric rice and egg stew	3.42	1.21	64.62	4991.98	4894.74	4943.36	1754.69	1.75
033	Vegetable and fish stew	4.82	1.11	76.97	5368.02	5240.79	5304.40	1219.87	1.22
034	Yumvita-rice				4442.72	4647.55	4545.13		
035	Cerelac- millet				4544.64	4762.53	4653.59		
036	Cerelac -rice				4505.52	4761.14	4633.33		
037	Cerelac-fruitmix				4687.41	4904.45	4795.93		
038	Gari and sugar and danu milk				3961.11	4156.85	4058.98		

R1-bomb calorimetry run 1, R2-Bomb calorimetry run 2, Dry sample- food sample after freeze drying, wet sample- actual food sample